

申 报	系列：教学科研
	专业：动物营养与饲料科学
	职称：教授

业绩成果材料

（申报人的业绩成果材料包括论文、科研项目、获奖以及其他成果等）

单 位（二级单位） 动物科学学院

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材料核对人：

单位盖章：

核对时间：

华南农业大学制

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一、教学研究业绩

项目编号: JG22 _ _ _

华南农业大学教育教学研究和改革项目

申 报 书

项目类别 重点项目

项目名称 《饲料学》线上线下一体化混合教学设计探究

项目负责人 朱 勇 文

职 称 副教授

手机号码 18818912892

工作单位 动物科学学院

申报日期 2023.12.1

华南农业大学 教务处 制

2023 年 10 月

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项目负责人（签章）：



2023 年 12 月 1 日

一、项目及项目负责人、项目组简况

项目 简 况	项目名称	《《饲料学》线上线下一体化混合教学设计探究					
	项目类别	<input type="checkbox"/> √1. 重点项目 <input type="checkbox"/> 2. 一般项目 <input type="checkbox"/> 3. 青年项目					
	起止年月	2023 年 12 月-2026 年 12 月					
项目 申 请 人	姓名	朱勇文		性别	男	出生年月	1986 年 7 月
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	所在单位 及联系方 式	单位名称	动物科学学院			手机号码	18818912892
		电子邮箱	zhuyw0724@scau.edu.cn				
	主要教学 工作简历	时间	课程名称	授课对象	学时	所在单位	
		2023-2024 年 度第 1 学期	饲料学	21 级本科	32	动物科学学 院	
		2022-2023 年 度第 2 学期	饲料学	20 级本科	32	动物科学学 院	
		2022-2023 年 度第 1 学期	饲料学	20 级本科	32	动物科学学 院	
		2021-2022 年 度第 2 学期	饲料学	19 级本科	32	动物科学学 院	
		2021-2022 年 度第 1 学期	饲料学	19 级本科	32	动物科学学 院	
		2022-2023 年 度第 1 学期	动物营养与 饲料实验课	20 级本科	112	动物科学学 院	
	主要教学 改革和科 学研究工 作简历	时间	项目名称				获奖情况
		2019-2021	线下示范课程—《饲料学》				校级质量工 程建设项目
		2017-2018	畜牧类专业《饲料学》课程过程考核评 价机制的探索与实践				学院自筹
		2021-2023 年	《富硒低胆固醇保健鸡蛋研制及其配 套技术开发》				广东大学生 科技创新攀 登计划项目
项目 组	总人数	职称			学位		
		高级	中级	初级	博士后	博士	硕士
	5	4	1			3	2

	主要成员 (不含申请者)	姓名	性别	出生 年月	职称	工作单位	分工	签名
		叶慧	女	1981. 1	高级实验师	动物科学学院	教学改革实施	
		杨琳	男	1963. 9	教授	动物科学学院	教学改革研究	
		王文策	女	1982. 10	教授	动物科学学院	教学改革研究	
		董泽敏	女	1977. 9	讲师	动物科学学院	教学改革实施	

二、立项依据（项目研究的意义、现状分析）¹

饲料是动物赖以生存和生存畜产品的物质基础。饲料学是一门研究饲料的学问，目的在于揭示饲料的化学组成、转化规律及动物营养需要之间的关系。饲料学对动物生产和饲料业的发展至关重要，它不仅是培养动物生产、动物营养与饲料科学专业人才的一门重要课程，也是推动动物生产和饲料业不断发展的理论和技术基础。动物科学专业是国家级特色专业和广东省名牌专业，也是国家级人才培养模式创新实验区的试点专业。而饲料学是动物科学专业的重要传统专业课程之一，该课程伴随畜牧系 1987 年成立至今一直在开设，助力培养动物科学专业杰出的复合应用型人才。但是饲料学课程持续建设的脚步缓慢，只是在教材方面进行了相应更新，而在互动式多媒体教学、慕课、微课等多元化现代教学手段和方式方面建设有所欠缺，远远落后于其它专业课程。但饲料学作为一门偏应用的课程，学院营养系的老前辈们花费了大量的时间和精力，收集了许多有意义和价值的实践视频资料和科研成果。这些技术资料为《饲料学》线上线下混合教学线上教学多元化立体设计打下了坚实的基础。此外，饲料研发方面与广东温氏集团等知名企业精诚合作，探索形成了

³ 表格不够，可自行拓展加页；但不得附其他无关材料。下同。

“产学研”紧密结合的发展思路，构建产学研合作平台，不断促进科技成果转化，为饲料学线下实践学习提供了很好的契机。同时，依托动物科学学院增城宁西教学基地、华南动物营养与饲料科学观测实验站，均可满足《饲料学》线上线下混合教学实操训练教学顺利开展所需条件。此外，通过基于《饲料学》线上线下混合式教学模式的探究，可辐射或新增动物营养学等教学实验项目，其中包括能充分锻炼学生综合实践能力的综合性实验、设计性实验和创新性实验，实现“以学定教”、“以学带教”、“多学少教”的有机结合。同时，制作多媒体课件或教学资源库，配合完成本科动物科学技能大赛，将很好地满足培养综合性卓越本科人才这一需求和目标。

三、项目实施方案及实施计划

1. 具体改革内容、改革目标和拟解决的关键问题

紧紧围绕全面提高本科教育和畜牧类人才能力的主题，以加强专业教学质量为目标，以探究线上线下混合式教学模式为切入点，以动物科学学院动物科学专业必修课程《饲料学》为示范点，结合数字课程、案例视频、虚拟仿真、饲料营养价值评定、豆粕生产工艺研学、饲料掺假鉴定、饲料青贮实操、饲料厂参观实习等线上线下虚实融合的手段，翻转传统课堂以实现立体混合式的智慧教学课堂，突破传统教学的时空限制，实现教与学的实时互动，满足不同场景的教学，充分发挥和挖掘学生这一主体在教学活动的基础，积极倡导“以学定教”、“以学带教”、“多学少教”的教学思维，积极探索和推进我校动物科学专业高质量卓越人才培养的改革，着力创新我校人才培养模式，形成多样化人才培养的新思路、新模式和新方法，着力培养“深基础、宽口径、高素质、强能力的复合型人才”，努力培养新农科复合型畜牧人才工作目标。

2. 实施方案、实施方法、具体实施计划（含年度进展情况）及可行性分析

（1）实施方法：基于线上线下混合教学模式，结合数字课程、案例视频、虚拟仿真、饲料营养价值评定、豆粕生产工艺研学、饲料掺假鉴定、饲料青贮实操、饲料厂参观实习等线上线下虚实融合的手段，以实现立体混合式的智慧教学课堂。

具体教学设计技术要点与方法如下：

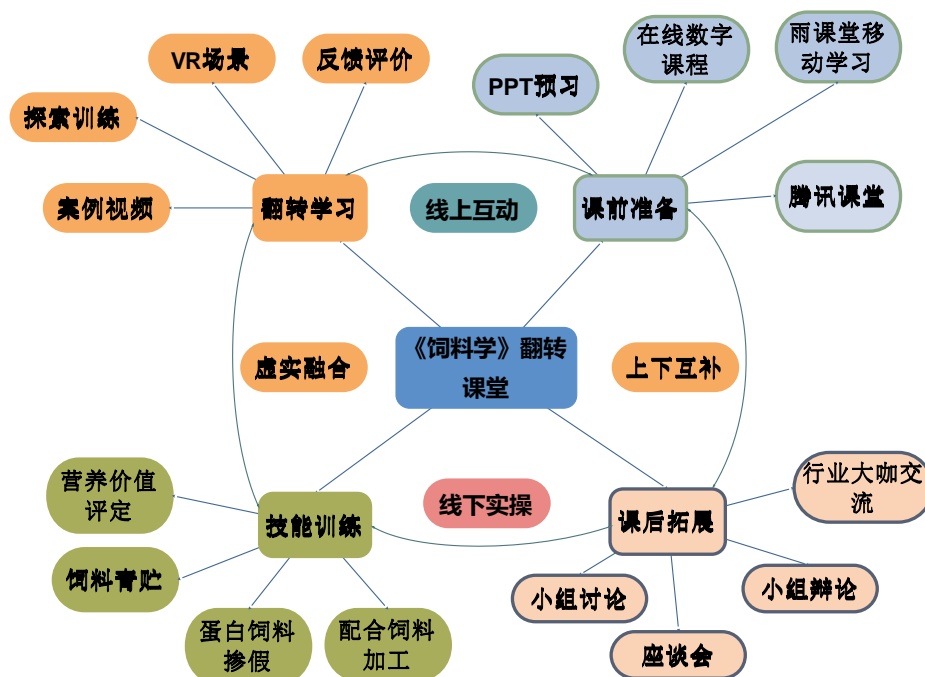


图 1 《饲料学》线上线下教学立体交叉网络图

(2) 实施方案

1. 线上学习

1) 登陆华南农业大学在线教育综合平台 <http://eol.scau.edu.cn/meol/course.do>，获取老师发布的饲料学课前教学资源 PPT 和视频等，启发学生发现问题并自主学习给出答案。



3) 通过雨课堂 APP 移动学习与问题讨论。



4) 通过中国农业教育在线网站 <https://www.ccapedu.com/selectProfessional.action>，在线学习饲料学国家精品课程。



5) 案例视频学习和虚拟场景训练。



饲料青贮案例



VR 实验场景模拟训练

2. 线下学习

1) 课堂教学改革—行业大咖+讨论互动式教学

首先选用智慧教室分桌式小组讨论教学，打破老师教学生听的传统死板教学，邀请行业大咖在饲料学课程上探索“个性化、互动式”教学模式改革。教学过程中，老师可以随时可以和学生近距离教学、互动和探讨；学生可针对老师提出的问题或感兴趣的话题，实现学习小组间互动交流，切实提升课堂教学质量。最终实现互动教学系统能够覆盖课本知识、行业大咖、生产实践和科研前沿等各个饲料学综合知识。



图 1 智慧课堂讨论互动式教学

2) 阶段性评价—注重实践和创新思维培养

饲料学作为动物营养学科核心课程，知识更新快，特别是饲料资源开发利用进展大，单独靠单一、老化的考试内容已经不能体现学生的知识掌握。通过过程性考核评价、阶段性考核评价和综合性考核评价相结合的手段进行了过程性考核制度改革探索，并取得了良好的效果。比如本项目在过程性阶段评价中布置了饲料厂布局创新设计大赛，学生们通过选题立项、试验分析、模型制作等实践环节是不断遇到问题、不断解决问题，将创新思维物化为模型、试验、行动的过程。选题及其后解决问题过程是激发创新思维和创新实践的过程，出现了很多优秀的作品。



图 2 饲料原料识别鉴定课堂现场

3) “理论讲授—实操训练—开放研讨”三位一体

我国现代饲料学要求本科人才需具备扎实的基础知识和实践技能，具有创新精神和实践动手能力。实操训练是培养学生创新意识和动手能力，提升学生综合素质的重要环节。本项目饲料营养价值评定环节，首先以教师为主讲授理论知识；然后设计实操训练

实验，并指导学生完成实验；最后根据该章节的知识点选择研讨主题，并引导学生完成研讨课。将理论知识具体化、形象化，构建“理论讲授—实操训练—开放研讨”三位一体教学模式。



图3 强饲法线上线下互动图

4) 实践教学—注重培养应用复合型人才

根据“新农科”人才要求，高校需明确规划动物科学专业建设目标，制定专业实践教学方案，优化课内教学内容，强化课外实践内容。饲料学课程对接“新农科”人才培养要求，独立开展饲料厂参观和实习内容，熟悉企业生产、加工、检测等工作。特别是对动物营养与饲料学感兴趣的学生，暑假安排到企业进行轮岗实习，由企业导师指导其在其各生产环节技能训练，一定程度上促进了动物科学专业应用型人才培养模式的创新。



图4 指导学生开展饲料营养评价实验

5) 开展过程性课堂效果评价

包括对课前任务、课堂任务、课堂提问、课后作业、期末考试等教学效果过程评价指标进行全面评价。

调查题目	调查结果(人数/比例)				
	很好	较好	一般	较差	很差
与传统课堂教学相比,在提高学习主动性方面的效果	17/45.9%	15/40.5%	2/5.4%	2/5.4%	1/2.7%
与传统课堂教学相比,在提高学习效率方面的效果	16/43.2%	16/43.2%	3/8.1%	1/2.7%	1/2.7%
与传统课堂教学相比,在提高对所学知识的掌握程度方面的效果	15/40.5%	16/43.2%	2/5.4%	2/5.4%	2/5.4%
与传统课堂教学相比,在提高自主学习的能力方面的效果	18/48.6%	14/37.8%	2/5.4%	1/2.7%	2/5.4%
与传统课堂教学相比,在提高分析问题和解决问题的能力方面的效果	14/37.8%	17/45.9%	3/8.1%	2/5.4%	1/2.7%

教学效果满意调查表

评价方式		评价项目	比例	备注
形成性评价	课前任务	学习总时长	2%	根据平台数据,由教师进行评分。
		课前讨论交流情况	2%	教师评分。
		课前测试成绩	5%	教师评分。
	课堂任务	上课出勤率	2%	教师评分。
		课堂参与活动的积极性	2%	教师评分。
		小组成员合作探究	3%	根据小组成员贡献及团队意识,由小组成员互评得分。
		上课发言情况	2%	根据回答问题的准确程度,由教师进行评分。
		课堂测试成绩	5%	教师评分。
	课后任务	课后总结与反思	2%	小组成员互评得分。
		课外拓展项目完成情况	5%	小组成员互评得分。
总结性评价	期末考试	期末考试成绩	70%	教师阅卷评分。
总体评价	形成性评价和总结性评价相结合		100%	教师综合评分。

教学效果过程评价指标

(6) 面对面多形式课后复习

- 1) **座谈会**: 与饲料学专业课在教和退休老师座谈,讨论饲料学学习方法与技巧;与高年级师兄师姐座谈,讨论和交流饲料学学习心得。
- 2) **交流会**: 邀请行业大咖分享饲料产业发展趋势和行业前景;邀请毕业师兄师姐回校学相关工作社会经验;学生自身未来规划。
- 3) **辩论会**: 就饲料行业热点辩证性话题,组织辩论赛,考查学生思维与思考方式。
- 4) **技能培训**: 依托本科实验室和试验基地,练习饲料裹包青贮、鱼粉掺假和配合饲料生产等饲料学实用试验技巧,提高本校动物科学饲料技能大赛的竞争力。

(3) 实施计划

第一阶段(2023年12月-2024年12月):依托本校动物科学专业,建设农科教人才培养基地的教学软件条件,建立《饲料学》线上线下立体混合式的智慧教学模式的的运行机制、培养方案、教学效果评价和反馈体系。

第二阶段(2024年12月-2025年12月)加大线上教学软硬件条件的建设,建立线下实操训练的场所和相应的配套设备;建设线上下虚实融合课程运行模式;建立反馈机制共享平台;整理评价效果和意见。

第三阶段(2026年1月-2026年6月):巩固线上线下课堂建设的成果,根据翻转效果存在的问题进一步优化线上下交叉运行的机制,完善课程立体多元教学模式,补充丰

富课堂教学资源。

第四阶段（2026 年 7 月-2026 年 12 月）：加强与动物营养与饲料实验课的合作力度，提高实践动手能力，提高学生动物科学饲料技能大赛的竞争力；同时对其他动物营养与饲料学类课程产生示范和辐射作用。

3. 项目预期成果及其实践运用预期（包括成果形式，预期推广、应用范围、受益面等）

1. **学科方面：**通过基于《饲料学》线上线下混合式教学模式的探究，制作多媒体课件或教学资源库 1-2 个，共享和配合姊妹课程《动物营养学》的多元化教学，提高动物营养与饲料科学学科的整体教学质量。

2. **实验教学方面：**实现线上线下混合教学线上线下虚实融合教学，辐射协助带动动物营养与饲料学等教学实验课有效开展，充分锻炼学生综合实践能力，提高本科动物科学技能大赛竞争力。

3. **创新创业或教改课题方面：**新增饲料学创新创业项目 1 项，发表教改论文 1 篇。

4. **实践教学：**配合农业部华南动物营养与饲料科学观测实验站建设，新增一个饲料配制教学示范基地 1 个。

4. 本项目的特色与创新点

（1）打造以学生为主体的线上线下课堂混合式教学模式，构建动物科学专业人才培养新模式，实现“专业知识+操作技能+创新能力”三位一体的多元化动物科学专业人才培养体系，实现“深基础、宽口径、高素质、强能力复合型人才”培养的整体优化。

（2）实现教学线上线下虚实融合，巩固学生的理论知识，使学生进一步加强对饲料学的认识、理解与深化。使学生学到的理论知识与生产实践相结合，在实践中不断总结知识点使之在理论学习中具体化，最终学以致用。

四、项目建设基础

1. 与本项目有关的工作积累和已取得的工作成绩

(1) 课堂教学改革—行业大咖+讨论互动式教学

探索性邀请温氏集团家禽饲料配方师刘松柏总经理饲料学历史、现状和发展趋势。针对“饲料营养价值评定”和“青贮饲料”教学环节，分别邀请了该领域专家广东省农科院李平老师和林学院张庆老师，进行了专场示范教学。



图 2 邀请行业大咖互动式教学

(2) 阶段性评价—注重实践和创新思维培养

通过和叶慧老师商量，把饲料原料识别和掺假鉴定搬到了课堂上，学生们把学到的理论知识得到现场应用。此外，通过凝练和总结探索过程考核评价改革，指导学生注重实践和创新思维培养，并获得广东省第一届动物科学技能创新大赛特等奖，第一作者发表 SCI 论文 1 篇。

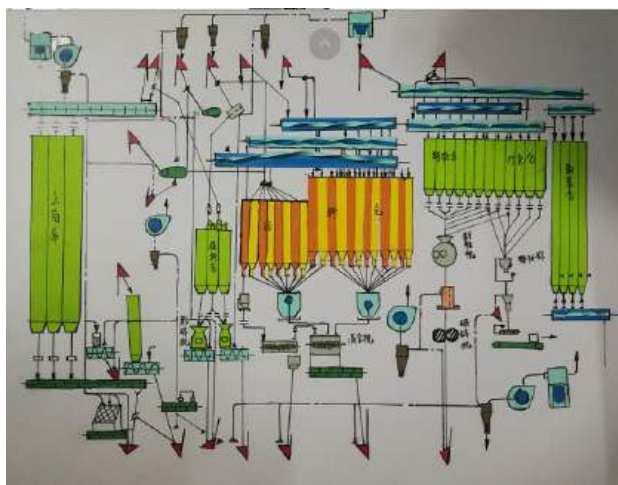


图 3 饲料厂布局设计大赛模型



图 5 指导学生获得第一届技能创新大赛特等奖

(4) 饲料厂参观实践教学—注重培养应用复合型人才

每年安排饲料厂参观实践教学，特别是对动物营养与饲料学感兴趣的学生，暑假安排到企业进行轮岗实习，由企业导师指导其在各生产环节技能训练，一定程度上促进了动物科学专业应用型人才培养模式的创新。



图 10 饲料厂现场培训和参观实景

2. 单位对项目的支持情况（含有关政策、经费及其使用管理机制、保障条件等，可附有关文件），尚缺少的条件和拟解决的途径

（1）动物科学学院营养系的老师们收集了许多有意义和价值的实践视频资料和科研成果。这些技术资料为《饲料学》线上线下混合教学线上教学多元化立体设计打下了坚实的基础。

（2）饲料学课程实践方面与广东温氏集团等知名企业精诚合作，探索形成了“产学研”

紧密结合的发展思路，构建产学研合作平台，不断促进科技成果转化，为饲料学线下实践学习提供了很好的契机。

(3) 依托动物科学学院增城宁西教学基地、华南动物营养与饲料科学观测实验站，均可满足《饲料学》线上线下混合教学实操训练教学顺利开展所需条件。

3. 项目负责人和项目组成员所承担的教学改革和科研项目情况

①主持的教学研究课题

(1) 2019 年校级质量工程建设项目：线下示范课程——《饲料学》(zlgc19056)。

(2) 2022 年度广东省大学生创新训练及创业实践项目《非淀粉多糖酶和蛋白酶组合在黄羽肉鸡玉米豆粕减量替代的应用效果研究》。

(3) 2017 年校级教改课题——畜牧类专业《饲料学》课程过程考核评价机制的探索与实践 (JG17122)

②发表教改论文

[1]朱勇文,董泽敏,付晓兰. 基于在线开放课程立体教学模式的探究——以华南农业大学饲料学为例. 黑龙江畜牧兽医, 2021(02): 152-154.

[2]朱勇文,叶慧,何丹林等. 动物生产类虚拟仿真实验教学的建设与实践——以黄羽肉鸡饲养及性能测定虚拟仿真实验为例. 广东饲料, 2022, 31(06): 26-28.

[3]朱勇文. 传统师德对当代高校师德建设的启发. 新一代:理论版, 2019(4): 54-54.

[4]朱勇文. 改革《饲料学》教学培养创新型适用人才. 高考, 2019(17): 16-16.

③获得的教学表彰/奖励

(1) 2020 年, 第一届广东省本科高校动物生产类大学生创新大赛特等奖指导老师。

(2) 2020 年, 华南农业大学动物科学学院 2019 年度青年教师教学观摩比赛一等奖。

(3) 2020 年, 华南农业大学动物科学学院 2019 年度青年教学十佳。

(4) 近三年指导本科生发表论文 4 篇, 其中 SCI 1 篇, 中文核心 2 篇。

五、经费预算

预算经费总额		10000（元）	
序号	支出科目	预算	支出用途
1	设备费	4000	购买线上线下课堂视频拍摄和制作相关设备。
2	材料费（含打印费、耗材费）	2000	
3	图书资料费	1000	用于专业资料和教学视频版权购买等。
4	软件服务费（含课堂录像及网站制作）	0	
5	交通费	1000	用于校外实践教学参观学习。
6	差旅费	1000	用于参加课程改革会议的车费和住宿费
7	会议费	1000	用于参加会议学习
8	出版/文献/信息传播/知识产权事务费		
9	专家咨询费		
10	其他费用		

六、单位意见²

所在单位意见：

该项目以《饲料学》课程为载体，开展线上线下
一体化混合式教学设计探索，项目改革内容
合理，实施方案可行，同意申请。

单位负责人签字：

2023 年 12 月 动物科学学院



基于翻转课堂立体教学模式的探究

——以华南农业大学饲料学为例

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摘要: 依据新农科动物生产类复合应用型人才的培养目标,畜牧类本科人才培养方案对专业课程教学内容、方式和方法的改革提出了新要求。笔者以动物科学专业必修课程饲料学为建设尝试点,从课程建设的必要性、任务目标、具体举措(线上线下虚实融合等)和进展效果(实现了“以学带教”和“多学少教”的有机结合)等方面探究基于翻转课堂立体教学模式的改革和实践的可行性。

关键词: 饲料学; 翻转课堂; 立体式教学; 虚实融合; 新农科; 课程改革

饲料是动物赖以生存和生产畜产品的物质基础。饲料学不仅是培养动物生产、动物营养与饲料科学专业人才的—门重要课程,也是推动动物生产和饲料业不断发展的理论和技术基础。近些年来,饲料科学理论和实践技术得到了长足的发展和进步,为了使—学生扎实掌握“无抗饲料、绿色饲料添加剂和饲料安全”等新理论和新技术,需要从教学手段、方法和方式等方面进行新的探索与实践。特别是依据新农科动物生产类复合应用型人才培养的新要求,饲料学课程教学也亟待深化教学模式的改革和创新^[1]。笔者以华南农业大学饲料学课程教学为例,在采用多媒体教学形式的基础上,通过师生角色、学习方式、课堂流程和评价体系等功能的翻转,并结合数字课程、案例视频、虚拟仿真等虚实融合的手段,翻转传统课堂以探究立体混合式智慧教学模式的可行性,希望为新农科动物生产类其他动物营养与饲料课程群的教学改革提供—定参考。

1 饲料学翻转课堂建设的背景

1.1 必要性和可行性分析

华南农业大学饲料学课程自畜牧系 1987 年成立起至今—直在开设,目前作为核心专业课程助力培养动物科学专业复合应用型新农科人才。但是饲料学课程持续建设的脚步缓慢,只是在教材方面进行了相应更新,而在互动式多媒体教学、慕课、微课等多元化

现代教学方式方面的建设有所欠缺,远远落后于其他专业课程。而饲料学作为—门应用性很强的课程,动物科学学院营养系的前辈们花费了大量的时间和精力,收集了许多有意义和价值的实践视频资料和科研成果,这些技术资料为饲料学翻转课堂线上教学多元立体化设计打下了坚实基础。此外,饲料研发方面与广东温氏集团等知名企业精诚合作,探索形成了“产学研”紧密结合的发展思路,构建“产学研”合作平台,不断促进科技成果转化,为饲料学线下实践学习提供了更多的机会。同时,动物科学学院广州增城宁西教学基地和华南动物营养与饲料科学观测实验站的设施设备,也能满足饲料学翻转课堂实操训练教学顺利开展所需的条件。

1.2 建设目标

紧密围绕全面提高本科教育和新农科人才培养的主题,以加强专业教学质量为目标^[2],以探究翻转课堂立体式教学模式为切入点,以动物科学专业必修课程饲料学为示范课,通过翻转传统课堂调动学生参与教学活动的积极性,充分发挥学生在教学活动中的主体作用^[3],有效推动华南农业大学动物科学专业高质量卓越人才培养的改革。同时,课程改革着力创新畜牧人才培养模式这—根本出发点,形成多样化人才培养的新思路、新模式和新方法,努力打造“深基础、宽口径、高素质、强能力”的复合型动物生产类人才^[2]。此外,基于翻转课堂的饲料学立体式教学模式的探究,可辐射或新增《动物营养学》等教学实验项目,其中包括能充分锻炼学生综合实践能力的综合性试验、设计性试验和创新性试验,实现“以学定教”“以学带教”“多学少教”的有机结合。同时,制作多媒体课件和教学资源库,配合完成本科生国家级和省

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级动物科学技能大赛 将很好地达成培养综合性卓越新农科本科人才这一目标^[4]。

2 饲料学翻转课堂建设的具体举措

通过师生角色、学习方式、课堂流程和评价体系

等功能的翻转^[5],并结合数字课程、案例视频、虚拟仿真、饲料掺假鉴定、饲料青贮实操等线上线下虚实融合的手段,通过翻转传统课堂途径打造立体混合式的智慧教学模式。见图1。

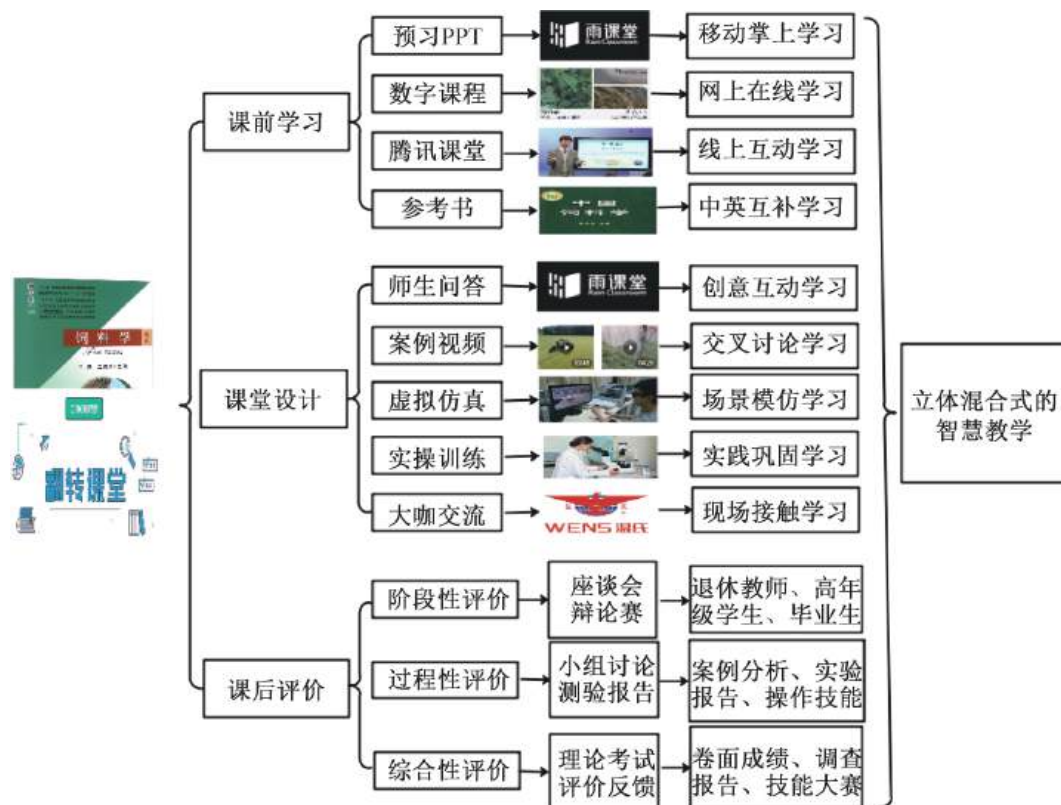


图1 基于翻转课堂饲料学立体混合式的智慧教学设计模式

2.1 线上课前学习

1) 学生登录华南农业大学在线教育综合平台,获取教师发布的饲料学课前教学资源 PPT 和视频等,发现问题并自主学习找出答案。学习网址为 <http://eol.scau.edu.cn/meol/course.do>。

2) 学生通过雨课堂 App 移动学习与讨论问题。

3) 通过中国农业教育在线网站,在线预习饲料学国家精品课程。学习网址为 <https://www.ccapedu.com/selectProfessional.action>。

4) 通过大学慕课和腾讯课堂形式拓展复习巩固饲料学知识点和试题。

2.2 课堂设计(线上线下虚实融合)

1) 师生问答。线上:通过雨课堂扫码签到、疑问反馈、弹幕互动、随堂练习等,实现创意互动;线下:小组讨论和 PPT 试讲。

2) 核心知识点学习。线上:案例视频学习和虚拟仿真场景训练;线下:实操训练和技能培养。

3) 课堂效果评价。主要采用线上线下结合方式从过程性评价、阶段性评价和综合性评价三个方面对饲料学课程进行过程性考核改革。见表1。

2.3 课后评价

2.3.1 座谈会 与饲料学专业课在教和退休教师座谈,讨论饲料学课程学习方法与技巧;与高年级学生座谈,讨论和交流饲料学学习心得。

2.3.2 交流会 邀请行业大咖分析饲料产业发展趋势和行业前景;邀请毕业生回校分享相关工作社会经验;学生相互分享未来规划。

2.3.3 辩论会 就饲料行业热点等辩证性话题组织辩论赛,考查学生思维方式和解决问题的能力。

2.3.4 技能培训 依托本科实验室和试验基地,练习饲料裹包青贮、鱼粉掺假识别和配合饲料生产等饲料学实用技巧,提高本校动物科学饲料类技能大赛竞赛能力。

3 实施进展

第一阶段:依托本校动物科学专业,建设农科教人才培养基地的教学软件条件,建立翻转课堂饲料学立体混合式的智慧教学模式的运行机制,确定培养方案,建立教学效果评价和反馈体系;此外,加强线上教学软硬件条件的建设,建设线下实操训练的场所和购置相应的配套设备;建设线上线下虚实融合课程运行模式;建立意见反馈共享平台;整理评价效果和意见。

表 1 教学效果过程考核评价表

考核类别	考核内容	考核方式
过程性评价	了解学科发展前沿的内容和拓展 辩证性话题,考查学生主动思考与逻辑思维方式 调查总结报告 文献综述	笔试 口试 实际操作 经典案例分析(线上线下融合)
阶段性评价	与在教和退休教师座谈,讨论饲料学学习方法与技巧 与高年级学生座谈,讨论和交流饲料学学习心得 邀请毕业生回来分享与饲料学相关工作社会经验	学习成果汇报交流 辩论赛
综合性评价	期末基本理论考试 评价反馈机制共享平台(线上):教师、高年级学生、其他专业相关教师评价	笔试成绩 调查报告撰写 实习和实践饲料企业评价反馈

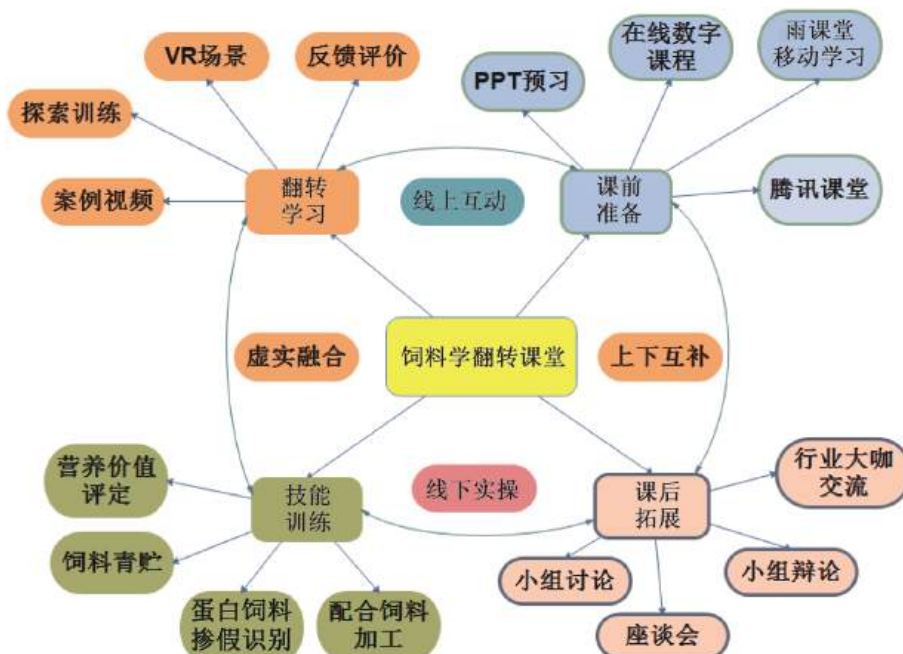


图 2 基于翻转课堂饲料学立体式交叉网络式教学模式

第二阶段:巩固翻转课堂建设的成果,根据翻转效果存在的问题进一步优化线上线下交叉运行的机制,完善课程立体多元化教学模式,丰富翻转课堂教学资源。加强与动物营养与饲料实验课的合作力度,提高实践动手能力,提高学生动物科学饲料技能大赛的竞争力;同时对其他动物营养与饲料学学科的课程起到示范和辐射作用。

目前,笔者已完成第一阶段全部和第二阶段大部分的进展工作,已基本实现教学线上线下的虚实融合;且参与设计“黄羽肉鸡饲养及性能测定”虚拟仿真项目,并获得2020年国家虚拟仿真项目;辅助华南农业大学学子在全国大学生动物专业技能大赛“饲料原料快速鉴定与掺假识别”项目获得优异成绩。

4 未来展望

未来两年内,进一步优化并完善线上线下交叉式立体多元化教学模式,加深学生对饲料学的认识与理解,使学生将学到的理论知识与生产实践相结合,在

实践中不断总结知识并使理论学习在实践中具体化,达到学以致用目的。最后,本次教学改革的长远目标期望打造基于以学生为主体的翻转课堂立体式交叉网络式教学模式(见图2),以加强专业教学质量为核心,构建动物科学专业人才培养新模式,实现“专业知识+操作技能+创新能力”三位一体的多元化动物科学专业人才培养体系,实现“深基础、宽口径、高素质、强能力专门人才”培养的整体优化。

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动物生产类虚拟仿真实验教学的建设与实践——以黄羽肉鸡饲养及性能测定虚拟仿真实验为例

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摘要:为探索动物生产类虚拟仿真实验教学的建设与实践,围绕南方特色黄羽肉鸡生产性能测定现实实验的关键内容,通过饲料营养价值评定、体尺测量、屠宰测定、人工授精和种蛋孵化5个关键核心环节的真实仿真,结合线上线下交互操作训练,巩固黄羽肉鸡生产全过程知识点。同时,针对可视化呈现并解决线下实践教学难以开展的场地选址及布局和废弃物处理等问题,充分调动学生的主观能动性,确保学生整体性熟悉并掌握黄羽肉鸡全生产流程。

关键词:动物生产;实践教学;虚拟仿真;黄羽肉鸡;课程改革

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黄羽肉鸡占我国肉鸡市场50%左右,是主要养殖和消费品种。因此,学会并掌握黄羽肉鸡科学养殖尤为重要。黄羽肉鸡饲养及性能测定虚拟仿真实验课程依托华南农业大学国家级特色动物科学专业优势和动物科学类省级实验教学中心,及中心已成立的省级虚拟仿真教学平台,围绕南方特色黄羽肉鸡生产性能测定现实实验的关键内容,通过引导学生虚拟仿真学习场地选址及布局和废弃物处理环节,打破传统黄羽肉鸡饲养实验空间跨度大、生物安全要求高和生产周期过长等制约,确保学生

整体性熟悉并掌握黄羽肉鸡全生产流程;通过对代谢实验、体尺测量、屠宰测定、人工授精和种蛋孵化5个关键核心环节的真实仿真,结合线上线下交互操作训练,克服部分实验设备要求特殊、家禽群体应激反应大及组织管理经费昂贵等不足,从而保证每个学生动手操作和熟悉各个实验环节,提高实验教学的有效度(李静等,2020)。主要知识点具体如下:

(1)场址规划:清楚场址科学选择和场区合理布局。(2)代谢实验:掌握代谢实验程序和要点,独立完成常规养分粗蛋白

质、总能等测定方法。(3)体尺测量:熟悉胸宽、胸深、胸围等部位的位置和测量方法。(4)屠宰测定:掌握屠宰分割、器官分离称重和器官指数计算等程序。(5)人工授精:熟悉和掌握人工采精、精液品质检查、输精等操作。(6)种蛋孵化:了解照蛋、孵化微生物检查、效果评估等。(7)废弃物处理:掌握粪污收集与好氧发酵、尸体无害化处理等手段。

1 黄羽肉鸡饲养及性能测定虚拟仿真实验课程建设的必要性

1.1 虚拟仿真学习的必要性

目前传统黄羽肉鸡饲养实验的场地选址及布局受到时间和空间跨度大等制约,以致高校教师无法开展相应的生产实践实验。此外,黄羽肉鸡生产过程

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中,废弃物处理实验危险性大、疫病传播风险高,粪污收集与好氧发酵、尸体无害化处理等实验在传统实验室无法完成;加上鸡场生物安全要求高,教师和学生也无法通过生产实践学习,因而实验实践教学被迫搁置或放弃。最为重要的是,黄羽肉鸡饲养试验是一个整体性试验,而传统饲养只是单一独立的试验,无法做到内容环环相扣和整个实验过程完整呈现,无法提高学生参与综合实践的兴趣和创造能力(熊宏齐,2020)。虚拟仿真技术可以让本实验课程具有可延续性,通过将新的实验模块和工作流程导入原有系统中,紧密联系实践,开展新的实验项目,更新的先进技术和手段(孙彦婷等,2019)。本课程确保学生整体性熟悉并掌握黄羽肉鸡生产流程。

1.2 线上线下交互学习的必要性

虽然目前动物生产专业综合实验课程开展了相应的人工授精、种蛋孵化、体尺测量、屠宰测定和代谢实验操作,传统实验教学中存在的教学方法单一,知识学习和实验动手操作相互分离,学生缺乏学习主动性和创造性,特别是在理论教学中,许多应用型知识受到限制而无法进行实践(谢晖等,2019)。此外,部分试验要求设备昂贵(如总能测定需要氧弹量热仪),不能保证每个学生动手操作实践。特别是人工授精和体尺测量实验容易引起黄羽肉鸡群体应激,增加了生产实践组织管理的难度,无法保证每个学生进入生产实践了解实验细节。目前,种蛋孵化和代谢强

饲实验只能以学生小组的形式开展,不能保证每个学生参与到各个环节中。动物生产类虚拟仿真实验教学的建设与实践弥补了现实传统实验存在的不足,线上线下混合实训最大限度发挥学生主观能动性和创新能力;并可一定程度上替代实验动物的使用,保障教学中的动物福利和伦理,有利于学生建立正确的对待动物的态度(蒋亚君等,2020)。

2 黄羽肉鸡饲养及性能测定虚拟仿真实验课程特色

2.1 实现自主-互动-探究的立体式教学模式

基于黄羽肉鸡生产实践实验中不能有效开展的鸡场地选址及布局和废弃物处理环节的问题,以学生为主体,教师为中心,通过仿真系统提供虚拟的实操与互作实验环境,倡导自主式、合作式、探究式学习,弥补黄羽肉鸡养殖生产实践实验中鸡场场址规划及布局和废弃物处理这一头一尾环节,做到内容环环相扣和整个实验过程完整呈现(图1),确保学生整体性熟悉并掌握黄羽肉鸡生产流程,大大调动学生的学习积极性和主动

性,达到事半功倍的学习效果(李改英等,2016)。

2.2 实现线上与线下交互式教学模式

传统实验教学中存在的教学方法单一,知识学习和实验动手操作相互分离,学生缺乏学习主动性和创造性(龚思颖等,2019)。本项目利用虚拟仿真技术开展从线上到线下全方位交互式教学实践,通过线上主动学习智能指导下人工授精、种蛋孵化、体尺测量、屠宰测定和代谢实验实操训练,实现线下教学的多向互动和多元互动,以实现师生从线上到线下进行实时交互的高效实验教学(图2)。

3 黄羽肉鸡饲养及性能测定虚拟仿真实验课程目标

3.1 最大程度实现实验教学理论与实践的融合

虚拟仿真实验可以实现对仪器设备的内部结构和实验对象的形态具有可视性,依靠科学真实的实验数据,通过对某些模拟手段“放大”观察项目,观察实验对象的相关因素变化的特征及规律(熊宏齐,2019)。本虚拟仿真项目通过传感技术、输入输出

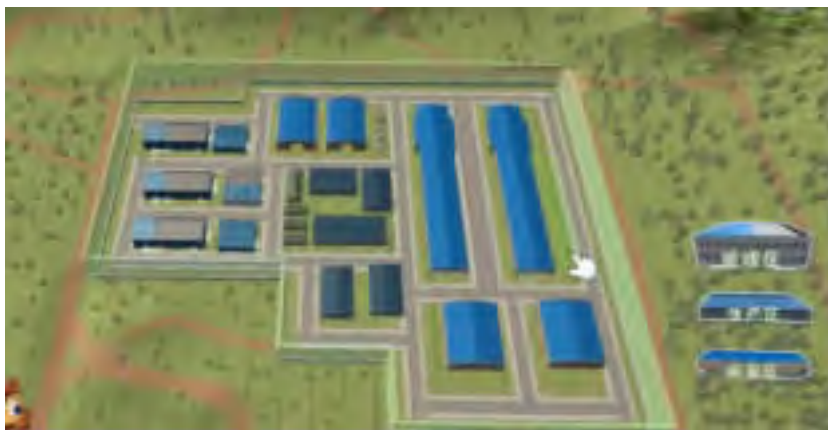


图1 虚拟仿真场址选择及布局效果图

技术将传统现实实验无法开展的场址规划和废弃物处理等关键技术环节可视化连续呈现,高度虚拟仿真还原黄羽肉鸡饲养及性能测定现实场景,实现仿真实践与理论实验教学资源的有机结合。加强实践教学体系的构建,强化学生的动手实践能力和专业综合能力的培养(王秋菊等,2020)。

3.2 首次尝试开展线上与线下交互实验教学

基于虚拟仿真项目开展线上线下相结合的交互式教学,实现线上操作预复习与线下实操环节进行深度融合,由上返下、由下及上的双线交替式教学,突破传统课堂教学模式,充分调动学生开展学习探究的积极性和主动性。

3.3 宏观上实现产学研相辅相成

校企合作、产学研紧密连结是动物专业人才培养的重要途径(陈伟国,2018)。本虚拟仿真项目基于华南农业大学动物科学学院张细权教授主持完成的“高档优质肉鸡新品种的培育与应用”广东省科学技术奖一等奖成果转化,也是我校动物科学学院与温氏集团产学研的亮点体现。本项目以黄羽肉鸡饲养及性能测定为平台,实现了科研反哺教学,教学促产业的良性循环发展局面。

4 黄羽肉鸡饲养及性能测定虚拟仿真实验课程效果

4.1 课程推广方面

将黄羽肉鸡的饲养和性能测定虚拟仿真实验教学项目纳入动物专业培养方案和教学课程必修环节,采用学时学分



图2 线上线下交互体尺测量



图3 动物生产类动科科学技能大学场面图

制考核,鼓励学生积极参加虚拟实验的学习。制订线上线下、虚实结合的学习效果评价办法,实现实验教学资源高效利用,促进学生创新能力的培养(图3)。此外,陆续向本校低年级生物专业的课外科研活动提供技术培训;为选修家禽生产学课程其他非专业学生开放提供教学辅导。后续陆续覆盖动物育种学、配合饲料学、饲料添加剂学等课程的相关实验项目,持续补充遗传育种、生物安全等模块。

4.2 高校推广方面

建立信息化管理共享平台,面向全国各大高校开放,支持和鼓励各地畜牧和兽医专业学生的学习,拓展相关专业院校之间的开放共享、共建共享资源。2019年3月已同广东海洋大学、佛山科学技术学院、仲恺农业工

程学院、韶关学院及石河子大学、安徽农业大学等高校签订共享协议,计划为上述学校的1000多名畜牧兽医专业学生开设共享服务。

4.3 社会推广方面

课程建设与广东温氏食品集团有限公司、珠海市裕禾农牧有限公司、广州驱动力饲料有限公司、茂名杨氏农业有限公司等省级教学实践基地建设统一规划实施,加强黄羽肉鸡的饲养和性能测定虚拟仿真开放平台的校企共享,目前已与6家家禽业协会和5家家禽生产企业提供开放的公共教育平台,逐步形成精品教学平台,深化校企产学研合作。此外,整合社会资源,依托本课程逐步向本市中小学生提供课外动物类科普平台。

参考文献:(略)

检索证明

根据委托人提供的论文材料，委托人华南农业大学动物科学学院 朱勇文 2 篇论文收录情况如下表。

序号	论文名称	发表刊物及发表的年月卷期/页码等	作者排名	论文等级	作者文中单位	收录情况	影响因素	中科院大类分区
1	基于翻转课堂立体教学模式的探究——以华南农业大学饲料科学为例	黑龙江畜牧兽医 出版年：2021 卷期： 页码： - 文献号： 文献类型：	1	C类	华南农业大学动物科学学院	北大核心	无	无
2	动物生产类虚拟仿真实验教学的建设与实践——以黄羽肉鸡饲养及性能测定虚拟仿真实验为例	广东饲料 出版年：2022 卷期： 页码： - 文献号： 文献类型：	1	普刊类	华南农业大学动物科学学院	CNKI	无	无

说明：论文等级和中科院大类分区按《华南农业大学学术论文评价方案（试行）》划分。

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荣誉证书

HONORARY CREDENTIAL

朱勇文 老师：

在华南农业大学 2022-2023 学年青年教师教学优秀奖
奖评选中荣获

二等奖

特发此证，以资证明！



二、科研项目



项目批准号	32472924
申请代码	C1705
归口管理部门	
依托单位代码	51064208A0499-0932



国家自然科学基金 资助项目计划书 (预算制项目)

资助类别：面上项目

亚类说明：

附注说明：

项目名称：肠道菌群通过OXLAMs调控肝脏线粒体能量代谢影响肉鸭饲料利用效率的“肠—肝”轴机制研究

直接费用：50万元 执行年限：2025.01-2028.12

负责人：朱勇文 BRID：06168.00.01981

通讯地址：天河区五山路华南农业大学动物科学学院520室

邮政编码：510642 电 话：18818912892

电子邮件：zhuyw0724@scau.edu.cn

依托单位：华南农业大学

联系人：唐家林 电 话：020-85280070

填表日期：2024年08月30日

国家自然科学基金委员会制

Version: 1.002.932



简表

项目负责人信息	姓 名	朱勇文	性 别	男	出生年月	1986年07月	民 族	汉族
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	工 作 单 位	华南农业大学						
	所 在 院 系 所	动物科学学院						
依托单位信息	名 称	华南农业大学					代码	51064208A0499
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	电 话	020-85280070			网站地址	http://kjc.scau.edu.cn/		
合作单位信息	单 位 名 称							
项目基本信息	项 目 名 称	肠道菌群通过OXLAMs调控肝脏线粒体能量代谢影响肉鸭饲料利用效率的“肠—肝”轴机制研究						
	资 助 类 别	面上项目				亚 类 说 明		
	附 注 说 明							
	申 请 代 码	C1705:动物营养学				C1706:饲料学		
	基 地 类 别							
	执 行 年 限	2025.01-2028.12						
	直 接 费 用	50万元						



项目摘要

中文摘要:

提高饲料利用效率是肉鸭产业降本增效的关键。目前提高饲料利用效率侧重肠道养分的消化吸收，对其在线粒体中代谢缺少报道。我们前期发现肉鸭饲料利用效率与靶组织肝脏线粒体代谢及肠道来源氧化亚油酸代谢产物（OXLAMs）含量密切相关，且OXLAMs可直接调控肝脏线粒体能量代谢。由此推测：肠道菌群可能通过OXLAMs调控肝脏线粒体能量代谢，影响肉鸭饲料利用效率。为验证假设，本项目通过构建伪无菌肉鸭、肝脏GPR120敲除小鼠和GPR120抑制表达原代鸭肝细胞模型，结合粪菌移植、*Streptococcus LOX*基因缺失株定植和体外盲肠发酵等技术，体内外解析肠道菌群通过OXLAMs介导GPR120信号调控肝脏线粒体能量代谢的“肠-肝”轴机制，并验证营养干预靶向调控肠道OXLAMs代谢提高肉鸭饲料利用效率的可行性。研究结果不仅有助解析肝脏线粒体能量代谢的“肠-肝”轴机制，也为改善肉鸭饲料效率提供新的营养策略。

Abstract:

Improving feed efficiency is an important avenue to achieve the beneficial effect of reducing the cost of ducks production in the context of high price of feed ingredients. Previous studies of feed efficiency focus on the digestion and absorption of nutrients in the intestine, but rarely on the metabolism and utilization of nutrients in mitochondria. Based on our previous findings of the positive correlation between the feed efficiency and hepatic mitochondrial energy metabolism as well as the role of screened oxidative linoleic acid metabolites (OXLAMs) on hepatic mitochondrial energy metabolism of meat ducks, it is speculated that the gut microbiota-driven OXLAMs on the regulation of hepatic mitochondrial energy metabolism plays an important role in feed efficiency of meat ducks through gut-liver axis. Therefore, the effect of gut microbiota-driven hepatic mitochondrial energy metabolism on efficiency are explored in vivo using the fecal microbiota transplantation in pseudo germ-free ducks and *Streptococcus LOX* gene deletion mutant colonized in germ-free mice. Then, the mechanism of OXLAMs mediated GPR120 signal on the regulation of mitochondrial energy metabolism are revealed using GPR120 silencing of the primary culture hepatocyte in vitro and hepatic GPR120 conditional knockout mice in vivo. Finally, the feasibility of the nutritional intervention on improving the feed efficiency via gut microbiota-driven hepatic mitochondrial energy metabolism in meat ducks is verified using cecal fermentation in vitro and feeding in vivo. The results of this study not only help to elucidate the "gut-liver" axis mechanism of mitochondrial energy metabolism in liver, but also provide a new nutritional strategy for improving feed efficiency of meat ducks.

关键词(用分号分开): 鸭; 微量元素; 线粒体能量代谢; 肠道菌群; 饲料利用效率

Keywords(用分号分开): meat ducks; trace elements; mitochondrial energy metabolism; gut microbiota; feed efficiency



项目组主要成员

编号	姓名	出生年月	性别	职称	学位	单位名称	电话	证件号码	项目分工	每年工作时间 (月)
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总人数		高级		中级		初级		博士后	博士生	硕士生
7		1		2					2	2



国家自然科学基金预算制项目预算表

项目批准号：32472924

项目负责人：朱勇文

金额单位：万元

序号	科目名称	金额
1	一、科学基金资助项目直接费用合计	50.0000
2	1、设备费	0.0000
3	其中：设备购置费	0.0000
4	2、业务费	44.2400
5	3、劳务费	5.7600
6	二、其他来源资金	0.0000
7	三、合计	50.0000

注：请按照项目研究实际需要合理填写各科目预算金额。



预算说明书

（请按照《国家自然科学基金项目申请书预算表编制说明》等的有关要求，按照政策相符性、目标相关性和经济合理性原则，实事求是编制项目预算。填报时，直接费用应按设备费、业务费、劳务费三个类别填报，每个类别结合科研任务按支出用途进行说明。对单价 ≥ 50 万元的设备详细说明，对单价 < 50 万元的设备费用分类说明，**对合作研究单位资质及资金外拨情况、自筹资金进行必要说明。**）

本项目直接经费申请总额为50.00万元。各项预算详细说明如下：

1. 设备费（0万元）

0万元。

2. 业务费（合计44.24万元）

(1) 试验动物相关费用（9.33万元）

试验总共购买雏鸭需6元/只 $\times 600$ 只=0.36万元；饲料：平均每只肉鸭饲养6周，饲料购买费需8.0 kg饲料/只 $\times 600$ 只 $\times 6.0$ 元/kg饲料=2.88万元；疫苗及消毒药：每只鸭疫苗及消毒药费用1.5元，用600只 $\times 1.5$ 元/只=0.09万元；条件性基因敲除小鼠：3对CK0纯合子+3对Flox纯合子共需6万元；共计9.33万元；

(2) 细胞培养和分子生物学试剂相关费用（合计18.01万元）

细胞培养方面：用于购买细胞培养板需35元/个 $\times 100$ 个=0.35万元，购买细胞培养液需100元/L $\times 100$ L=1.00万元，购买胎牛血清需4000元/L $\times 6$ L=2.40万元，购买胶原酶2500元/g $\times 8$ g=2.0万元。用于购买siRNA转染试剂盒2000元/个 $\times 5$ 个=1.0万元；Seahorse检测线粒体功能相关试剂2万元/套 $\times 2$ 套=4.0万元。mRNA和蛋白水平检测：购买Trizol试剂需2000元/个 $\times 5$ 个=1.0万元，购买反转录试剂盒和荧光定量试剂盒需3800元/个 $\times 5$ 个=1.90万元，一抗共需3000元/个 $\times 7$ 个=2.10万元，购买二抗500元/个 $\times 4$ 个=0.20万元，购买超敏发光液需1200元/个 $\times 5$ 个=0.60万元，购买PVDF膜需1500元/卷 $\times 4$ 卷=0.60万元，购买蛋白浓度测定试剂盒1000元/个 $\times 3$ 个=0.30万元，购买转膜液和TBST缓冲液100元/瓶 $\times 36$ 瓶=0.36万元，购买标准蛋白Marker需400元/个 $\times 5$ 个=0.20万元。总共18.01万元

(3) 常规试剂耗材费用（合计4.0万元）

用于购买实时荧光定量PCR专用板需1000元/盒 $\times 20$ 盒=2.00万元，荧光定量透明封板膜2500元/盒 $\times 2$ 盒=0.5万元。购买样品消化及分子生物学指标分析用离心管、加样枪头、手套、口罩等耗材共需1.50万元。共4.0万元。

(4) 测试化验加工费（合计6.5万元）

GPCR120信号通路相关基因干扰序列合成：300元/条 $\times 10$ 条=0.30万元；靶向和非靶向代谢组学检测：800元/个样品 $\times 40$ 个=3.20万元；肠道菌群16S检测：200元/个样品 $\times 30$ 个=0.60万元；肠道微生物高通量测序1200元/个样品 $\times 20$ 个=2.40万元；共计6.5万元。

(5) 差旅/会议/国际合作与交流费（合计4.0万元）

用于课题组人员参加国内学术交流会议的食宿及往返路费，共需0.50万元/人 \cdot 次 $\times 2$ 人 \cdot 次/年 $\times 4$ 年=4.0万元。共计4.0万元。

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文献检索费需50元/篇 $\times 20$ 篇/年 $\times 4$ 年=0.40万元；论文版面费需1.0万元/篇SCI论文 $\times 2$ 篇=2.0万元；共计2.40万元。

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用于直接参加项目研究的4名博硕士生劳务费，按照平均每月600元计，4人 $\times 600$ 元/人/月 $\times 6$ 个月/年 $\times 4$ 年=5.76万元。

国家重点研发计划项目 子课题任务书

子课题名称/编号: 提高种禽输卵管黏膜免疫力和修复能力的营养调控技术
/2022YFD1300405-11

子课题承担单位: 华南农业大学

子课题负责人: 朱勇文

课题名称/编号: 种畜禽生殖健康与繁殖性能的营养调控技术 2022YFD1300405

课题承担单位: 华中农业大学

所属项目: 畜禽胃肠道和生殖道健康与营养调控技术

所属专项: 畜禽新品种培育与现代牧场科技创新

执行期限: 2022 年 12 月至 2027 年 11 月

填写说明

- 一、任务书甲方即课题牵头承担单位，乙方即子课题承担单位。
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- 六、《课题任务书》是本任务书填报的重要依据，任务书填报不得降低考核指标，不得自行对主要研究内容作大的调整。《课题任务书》和本任务书将共同作为子课题过程管理、验收和监督评估的重要依据。

子课题基本信息表

子课题名称	提高种禽输卵管黏膜免疫力和修复能力的营养调控技术					
所属课题	幼龄畜禽消化道发育及微生物的调控机制					
所属项目	畜禽胃肠道和生殖道健康与营养调控技术					
所属专项	畜禽新品种培育与现代牧场科技创新					
密级	<input checked="" type="checkbox"/> 公开 <input type="checkbox"/> 秘密 <input type="checkbox"/> 机密					
经费预算	总预算 30 万元，其中中央财政专项经费 30 万元					
子课题 承担 单位	单位名称	华南农业大学			单位性质	大专院校
	单位所在地	广东广州			组织机构代码	124400004554165 634
	银行账号	3602002609000310520			法定代表人姓名	刘雅红
	单位开户名称	华南农业大学				
	开户银行(全称)	中国工商银行广州五山支行				
子课题 负责人	姓 名	朱勇文	性 别	<input checked="" type="checkbox"/> 男 <input type="checkbox"/> 女	出生日期	1986. 7
	证件类型	身份证	证件号码	431122198607243414		
	所在单位	华南农业大学				
	最高学位	<input checked="" type="checkbox"/> 博士 <input type="checkbox"/> 硕士 <input type="checkbox"/> 学士 <input type="checkbox"/> 其他				
	职 称	<input type="checkbox"/> 正高级 <input checked="" type="checkbox"/> 副高级 <input type="checkbox"/> 中级 <input type="checkbox"/> 初级 <input type="checkbox"/> 其他			职务	
	电子邮箱	zhuyw0724@sacu. edu. cn		移动电话	18818912892	
子课题 联系人	姓 名	王丽华	电子邮箱	wanglihua4822@163. com		
	固定电话	020-38882017	移动电话	15004934822		
	证件类型	身份证	证件号码	152322199511022720		
子课题	姓 名	何旭日	电子邮箱	hxr17688209125@yeah. net		

财务 负责人	固定电话	020-38882017	移动电话	17688209125
	证件类型	身份证	证件号码	441781200002150113
子课题 参加人 数	5 人。其中：	高级职称 2 人，中级职称 1 人，初级职称 0 人，其他 0 人；		
		博士学位 1 人，硕士学位 1 人，学士学位 0 人，其他 0 人。		

一、子课题研究目标、内容及考核指标

(一) 主要研究内容及目标

(要解决的关键科学问题、关键技术问题,针对这些问题拟开展的主要研究内容。研发主要针对什么问题和需求;将要解决哪些科学问题、突破哪些核心/共性/关键技术;预期成果及应用等。)

首先,采用多组学联合分析产蛋前、中和后期种禽输卵管各部位(膨大部、峡部和子宫部)炎症的发生规律,明确种禽输卵管炎症发生的关键节点与窗口期;然后,结合微生物组学和代谢组学等技术分析种禽输卵管中菌群及其代谢产物的时空变化规律,明确种禽输卵管微生物组成变化与炎症发生的相关性;最后,筛选出关键候选营养素介导微生物途径提高种禽输卵管黏膜免疫力和修复能力,解析其缓解种禽输卵管炎症的作用机制,形成相关营养调控技术。

(二) 考核指标

(考核指标:指相应成果的数量指标、技术指标、质量指标、应用指标和产业化指标等,其中,数量指标可以为论文、专利、产品等的数量;技术指标可以为关键技术、产品的性能参数等;质量指标可以为产品的耐震动、高低温、无故障运行时间等;应用指标可以为成果应用的对象、范围和效果等;产业化指标可以为成果产业化的数量、经济效益等。

筛选出种禽输卵管提高黏膜免疫力和修复能力的关键候选营养素,解析其缓解种禽输卵管损伤的作用机制,形成相关营养调控技术。考核指标:揭示种禽输卵管损伤营养修复的分子机制,提出修复种禽输卵管炎症的营养调控技术1项,开发相关产品1个,发表高水平论文1篇。

二、子课题年度计划

按每6个月制定形成课题的计划进度，应将课题的考核指标分解落实到年度计划中。

1、2023年度：2023年1月—2023年5月

任务：研究不同生理阶段产蛋种禽输卵管炎症发生规律

考核指标：挖掘出种禽输卵管炎症发生的关键部位与窗口期

成果形式：研究报告

2、2024年度：2023年6月—2023年11月

任务：研究种禽输卵管中菌群及其代谢产物的时空变化规律

考核指标：明确种禽输卵管微生物组成变化与炎症发生发展的相关性

成果形式：年度报告

3、2024年度：2024年1月—2023年5月

任务：研究关键候选营养素对产蛋种禽繁殖性能和输卵管免疫力的影响。

考核指标：筛选并获得修复种禽输卵管炎症的关键营养素1种

成果形式：研究报告

4、2024年度：2024年6月—2023年11月

任务：解析关键候选营养素提高种禽输卵管免疫力的作用机制

考核指标：筛选并获得修复种禽输卵管炎症的关键营养素1种

成果形式：年度报告、发表论文1篇

5、2025年度：2025年1月—2025年5月

任务：研究关键候选营养素对种禽输卵管微生物及代谢产物的影响

考核指标：筛选出影响种禽输卵管免疫力的关键代谢产物

成果形式：研究报告

6、2025年度：2025年6月—2025年11月

任务：解析关键候选营养素介导关键代谢产物调节种禽输卵管免疫力的作用机制

考核指标：揭示种禽输卵管损伤营养修复的分子机制

成果形式：年度报告、发表论文1篇

7、2026年度：2026年1月—2026年5月

任务：体外研究关键候选营养素对输卵管上皮细胞免疫性能的影响

考核指标：体外验证关键候选营养素对输卵管的修复作用

成果形式：研究报告，开发产品 1 个

8、2026 年度：2026 年 6 月—2026 年 11 月

任务：体外研究营养素和差异代谢产物共培养对输卵管上皮细胞免疫性能的影响

考核指标：体外验证关键候选营养素介导微生物途径对输卵管的修复作用

成果形式：年度报告

9、2027 年度：2027 年 1 月—2027 年 5 月

任务：营养调控技术（产品）对种禽繁殖性能和输卵管免疫力的影响。

考核指标：提出修复种禽输卵管炎症的营养调控技术 1 项

成果形式：研究报告

10、2027 年度：2027 年 6 月—2027 年 11 月

任务：验证营养调控技术（产品）对种禽繁殖输卵管免疫力和修复力的应用效果评价

考核指标：完成修复种禽输卵管炎症的营养调控技术 1 项

成果形式：结题报告

课题承担单位（甲方）：

单位负责人签字（签章）：

李锐



课题负责人签字（签章）：

李锐

年 月 日

子课题承担单位（乙方）：

单位负责人签字（签章）：

刘雅红



2023年3月13日

子课题负责人签字（签章）：

朱恩子

2023年3月13日

受理编号: c191405000000006

项目编号: 2019B1515210031

文件编号: 粤基金字(2020)6号

广东省基础与应用基础研究基金项目 任务书

项目名称: 钙通过HIF-1 α 介导的糖酵解途径影响番鸭胚胎发育的作用机制

项目类别: 企业(温氏)联合基金-重点项目

项目起止时间: 2020-01-01 至 2022-12-31

管理单位(甲方): 广东省基础与应用基础研究基金委员会

依托单位(乙方): 华南农业大学

通讯地址: 广东省广州市天河区五山路483号

邮政编码: 510642

单位电话: 020-85283435

项目负责人: 朱勇文

联系电话: 18818912892



(广东科技微信公众号)



(查看任务书信息)



(受理纸质材料二维码)

广东省基础与应用基础研究
基金委员会
二〇二〇年制

填写说明

一、项目任务书内容原则上要求与申报书相关内容保持一致，不得无故修改。

二、项目承担单位通过广东省科技业务管理阳光政务平台下载项目任务书，按要求完成签名盖章后提交至省科技厅受理窗口。

三、签名盖章说明。请分别在单位工作分工及经费分配情况页、人员信息页、签约各方页等地方按要求签字或盖章，签章不合规或错漏将不予受理。其中，人员信息页要求所有参与人员本人亲笔签名，代签或印章无效，漏签将不予受理。

四、本任务书自签字并加盖公章之日起生效，各方均应负本任务书的法律责任，不应受机构、人事变动影响。

2019B1515210031

一、主要研究内容和要达到的目标

研究内容：

（1）不同胚龄鸭胚低氧糖酵解水平及HIF-1 α 表达变化规律的研究

采用qPCR和WB技术检测不同胚龄鸭胚组织低氧糖酵解水平及HIF-1 α 表达水平，探究鸭胚糖酵解供能与HIF-1 α 信号通路的相关性；比较初生健雏和弱雏仔鸭组织中糖酵解及HIF-1 α 通路表达水平的差异性，结合阐明HIF-1 α 通路、糖酵解及胚胎发育之间相互关系。

（2）HIF-1 α 通路调节原代培养鸭胚肝细胞低氧糖酵解的信号机制研究

以原代培养鸭胚肝细胞为模型，体外模拟胚蛋低氧生理环境条件，研究孵育时间对细胞HIF-1 α 通路基因表达的影响，以确定低氧环境细胞最适培育时间；随后，采用HIF-1 α 通路过表达或者RNAi干扰处理，探究HIF-1 α 信号通路调节鸭胚肝细胞低氧糖酵解的作用机制；然后，采用Akt和p53阻断剂联合孵育，体外证实HIF-1 α 介导PIK3/Akt/P53途径调节低氧糖酵解作用的可能机制。

（3）卵黄囊和蛋壳钙动员对鸭胚肝脏HIF-1 α 介导的糖酵解水平的影响

研究不同胚龄鸭胚卵黄囊和蛋壳钙动员供给变化规律及其对鸭胚肝脏HIF-1 α 介导的糖酵解水平的影响；接着，采用胚蛋注射手段，研究促进或阻断卵黄囊钙供给（蛋壳钙供给）对鸭胚肝脏糖酵解和HIF-1 α 介导的PIK3/Akt/P53信号通路及其靶基因表达的影响，明确卵黄囊和蛋壳钙营养供给通过HIF-1 α 信号介导的糖酵解影响鸭胚发育的作用机制。

（4）饲料钙营养通过HIF-1 α 通路介导的糖酵解途径对鸭胚发育的影响

采用母代自然饲喂方式，研究种鸭饲料不同钙源和钙水平对鸭胚生长发育、低氧糖酵解水平及HIF-1 α 信号通路功能基因表达的影响，最终证实钙营养干预介导HIF-1 α 通路-糖酵解途径改善鸭胚生长发育的可能性。

研究目标

揭示低氧下HIF-1 α 通路介导糖酵解途径调节鸭胚胎发育及钙营养干预机制。

二、项目预期获得的研究成果及形式

论文及专著情况	国家统计源刊物以上刊物 发表论文（篇）		3		科技报告（篇）		0	
	其中被SCI/EI/ISTP收录 论文数（篇）		2		培养人才（人）		1	
	专著（册）		0		引进人才（人）		0	
专利情况(项)	发明专利		实用新型专利		外观设计专利		国外专利	
	申请	授权	申请	授权	申请	授权	申请	授权
	1	0	0	0	0	0	0	0
其他								

三、项目进度和阶段目标

(一) 项目起止时间: 2020-01-01 至 2022-12-31		
(二) 项目实施进度及阶段主要目标:		
开始日期	结束日期	主要工作内容
2020-01-01	2020-12-31	完成不同胚龄鸭胚低氧糖酵解水平及HIF-1 α 表达变化规律的研究(第一部分)所有种蛋孵化、样品采集与制备工作及样品分析工作,并建立鸭胚原代培养肝细胞模型(第二部分的准备工作)。
2021-01-01	2021-12-31	HIF-1 α 通路介导原代培养鸭胚肝细胞低氧糖酵解的信号机制研究(第二部分)和卵黄囊和蛋壳钙动员对鸭胚肝脏HIF-1 α 介导的糖酵解水平的影响(第三部分)的种蛋孵化、胚蛋注射、样品采集及样品分析工作。
2022-01-01	2022-12-31	完成饲料钙营养通过HIF-1 α 通路介导的糖酵解水平对鸭胚发育的影响(第四部分)中的所有动物饲养、种蛋孵化、样品采集及样品分析工作,并对所有数据进行整理加工和统计分析,最后撰写并提交项目总结报告。

四、项目总经费及省基金委经费预算

(一) 省基金委经费下达总额: (大写) 贰拾万圆整; (小写) 20.00万元;					
(二) 省基金委经费年度下达计划:					
年度	2019 年	年	年	年	年
经费(万元)	20.00				
(三) 总经费及省基金委经费开支预算计划:					
经费筹集情况:					(单位: 万元)
省基金委经费	自筹资金				合计
	自有资金	贷款	地方政府投入	其它	
20.00	0	0	0	0	20.00
政府部门、境外资金及其他资金投入情况说明:	无				

经费预算			(单位: 万元)	
	总投入经费		省基金委经费	
支出经费	经费额	用途说明	经费额	用途说明
基建费:				
1、直接费用:	17.50		17.50	
(1) 设备费:				
(2) 材料费:	13.50	试剂耗材	13.50	试剂耗材
(3) 测试化验加工外协费:	1.00	委托测序	1.00	委托测序
(4) 燃料动力费:				
(5) 差旅费/会议费/国际合作与交流费:	1.00	会议费和交通费	1.00	会议费和交通费
(6) 出版/文献/信息传播/知识产权事务费:				
(7) 劳务费:	2.00	研究生劳务	2.00	研究生劳务
(8) 人员费:				
(9) 专家咨询费:				
(10) 直接费用其他支出:				
(11) 科技金融服务体系其他费用:	0.00		0.00	
①信用评级补贴:				
②大赛场租:				
③特派员奖励与补贴:				
2、间接费用:	2.50		2.50	
(1) 间接成本:				
(2) 管理成本:	1.00	学校管理	1.00	学校管理
(3) 绩效支出:	1.50	绩效支出	1.50	绩效支出
合计:	20.00		20.00	
与本项目相关的其他经费来源		(单位: 万元)		
其他计划资助经费:				
单位配套经费:				
其他经费资助:				
其他经费来源合计:				

五、人员信息

项目负责人

姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
朱勇文	431122198607243414	34	男	副教授	博士研究生	项目负责人	华南农业大学	

项目组主要成员

姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
杨琳	230106196309292114	57	男	教授	博士研究生	技术指导	华南农业大学	
刘闯	410411198108053512	39	男	高级畜牧师	硕士研究生	种番鸭饲养技术指导	云浮市云城温氏畜牧有限公司	
耿伟	410181198201090070	38	男	高级畜牧师	硕士研究生	种蛋孵化技术指导	云浮市云城温氏畜牧有限公司	
叶慧	440106198010011849	40	女	高级实验师	硕士研究生	指标分析	华南农业大学	
王悦名	371325199411031936	26	男	畜牧师	硕士研究生	肉鸭生产技术管理	云浮市云城温氏畜牧有限公司	
劳永聪	440881199506262919	25	男	畜牧师	硕士研究生	种蛋孵化试验	云浮市云城温氏畜牧有限公司	
汪珩	42900619940111091X	26	男	未取得	硕士研究生	试验开展、样品收集和分析	华南农业大学	
唐凤坚	440682198011286047	40	男	兽医师	硕士研究生	种鸭饲养管理	云浮市云城温氏畜牧有限公司	

六、工作分工及经费分配

承担/参与单位名称 (盖章)	工作分工	总经费分摊 (万元)	省基金委经费分配 (万元)
华南农业大学	负责项目“钙通过HIF-1 α 介导的糖酵解途径影响番鸭胚胎发育的作用机制”涉及的胚蛋注射和原代鸭肝细胞培养试验及样品采集、样品分析和报告总结撰写等工作。	13.00	13.00
云浮市云城温氏畜牧有限公司	负责项目“钙通过HIF-1 α 介导的糖酵解途径影响番鸭胚胎发育的作用机制”涉及的种鸭饲养和种蛋孵化试验，协助样品采集和分析工作。	7.00	7.00
	合计	20.00	20.00

八、本任务书签约各方

管理单位（甲方）：广东省基础与应用基础研究基金委员会（盖章）

法定代表人（或法人代理）：曾路（签章）

年 月 日

依托单位（乙方）：华南农业大学（盖章）

法定代表人（或法人代理）：刘雅红（签章）

联系人（项目主管）姓名：郑鹏（签章）

Email: kjcgxk@scau.edu.cn

电话：020-85283435

开户单位名称：华南农业大学

开户银行名称：广东广州工行五山支行

开户银行账号：3602002609000310520

年 月 日

联系人（项目负责人）姓名：朱勇文（签名）

Email: zhuyw0724@scau.edu.cn

电话：18818912892

年 月 日

合同编号：2024CXTD20

2024年度乡村振兴战略专项资金——省级现代 农业产业技术体系创新团队建设项目

任 务 书

项目名称：以农产品为单元的广东省现代农业产业技术体系创新
团队建设项目（家禽产业技术体系）

岗位名称：营养与饲料科学

专家姓名：朱勇文

依托单位（公章）：华南农业大学

依托单位法定代表人：薛红卫

项目牵头单位（公章）：华南农业大学

牵头单位法定代表人：薛红卫

日期：2025年5月

广东省农业农村厅制

一、目的及意义

主要说明项目的建设目的、研究价值和意义。

广东省家禽品种多样化且消费需求多元化。而不同家禽品种不仅营养需求差异很大，饲料营养价值评价方法不成熟，饲料营养数据库存在一定的滞后性。因此，如何快速、准确地评估不同品种家禽对饲料原料的营养价值和精准营养需要量，是产业的痛点问题之一。随着家禽品种的改良和智能养殖模式的引入，现代家禽品种对特定营养素的需求也随之变化；但现有的饲料配方营养推荐量往往基于较早的品种和饲养模式的数据，未能精确匹配动物的动态需求量，难以充分发挥家禽的最大生产潜能。特别在追求高生产效率的同时，如何平衡营养供给与保持家禽产品的胴体质量、营养风味和安全品质，成为家禽产业当前另一痛点问题。

因此，针对不同家禽品种消化生理特点，在完善传统动物代谢试验评价饲料营养价值的基础上，构建针对不同类型饲料原料和配方结构的快速准确的体外仿生评定方法，建立不同家禽品种的饲料营养数据库，为家禽精准营养需要评估提供方法支撑和数据参考。在此基础上，研发基于广东省主要家禽品种不同生长阶段和饲养模式的动态营养配方技术体系，利用预测模型精准评估其营养需求，从而动态优化饲料配方，提高家禽生产效率和产品质量，实现饲料产业的提质增效。

二、项目建设内容

详细说明项目建设内容（项目需求或项目建设任务）。

（1）家禽饲料营养价值评定的新技术方法研究

研究不同饲料类型对家禽消化道 pH、消化酶活性、离子浓度和微生物组的影响；建立并完善主要番鸭饲料养分利用率的快速准确体外仿生消化评定新技术方法，建立家禽代谢能、酶水解能等营养价值评定技术规程。

（2）家禽精准营养需要与动态预测模型研究

以广东省养殖量大和消费高的主要家禽品种（麻黄鸡、番鸭、肉鹅等）为研究对象，探究不同品种家禽差异化发育特性，开展不同生长阶段和养殖模式下能量、蛋白质、氨基酸等营养素的需求模式研究，确定适宜的营养需要量，建立满足不同品种不同生长阶段低蛋白日粮的精准营养供给技术体系。

（3）家禽产品品质及其营养调控研究

探究相同饲养条件下家禽皮肤质量和羽毛生长发育的规律；在此基础上，探究不同饲料配方的营养水平、结构组成和功能性添加剂等对家禽胴体品质、羽毛发育、皮肤质量和肉质风味的改善效果，形成广东特色家禽皮肤品质的营养调控技术。

备注：项目建设内容（项目需求或项目建设任务）按《项目申报书》内容填写。

三、项目绩效目标

主要说明项目实施后,预期达到的目标和产生的效果,相关表述应量化。

(1) 研发家禽体外仿生消化饲料营养价值评价技术规程 1 项,探究饲料营养对肉品质及皮肤质量的改善作用,形成营养调控技术方案 1 套;

(2) 推广笼养模式下黄羽肉鸡豆粕减量替代及产品品质调控的日粮配制技术的应用与示范 1-2 项;

(3) 年度检测饲料养分利用率、肉品质和样品 300 份以上;开展技术培训与交流 5 场,培训农民或技术人员 150 人以上;申报专利 3 件;

(4) 团队成员均为“百千万工程”农业科技特派员,对接参与“百千万工程”农业科技特派员乡村行服务活动 10 人次以上,对接服务农技推广服务驿站、现代农业产业园、新型农业经营主体等 10 次以上,解决产业发展瓶颈问题 1-2 个;

(5) 完成省农业农村厅安排的防灾减灾、救灾复产等应急救灾工作任务;至少召开创新团队工作部署及总结会议 1 次。

四、项目进度安排

(1) 2024 年 10 月—2024 年 12 月：开展番鸭体外仿生消化能评定技术方法研究；探究笼养模式下麻黄鸡不同生长阶段营养需要量研究，对接参与“百千万工程”农业科技特派员乡村行服务活动 5 人次以上，开展技术培训 25 人次以上；线上线下开展技术培训与交流 1 场，培训农民或技术人员 50 人以上。

(2) 2025 年 1 月—2025 年 5 月：采用体外仿生法评价不同饲料原料的能量利用率；推广笼养模式下饲料营养改善快大鸡胴体品质和肉质，对接服务农技推广服务驿站、现代农业产业园、新型农业经营主体等 10 次以上，解决产业发展瓶颈问题 1-2 个。

(3) 2025 年 6 月—2025 年 10 月：联合联合站推广笼养模式下黄羽肉鸡豆粕减量替代及产品品质调控的日粮配制技术的应用与示范，对接参与“百千万工程”农业科技特派员乡村行服务活动 5 人次以上，开展技术培训 25 人次以上；线上线下开展技术培训与交流 1 场，培训农民或技术人员 50 人以上。

备注：项目绩效目标按《项目申报书》内容填写。

五、项目主要合作、参与单位（含牵头承担单位）					
单位名称	单位性质	统一社会信用代码	通讯地址		
华南农业大学	高校	124400004554165634	广州市天河区五山路 483 号		
佛山大学	高校	1244060045607389XC	广东省佛山市禅城区江湾一路 18 号		
六、项目组主要成员（含项目负责人）					
姓名	性别	身份证号	单位	职称/职务	电话
朱勇文	男	431122198607243414	华南农业大学	副教授	18818912892
柴启恩	男	362204198710127839	佛山大学	副教授	13415735577
朱翠	女	441625198510043828	佛山大学	副教授	13560059496
冯鑫	男	130185198611241845	佛山大学	副教授	18824838157
叶慧	女	440106198010011849	华南农业大学	高级实验师	15989221997
董泽敏	女	420111197208045027	华南农业大学	讲师	13632363756
姚斌华	男	432321197503072391	华南农业大学	中级实验师	13808882290

七、资金使用预算

主要说明资金使用的范围或方向及资金使用进度安排。

序号	支出类别	预算金额（万元）	支出范围说明
	（一）直接经费	9.4	
1	材料费	5	试剂耗材、试验动物、饲料原料、添加剂等原料采购
2	测试费	2	外部检测服务（肉质风味、电镜切片等）
3	差旅费	0.6	调研、学术会议、住宿费用等
4	劳务费	0.9	研究生助研津贴、临时聘用人员工资
5	会议费	0.4	学术研讨会、成果交流会等费用
6	出版/文献	0.5	论文发表、专利申请、文献检索等

	费		
	(二) 间接 费	0.6	
1	管理费	0.6	用于高校科研管理支出

八、保障措施

说明围绕完成项目任务、目标所要采取的具体措施。

研究涵盖了家禽饲料的营养价值评定、精准营养需要与动态预测模型、以及家禽产品品质及其营养调控等多个方面，形成了一个完整的研究体系。在建立不同家禽品种饲料营养价值评定技术的基础上，构建基于广东省主要家禽品种不同生长阶段和饲养模式的动态营养配方技术体系，利用预测模型精准评估其营养需求，从而动态优化饲料配方，提高家禽生产效率和产品质量，实现饲料产业的提质增效。

通过研发新技术、建立数据库、优化评定方法、开展深入研究、构建预测模型、更新推荐量、探究品质形成规律、设计营养调控方案以及利用多组学等手段的集成创新，将有助于推动家禽饲养业的技术进步和产业升级，提高生产效率和产品品质，提升消费者满意度和信任度。

广东省农业农村厅

粤农农计〔2021〕59号

关于下达 2021 年省级乡村振兴战略专项 ——种植业类项目任务清单的通知

各有关单位：

2021 年省级乡村振兴战略专项省级组织实施项目资金（第 1 批）已以《广东省财政厅关于安排下达 2021 年省级乡村振兴战略专项省级组织实施项目资金（第 1 批）的通知》（粤财农〔2021〕37 号）下达。请严格按照文件要求，抓紧做好项目组织实施等工作。

结合我省实际，现将 2021 年省级乡村振兴战略专项（种植业类项目）任务清单下达给你们。请及时与我厅种植业管理处项目经办人对接，研究制定项目实施方案，并于 6 月 30 日前在广东省农业农村厅专项资金管理系统（<http://183.62.243.12:8001/nytzj-web/minstone/login>）填报该项目实施方案，正式文件报送我厅（种植业管理处）备案。

(此页无正文)



(联系人：邝舒，联系电话：020-37288270)

公开方式：依申请公开

序号	项目名称	项目承担单位	建设内容	绩效目标	资金额度	联系人
31	秸秆饲料化应用全产业运行技术支撑	华南农业大学	开展秸秆收储体系模式探索与示范，研究水稻秸秆青贮及生物发酵、饲用养殖废弃物处理等技术并进行示范推广，对秸秆饲料化应用全产业链生态经济效益进行综合评价。	制定秸秆收储运和预加工技术体系 1 套；建立秸秆饲料化整体应用模式技术规程 1 套；建立秸秆饲料化整体应用示范点 1 个以上，每个示范点作物面积 50 亩以上，秸秆综合利用率 90% 以上；提供有机肥产品经检测报告，有机肥回田应用面积 50 亩以上。	45	张育灿
32	全生物降解地膜研发与示范应用	广东省科学院生物工程研究所	1. 开展全生物降解地膜研发及应用试验研究，解决产品研发和应用过程中的关键技术问题；2. 建立示范区，综合评估全生物降解地膜产品的安全性、可控性、环境效应和增产作用等，形成全生物降解地膜应用技术规程。	针对不同地区不同作物不同生产者耕作条件下，研发合适的全生物降解地膜 8 种以上，每种产品制定 1 套应用技术规程，建立 1 个示范区，每个示范区面积 100 亩以上。	45	张育灿
33	严格管控类耕地种植结构调整模式研究与示范	华南农业大学	开展种植业结构调整模式探索，分析土壤、作物污染状况，筛选低累积作物，结合当地农民的种植意愿和产业发展，筛选出适宜推广的调整模式。	形成严格管控类土壤主要污染物调查分析报告 1 份；筛选污染物低累积作物品种 2-3 种；形成 1-2 种植结构调整模式并进行示范。	45	张育灿
34	汕头市濠江区东陇社区广佛手产业示范基地建设项目	汕头市濠江区农业农村局	扶持汕头市濠江区东陇社区建设一个 3000 平方米的广佛手产业加工基地，完善基地硬件设施，购置加工装备机械等。	建成一个 3000 平方米的广佛手产业加工基地。	200	邝舒
35	广东适用特优草莓品种（系）及配套轻简绿色栽培技术的示范推广项目	华南农业大学	建设一个广东适用特优草莓品种（系）示范基地，示范特优新品种（系）华香 6 号地栽和基质栽的轻简绿色栽培技术	建成 1 个 20 亩的广东适用特优草莓品种（系）示范基地，制定特优新品种（系）华香 6 号地栽和基质栽的轻简绿色栽培技术规程，项目推广的技术集成提质增效 15%。	45	邝舒

其他联系人联系方式：张育灿 37288089、张小强 37288269、郭奕生 37289240、余平 37288276。

附件 4:

广东省农业科研项目和农业技术推广项目 实施方案

项目名称：	秸秆饲料化应用全产业运行技术支撑
项目建设期限：	2021 年 1 月-2021 年 12 月
所属项目：	广东省乡村振兴战略专项资金项目
申报单位：	华南农业大学（公章）
项目负责人：	朱勇文
项目申报日期：	2020 年 11 月至 2020 年 12 月

广东省农业农村厅制

二〇二 年 月 日

一、项目单位概况

包含单位性质、隶属关系、相关职能业务范围；人员状况，包括项目和子项目的负责人及骨干人员情况和职责，从事过的相关研究和承担的同类科研任务、发明专利及获奖其概况，在国内外主要刊物上发表的与本项目相关的研究成果情况，人员情况应增加列表说明；财务收支和资产负债简况；平台、基础设施和配套仪器设备、专业技术水平和组织管理能力等现有条件基础等内容。)

1. 项目单位概况

本项目申请单位华南农业大学作为全国重点大学,广东省和农业部共建的“211 工程”大学,广东省高水平大学重点建设高校,拥有开展科学研究和示范推广的良好条件。现有亚热带农业生物资源保护与利用国家重点实验室、国家生猪种业工程技术研究中心等 8 个国家级科研平台,教育部重点实验室 3 个,农业农村部重点实验室 9 个,农业农村部科学观测实验站 3 个,农业农村部科研基地 2 个等,其先进基础设施和配套仪器设备均保证了本项目的顺利实施。华南农业大学师资力量雄厚,学科门类齐全,国家省部级重点学科覆盖本项目涉及的农学、畜牧学、环境科学与生态学、农业工程学等专业背景,充分强大的专业技术水平和综合实力为本项目高质量完成提供技术支撑。本单位在财务收支和资产负债方面均符合事业单位会计准则和规定,配备专职财务和科研项目管理人员跟踪管理。

2. 项目团队介绍

本项目参与人员共 9 名，其中教授 2 名，副教授 3 名，讲师 2 名，高级实验师 2 名，人员组成结构合理，梯队比例合适；项目邀请生产实践经验的教授专家作为技术指导。人员组成结构合理，梯队比例合适；项目邀请生产实践经验的教授专家作为技术指导。项目人员的专业背景覆盖各个子项目涉及的作物栽培学与耕作学、饲料资源开发与利用、饲料发酵微生物、生态循环农业等专业背景，充分体现了学科交叉和专业优势互补，有利于从整体上高质量完成产业链秸秆高效饲用技术的解决方案。

项目负责人朱勇文副教授及所在团队长期从事非常规饲料的资源评价与饲料配制技术方面的研究。目前承担或参与与本项目相关的多项国家和省部级自然科学基金项目、国家农业产业体系项目、国家重点研发计划子课题，在非粮饲料资源开发与利用和菌酶协同生物发酵等研究领域已有探索性的积累和单项技术成果的储备。“十三五”期间，本单位承担了“肉鸭高效低排饲料配制技术应用与示范”、并参与了“国家水禽产业体系—饲料资源开发与利用”，采用体外生物法、体外仿生消化法及生物发酵预处理等手段，体内外系统评价了 10 多种非常规饲料原料的养分利用效率与饲用价值，构建了以南方经济作物及其加工副产物为基础的饲料营养价值数据库，在非粮饲料资源开发与高效利用及饲料精准配方和加工技术等方面拥有较为深入的技术基础。相关成果在 *Animal Feed Science and Technology*、*Poultry Science*、*动物营养学报* 等主流经典期刊发表论文 20 余篇，申请或授权发国家明专利 4 项，获得企业标准 1 项。

子项目负责人张庆副教授及所在团队长期从事饲料发酵微生物调控优质饲料添加剂开发方面的研究。前期在非粮型木本饲料资源加工利用开展了大量的基础性工作，特别采用混合青贮和添加剂青贮手段，实现了高蛋白木本饲料（构树叶、辣木叶、黄梁木叶等）的高效利用，为合理利用非粮饲料资源、提高粗饲料利用等方面提供了技术支撑，也为本项目稻秸饲料在畜牧养殖业中的高效利用、减少饲料粮消耗和饲料成本提供了新方法和新参考。近年来先后主持国家自然科学基金青年基金项目等课题多项，在 *Bioresource Technology*, *Journal of Dairy Science*, *Microbial Biotechnology*, *mSphere*, *草业学报* 等国内外重要期刊发表论文 40 余篇，申请国家发明专利 2 项。

子项目负责人王小龙副教授及所在团队长期从事“种养循环”全产业链生态、经济效果研究。前期通过开展不同有机废弃物还田长期定位试验，研究发现在等碳或减氮条件下，合理配施养殖业废弃物，可以促进广东省红壤区农田生产力提高并减少潜在环境影响；采用碳足迹、生命周期评价、能值分析等相关研究方法对我国典型农业系统（包括种植业、养殖业和沼气工程）生态经济效果进行分析，提出了不同产业链优化的技术方案。目前，主持和参与国家自然科学基金、广东省自然科学基金等各类项目多项，所得研究成果在 *Journal of Cleaner Production*、*Resources, Conservation and Recycling*、*生态学报*、*中国生态农业学报* 等学术期刊上发表相关论文 24 篇，参编专著和教材 4 部，获得实用新型专利 1 项。

子项目负责人郑鹏博士及所在团队主要从事茶树栽培、农业废弃物资

源化利用相关研究工作。特别是在废弃物资源化利用和粪肥有机化还田技术方面具有一定的技术基础。目前主持、参加各级科研项目 10 余项。发表科技论文 33 篇。获得授权发明专利 9 项、软件著作权 4 项。参与制定国家标准、地方标准各 1 项。

子项目负责人段美洋博士及所在团队主要围绕广东省丝苗香米现代农业发展的需求，以“浓香、绿色、优质、高产、高效和机械化、智能化、名牌化”为目标，构建适应省内不同稻作区不同类型的丝苗香稻绿色高效种植综合集成技术和品牌丝苗香米加工技术，及丝苗香稻高效种植技术成果转化和推广体系，同时开展丝苗香稻栽培基础理论研究，形成丝苗香米产业可持续创新能力，也为本项目稻秸收储和有机肥还田技术示范推广提供了技术支持和场地保障。主持和主要参加国家或省部级项目 10 多项，主要参与授权发明专利 6 项，参与制定广东省地方标准 1 项，以第一作者（通讯作者）发表学术论文 8 篇。

表 1 项目人员主持或参与的科研课题情况

序号	项目名称及编号	项目级别 (A、B、C)	项目来源	立项时间	项目人 员	是否结题	备注
1	金属硫蛋白调节氧化应激下鸡 胚肝脏能量代谢的作用机制	A	国家自然科 学基金	2019	朱勇文	否	主持人
2	肉鸭高效低排饲料配制技术应 用与示范	A	国家重点研 发	2018	朱勇文	否	主持人
3	青贮辣木叶水解单宁降解乳酸 菌筛选及作用机理研究	A	国家自然科 学基金	2019	张庆	否	主持人
4	适用于循环农业系统评价与调 控的能值方法改进及实证研究	A	国家自然科 学基金	2019	王小龙	否	主持人
5	国家水禽产业体系—饲料资源 开发与利用	A	现代农业产 业体系	2017-2020	杨琳	是	主持人
6	种鸭缺锌临界值预测及其影响 胚胎发育氧化损伤机制研究	B	广东省自然 基金	2017	朱勇文	否	主持人
7	金属硫蛋白表达介导鸡胚肝脏 线粒体锌稳态和能量代谢分子	B	广东省自然 基金	2018	朱勇文	否	主持人
8	广东夏季三黄鸡高效健康养殖 技术的应用示范	B	广东省科技 特派员项目	2018	朱勇文	否	主持人
9	黄梁木叶提取物对青贮饲料不 良微生物的抑制及其机理研究	B	广东省自然 基金	2019	张庆	否	主持人
10	秸秆添加剂青贮技术推广应用	B	广东省科技 特派员项目	2018	张庆	否	主持人
11	广东省农作物品种试验	B	广东省农业 农村厅	2016	段美洋	是	主持人
13	基于 LCA 的不同循环农业路径 “节能 - 减排 - 经济”综合效益	B	广东省自然 基金	2017	王小龙	否	主持人
14	减量施氮与间作大豆对甜玉米 土壤丛枝菌根真菌与氮转化微 生物互作关系影响的机理	A	国家自然科 学基金	2018	王小龙	否	排第 2

15	香稻香气 2-乙酰-1-吡咯啉的有机碳营养调控机理研究	A	国家自然科学基金	2016	段美洋	是	排第 2
16	亚精胺参与牛筋草抗性调控的机理研究	A	国家自然科学基金	2016	段美洋	是	排第 2
18	畜禽废水在枇杷优良品种种植中的高效利用技术推广及应用	B	广东省科技厅	2018	郑鹏	是	排第 2
19	单丛茶生态栽培、优质加工及品牌包装	B	广东省科技厅	2019	郑鹏	否	排第 2

项目预算汇总表

表B3 项目编号: 项目名称: 金额单位: 万元

序号	预算科目名称	合计	省级财政专项资金	其他来源资金
	(1)	(2)	(3)	(4)
1	一、资金支出			
2	(一) 直接费用	41.4	41.4	
3	1. 设备费	7.2	7.2	
4	2. 材料费	13.95	13.95	
5	3. 测试化验加工费	3.6	3.6	
6	4. 燃料动力费	3.6	3.6	
7	5. 出版/文献/信息传播/知识产权事务费	2.9	2.9	
8	6. 会议/差旅/国际合作交流费	3.14	3.14	
9	7. 培训费	0.9	0.9	
10	8. 劳务费	4.24	4.24	
11	9. 专家咨询费	0.52	0.52	
12	10. 其他支出	1.35	1.35	
13	(二) 间接费用	3.6	3.6	
14	二、项目资金来源			
15	(一) 省级财政专项资金	45	45	/
16	(二) 其他来源资金		/	
17	1. 单位自筹资金		/	
18	2. 其他资金		/	

注: 1、请提供表B4-间接费用无需编制预算说明;

华南农业大学新农村发展研究院 农业科技合作共建项目 合同书

项目名称： “东西部协作”助力贵州纳雍家禽产业提质增效
项目类别： 建设永根科技站
项目负责人： 朱勇文
联系方式： 18818912892
所在单位： 华南农业大学动物科学学院
电子邮箱： zhuyw0724@scau.edu.cn
填报日期： 2023.10.9

华南农业大学科学研究院（新农村发展研究院）

二〇二三年五月

一、项目基本情况

项目名称	“东西部协作”助力贵州纳雍家禽产业提质增效					
永根科技站名称	贵州纳雍家禽永根科技站					
项目负责人(专家团队首席)	朱勇文		职称/职务		副教授	
承担单位(所在学院)	动物科学学院		资助经费(万元)		15	
研究领域	家禽饲料与营养					
服务产业	家禽产业					
依托企业名称	贵州纳雍源生牧业股份有限公司					
科技站地点(企业地址)	贵州省毕节市纳雍县经济技术开发区D区					
建站依托的平台	<input type="checkbox"/> 地方产业研究院 <input type="checkbox"/> 省现代农业产业园 <input type="checkbox"/> 科技小院 <input checked="" type="checkbox"/> 高校服务乡村振兴共同体 <input type="checkbox"/> 其它					
项目起止时间	2023年5月1日至2025年4月30日					
团队共建单位	纳雍农业技术推广中心/贵州省畜牧兽医研究所					
团队 成	姓名	出生年月	职称	所在单位	研究领域	任务分工
	朱勇文	1986.7	副教授	华南农业大学	家禽饲料与营养	项目执行
	谢青梅	1972.9	教授	华南农业大学	家禽生产与管理	项目指导
	冯宇隆	1988.3	副研究员	贵州畜牧兽医研究	家禽饲料与营养	项目骨干

员				所		
	顾怀龙	1978. 10	科长	纳雍农业 技术推广 中心	畜牧生产	项目骨干

二、项目的概述

加强东西部协作，是党中央着眼全局作出的重大决策，是推动区域协调发展，缩小发展差距、实现共同富裕的重要举措。广东省委乡村振兴战略小组指出支持贵州省国家乡村振兴重点帮扶县巩固拓展脱贫攻坚成果同乡村振兴有效衔接。“永根科技站”是以中国科学院院士、全国优秀共产党员、“时代楷模”、原华南农业大学校长卢永根命名，是华南农业大学面向全社会设立的科技服务站点。贵州纳雍家禽永根科技站围绕纳雍县生态家禽产业的主要养殖品种和上下游段生产企业的痛点和难点问题开展技术帮扶，涵盖优质肉鸡、蛋鸡、地方特色鸡等多个品种，覆盖种鸡繁育、饲料加工、生态养殖和生鲜产品加工等全产业链多个环节，聚焦技术攻关、成果转化、指导培训三大服务，切实解决实际问题。

三、实施方案（技术简述、服务内容、实施进度安排等）

1. 技术简述

围绕纳雍县生态家禽产业种源、养殖技术、饲料生产、产品加工销售等痛点问题开展调研和技术帮扶工作。主要包括：（1）调研产业现状和问题，形成产业报告和帮扶计划：围绕养殖方式，技术力量，专业人才等方面开展调研。（2）开展技术培训，提高养殖标准化程度。针对生产大多以专业合作社主培训，提高生产技术规范程度。（3）协助拓宽销售

渠道，提高产品品牌价值。实现产品优质优价，拓宽销售渠道。

2. 服务内容

主要针对纳雍县生态家禽产业的重点和难点问题有效开展系列工作。

(1) 开展家禽产业调研，完成产业调研报告 1 份：通过实地走访、问卷调查、搜集资料三种方法调研，系统了解家禽产业种质资源的保护与利用、养殖设施、饲养技术、疫病防控和产品销售等产业现状和问题，完成产业调研报告 1 份。(2) 开展标准化生产技术培训，形成帮扶技术方案 1 套：整合乡村振兴促进团和国家科技特派团（员）机遇，力量开展家禽产业技术服务，对农民专业合作社、养殖企业的人员培训 5 场次，每场 60 人/次以上，形成标准化生产规程 3 套。(3) 协助产品销售市场拓展和品牌塑造。根据产业存在问题，提供数字化销售平台技术支持，拍摄品牌宣传片，拓宽线上销售渠道。

3. 实施进度安排

(1) 2023.5-2023.10：系统调研纳雍县生态家禽产业现状和问题，撰写产业报告 1 份，为地方政府政策制度提供技术支撑；(2) 2023.11-2025.4，针对产业存在的问题开展标准化生产技术培训和帮扶工作，形成产业企业帮扶规划 1 份；(3) 2023.11-2025.4，“线上线下”形式协助打造品牌价值和拓宽产品销售渠道，具体包括线上提供电子商务腾讯、抖音等提供数字化和销售平台；线下协助推介参加国内国际推介会、展销会、博览会等。

四、考核指标：

包括示范推广新品种、新产品、新工艺、新技术等；开展技术咨询和技术服务内容、次数、人数；开展服务培训/指导内容、天数、次数、人数；培养或指导乡村振兴人才数量；预期经济、社会、环境效益（产业贡献、推广规模、节本增效、带动就业、提高生活水平、保护环境等）；发布相关新闻报道等。

1. 组建贵州纳雍家禽永根科技站专家团队一支；
2. 示范推广优质肉鸡和蛋鸡标准化生产技术和方案 3 项；
3. 开展技术服务或咨询活动不少于 20 次；
4. 建立技术示范基地 3 个；
5. 培养“永根科技站”硕士研究生人数 2 人；
6. 培训“新农人”（含农民）300 人次；
7. 开展家禽产业调研，完成产业调研报告 1 份；
8. 在各类媒体平台发布宣传“永根科技站”的报道 6 篇。

五、经费预算

单位：万元

序号	预算科目名称	经费	备注
1	差旅费	5	赴贵州家禽产业调研帮扶产生的住宿和飞机票等费用
2	材料费	7	协助家禽饲料、肉质和抗体检测等费用
3	测试化验加工费	1	用于委托检测等费用
4	会议培训费	1	用于指导培训等费用
5	资料费	1	培训资料打印等费用
6	劳务费	0	研究生调研等支出
7	专家咨询费	0	
8	其他支出	0	
合计		15	

五、所在学院意见

同意

项目负责人（签字）：朱书

单位负责人（签字）：张哲

2023年9月12日



六、新农村发展研究院意见

单位（公章）：

年 月 日





编号: STKJ202209046

专题编号: 20220501

专题名称: 专题十: 农村科技特派员驻镇帮镇扶村专题

2022年省科技创新战略专项 (“大专项+任务清单”)项目 任务书 (社会发展类项目)

项目名称: 汕头市农村科技特派员驻镇帮镇扶村项目(澄海区莲下镇)

专项资金类别: 2022年省科技创新战略专项(“大专项+任务清单”)项目

业务类型: 五、支持科技特派员对接帮扶镇村

申请单位: 华南农业大学

通讯地址: 五山路483号

邮政编码: 510642 单位电话: 020-38632413 传真: 020-85281885

项目负责人: 朱勇文 联系电话: 020-3772002 手机: 18818912892

项目联系人: 朱勇文 联系电话: 020-3772002 手机: 18818912892

电子邮箱: kjcgxk@scau.edu.cn

主管部门: 汕头市科学技术局

申报日期: 2022-07-26

汕头市科学技术局

二零二二年六月制

一、项目基本情况表

项目名称	汕头市农村科技特派员驻镇帮镇扶村项目（澄海区莲下镇）		
项目起止时间	2022-01-01 至 2024-12-31		
项目总经费预算	15.00（万元）	申请经费资助	15.00（万元）
项目摘要（200字以内）：			
<p>本项目针对目前狮头鹅生产技术落后、养殖污染严重、标准化和规模化程度低等现状，研究育雏期狮头鹅高效饲养技术、夏季高温狮头鹅高效饲养技术及狮头鹅种养结合生态养殖技术的推广与应用，形成狮头鹅绿色高效养殖技术的集成示范，降低育雏期狮头鹅痛风发生率和死亡率，大大节约狮头鹅养户饲料成本，从而提高狮头鹅的生产效率，减少养殖废弃物的排放，达到绿色安全高效和可持续循环的生产目的。</p>			
项目实施必要性（200字以内）			
<p>作为广东省农村科技特派员团队前期与汕头市澄海区莲下镇对接帮扶，详细调研了解莲下镇地区狮头鹅养殖技术需求情况，采用“农户+基地+公司+高校技术帮扶”的模式，期望打造种养结合立体生态狮头鹅绿色健康养殖模式，在构建“狮头鹅养殖—废弃物饲料化—粪便肥料回田—牧草种植”循环生态模式方面达成共识。</p>			

二、项目内容

(一) 项目实施绩效 (单位: 项数)				
1. 主要成果指标				
成果形式	成果数量	成果形式		成果数量
专利 (件)	1	引进人才 (人)		0
其中: 发明	0	培养人才 (人)		1
实用新型	1	科技人才奖励 (人)		0
其中: 国际专利		技术标准制定	牵头 (个)	
软件著作权 (项)			参与 (个)	
获国家级奖项 (项)		论文论著 (篇)		2
获省级奖项 (项)		被收录论文数 (篇)	SCI	
新产品 (个)			EI	
新材料 (种)			ISTP	
新装备 (台/套)		新服务 (项)		
新工艺 (或新方法、新模式) (个)				

其他将提供的研究开发成果及形式（创新特色、成果宣传推介措施等，限200字）：
形成提高育雏期狮头鹅成活率的高效饲养技术1套；建立缓解夏季狮头鹅热应激的高效饲养技术1套。
2. 本项目完成后预计经济、社会效益情况（限300字）：
提高育雏期和夏季高温狮头鹅成活率10%以上，实现种养结合绿色发展新模式，通过推广应用节约狮头鹅饲料成本10%以上；减少环境碳、氮、磷等有机物排放10%以上，实现农民增收10%以上。
3. 其他主要技术经济指标及社会效益说明（限500字）：
开展技术咨询、技术服务 4 次/年以上，举办技术培训、现场会、展示会等活动 2 次/年以上，在省、市级电视台、报纸、网站、电子刊物、官方公众号等媒体上发布特派员工作报道 2 篇以上。培训技术人员30人次，技术辐射澄海区莲下镇周围地区。

(二) 组织实施和保障措施 (400字以内)

- 1、从技术层面，研究所和高校强强联合和产学研合作，保障狮头鹅技术创新平台的创建和升级。联合各参与单位的水禽产业体系研发平台和技术优势，开展产学研合作及技术转化和创新，因地制宜发展规模化、标准化、生态化养殖，实行科学养殖，提高产品质量；同时以市场为导向，满足多元化消费需求。
- 2、从研究团队层面，项目（任务）参与人有主持省部级科技项目的经验，项目骨干多年从事狮头鹅养殖技术领域的工作，具有丰富的科研经验和知识能力储备，国际学术联系广泛，对国内外相关领域的发展动态十分熟悉。研究人员技术水平可以保障项目的顺利进行。
- 3、从研究平台层面，依托国家水禽产业体系岗位技术和资源优势，进一步加大研发和设施装备投入，不断提升狮头鹅高效养殖的技术水平和竞争优势，并探索推广规模化、标准化的饲养模式。

(三) 项目计划进度

起止时间	主要工作内容 (限500字)
2022-01-01 至 2022-12-31	详细调研汕头市澄海区莲下镇等狮头鹅养殖产业状况，实地考察和协助帮助解决狮头鹅痛风死亡率高的问题，提供相应缓解痛风的综合措施。
2023-01-01 至 2023-12-31	研究棚舍内环境控制、有害因子浓度、光照程序等技术参数；优化夏季饲料配制和环境控制等饲养管理关键技术参数，提供了相应缓解夏季热应激的综合措施。
2024-01-01 至 2024-12-31	开展生态发酵床养殖关键技术；推广鹅粪+农作物桔杆混合发酵生产有机肥技术；开展草-鹅生态种养模式。

三、经费情况表

(一) 拟申请财政科技资金总额: (大写): 壹拾伍万元零仟零佰圆整; (小写): 15.00万元						
(二) 总经费及拟申请财政科技资金情况 (单位: 万元)						
项目总经费投入	市级财政科技资金	自筹经费				合计
		自有资金	贷款	其他政府部门投入	其它	
	15	0	0	0	0	15.00
自筹资金投入情况说明: (限1000字)						
无						

项目经费预算：			(单位：万元)	
	项目总经费		其中：市科技局经费	
支出经费	经费额	用途说明	经费额	用途说明
(一) 直接费用	12.00		12.00	
1、设备费	0		0	
2、业务费	8		8	
3、直接人力资源成本	4		4	
(二) 间接费用	3.00		3.00	
1、绩效支出	1.5		1.5	
2、管理费用	1.5		1.5	
3、其它间接费用				
合计	15.00		15.00	
其他需说明的情况：				
填报说明： <p>(1) 直接费用</p> <p>a、设备费：与科研项目直接相关的计算类仪器设备、软件工具，及仪器设备的租赁、现有仪器设备的升级改造等支出。</p> <p>b、业务费：项目研究过程中发生的与之直接相关的除设备费、人力资源成本费用以外的其他支出。</p> <p>c、直接人力资源成本：参与项目研究的科研人员、科研辅助人员的工资性支出或劳务支出。</p> <p>(2) 间接费用</p> <p>间接费用是指项目承担单位在组织实施项目过程中发生的无法直接列支的相关费用，主要用于补偿项目承担单位为了项目研究提供的现有仪器设备、房屋、水、电、气、暖消耗，有关提高科研管理、服务能力等费用，以及绩效支出等。</p>				

五、主承担单位及参与单位分工及经费分配情况

承担单位（名称及盖章）	华南农业大学			
工作分工	全面负责狮头鹅高效养殖技术的整体实施和推广。			
经费预算分配情况	总经费分摊 （万元）	7.5	市级财政科技资金分配 （万元）	7.5
参与单位（名称及盖章）	汕头市白沙禽畜原种研究所			
工作分工	负责狮头鹅高效健康养殖技术推广，开展技术咨询、技术服务 1次/年以上，举办技术培训、现场会、展示会等活动1次/年以上，在省、市级电视台、报纸、网站、电子刊物、官方公众号等媒体上发布特派员工作报告 1篇。			
经费预算分配情况	总经费分摊 （万元）	3.75	市级财政科技资金分配 （万元）	3.75
参与单位（名称及盖章）	广东省农业科学院			
工作分工	负责狮头鹅高效健康养殖技术推广，开展技术咨询、技术服务 1次/年以上，举办技术培训、现场会、展示会等活动1次/年以上，在省、市级电视台、报纸、网站、电子刊物、官方公众号等媒体上发布特派员工作报告 1篇。			
经费预算分配情况	总经费分摊 （万元）	3.75	市级财政科技资金分配 （万元）	3.75

六、项目组人员情况

项目负责人								
序号	姓名	职称	职务	最高学位	现从事专业	所在单位	身份证件号码	在本项目中承担的工作任务
1	朱勇文	副教授	无	博士	家禽饲料配制技术、家禽营养代谢与调控	华南农业大学	431122198607243414	全面负责狮头鹅高效健康养殖技术推广与应用。
主要参与人员								
序号	姓名	职称	职务	学位	现从事专业	所在单位	身份证件号码	在本项目中承担的工作任务
1	陈俊鹏	高级畜牧师	股长	硕士	狮头鹅养殖技术	汕头市白沙禽畜原种研究所	445121198312203617	负责狮头鹅高效健康养殖技术推广
2	魏文康	研究员	所长	博士	畜禽疫病监测与综合防控	广东省农业科学院	441423197105151018	负责狮头鹅疫病监测与综合防控

八、项目重复申报/立项情况自查

本项目（或其主要内容）是否同时申报了本年度市科技局其他类别的科技计划项目：							否	
序号	名称或主要内容与本项目相同的项目	业务类别	申报人	申报单位	申请金额（万）	否		
合作单位或参加人员是否在本年度申报的市科技局其他科技计划项目中包含了本项目主要内容：								
序号	名称或主要内容与本项目相同的项目	业务类别	申报人	申报单位	申请金额（万）	否		
本项目（或其主要内容）是否包含于已获市科技局资助的其他科技计划项目中（含合作单位、参与人员）：								
序号	名称或主要内容与本项目相同的已获资助项目	业务类别	负责人	承担单位	项目编号	立项年度	立项金额（万）	完成情况

九、本任务书签约各方

管理单位（甲方）：汕头市科学技术局		(盖章)
单位地址：汕头市海滨路12号汕头科技馆九、十楼		(盖章)
法定代表人（或授权代表）：		(盖章)
联系人（经办人）姓名：		(盖章)
E-mail: stkjzypzk@163.com		
电话：		
		2022年 10月 13日
承担单位（乙方）：华南农业大学		(盖章)
单位地址：五山路483号		
法定代表人（或法人代理）：		刘雅红 (盖章)
联系人（项目主管）姓名：		董梅 (盖章)
E-mail: kjcgxk@scau.edu.cn		
电话：13602414573		
开户单位名称：华南农业大学		
开户银行：中国工商银行		
开户帐号：3602002609000310520		
		2022年 9月 30日
乙方主管部门（丙方）：汕头市科学技术局		(盖章)
单位地址：汕头市海滨路12号汕头科技馆九、十楼		
法定代表人（或法人代理）：		(盖章)
联系人（项目主管）姓名：		(盖章)
E-mail: stkjzypzk@163.com		
电话：		
开户单位名称：		
开户银行：		
开户帐号：		
		年 月 日

附件 2

项目编号: B Q W Z X - 2 0 2 4 - ____ - ____

华南农业大学 2024 年服务“百千万工程” 项目合同书

项 目 名 称:	阳山鸡健康高效标准化养殖技术应用与示范
团 队 名 称:	
申 请 单 位:	(公章) 动物科学学院
项 目 负 责 人:	朱勇文
联 系 电 话:	18818912892
邮 箱:	408034085@qq.com
填 报 日 期:	2024.9

华南农业大学新农村发展研究院

二〇二四年九月

项目名称		阳山鸡健康高效标准化养殖技术应用与示范				
团队名称						
申请单位		动物科学学院		申请经费（万元）		9
项目负责人		朱勇文		职称	副教授	
所学专业		动物营养与饲料科学	学位	博士研究生		
现从事专业		家禽健康高效养殖技术、家禽营养与饲料配制				
服务地区/单位		清远市阳山县/阳山润翔养殖发展有限公司和阳山县天瑞然农业有限公司				
项目类型		<input type="checkbox"/> 专题一 服务现代农业产业高质量发展专题 <input type="checkbox"/> 专题二 全国农业科技现代化先行县建设专题 <input checked="" type="checkbox"/> 专题三 定点帮扶专题 <input type="checkbox"/> 专题四 绿美广东生态建设专题				
申报方向		支持方向 2. “双百行动”紫金阳山结对专项				
起止时间		2024 年 10 月 1 日至 2025 年 12 月 31 日				
团队 成 员	姓名	性别	出生年月	技术职称	所在单位	任务分工
	叶慧	女	1980.10.8	高级实验师	动物科学学院	肉鸡营养与料配制
	王伟唯	男	1990.8.18	副研究员	动物科学学院	肉鸡疫病防控
	董泽敏	女	1977.5.8	讲师	动物科学学院	肉鸡养殖技术
	陈泓睿	男	2000.6.12	硕士研究生	动物科学学院	样品收集与分析
	孙广杰	男	2001.7.5	硕士研究生	动物科学学院	样品收集与分析

编写提纲

(可根据项目具体情况进行适当调整)

一、项目概述

2023 年肉鸡出栏量超过 100 亿只,其中黄羽肉鸡达 35.95 亿只,产生了巨大的社会与经济效益。阳山鸡产业是广东省清远市阳山县的一大特色产业,阳山鸡具有较强的抗病能力和适应性,肉质滑嫩、皮爽、味道鲜美,适合在多种环境下饲养。与清远鸡、胡须鸡和文昌鸡相比,阳山鸡的养殖方式与技术更新相对滞后。在当前高质量畜牧业发展理念的倡导下,绿色、健康、高效养殖技术已经成为黄羽肉鸡产业转型升级、提质增效的关键。为此,本项目针对目前养殖效率低、生产技术相对落后、标准化和规模化程度偏低等现状,基于前期阳山鸡产业调研,研究集成阳山鸡保种技术、笼养及生物床发酵饲养技术、高效低排饲料配制技术开发及养殖废弃物资源化处理技术等技术体系,形成阳山鸡绿色高效养殖技术创新与集成示范,提高阳山鸡产业的生产效率,减少养殖废弃物的排放,达到绿色安全高效和可持续循环生产的目的,推动阳山鸡生产向规模化、集约化、标准化和产业化方向升级发展,助力乡村振兴。

二、项目实施方案(技术简述、服务内容、实施进度等)

(一) 技术简述

通过技术优化、组装集成和示范推广实现阳山鸡的绿色、高效养殖,构建阳山鸡保种技术、优化饲料营养与养殖技术、粪污处理技术,构建品种高繁、养殖过程精准控制、末端充分利用的三位一体化的阳山鸡高效养殖技术。然后,建立标准化、规范化养殖技术,在大型养殖企业示范推广,全面促进清远市阳山鸡产业转型升级

级，实现产业振兴和农村致富。

（二）服务内容

1、阳山鸡保种技术体系的构建与推广

研究对阳山鸡的分布、数量、遗传多样性进行资源普查，收集历史资料和现有样本。通过分子生物学技术评估种群的遗传多样性，识别关键遗传位点。建立现代化的繁殖基地，采用封闭式管理，确保种群的纯度和健康。

2、阳山鸡笼养及生物床饲养技术集成与示范

构建阳山鸡笼养养殖及生物床饲养模式，一方面优化笼养养殖舍内、外设施、垫片结构等参数；开展生态养殖发酵床设计、发酵菌种组合、垫料选择、制备及配比、营养素添加量与翻堆频率等生物发酵床养殖关键技术与相关配套技术研究，最终集成阳山鸡标准化笼养及生物床养殖技术方案。

3、阳山鸡高效低排饲料配制技术开发与应用

开展阳山鸡常用饲料原料营养与饲用价值评定；针对稻谷和小麦肉鸡非常规能量饲料抗营养因子特点，优化基于酶制剂的稻谷为主体能量饲料的肉鸡日粮配制，构建阳山鸡高效低排饲料配制技术体系。

4、阳山鸡养殖废弃物资源化处理技术集成与示范

评价阳山鸡粪污特征参数及粪污产生量，研究粪尿废弃物收集技术，优化鸡粪堆肥发酵技术、鸡粪+农作物桔杆混合发酵生产有机肥技术和还田生态种养模式，促进绿色有机循环农业的发展。

（三）实施进度

2024.10-2024.12：调研阳山鸡的分布、数量、遗传多样性，选择并建立现代化的繁殖基地，采用封闭式管理，确保种群的纯度

和健康。

2025.1-2025.6：开展阳山鸡笼养及生物床饲养技术参数探究，形成配套技术方案。

2025.7-2025.12：基于笼养及生物床饲养模式，开展阳山鸡饲料营养与饲料配制技术的研究，

2026.1-2026.12：优化鸡粪堆肥发酵技术、鸡粪+农作物桔杆混合发酵生产有机肥技术，提高养殖废弃物资源化处理利用度。

三、合作基础（合作共建单位、服务地区/单位、前期基础等）

申请人及其所在团队于 2023-2024 年度积极对接了合作共建单位清远市阳山润翔养殖发展有限公司和阳山县天瑞然农业有限公司，前期开展了阳山鸡养殖情况调研，在育种、饲养技术、市场销售和人才与培训等产业状况摸清了产业需求和存在的问题，为清远市农业农村局针对性制定一企一策的帮扶规划 3 份，建立了阳山鸡全程科学饲养规程，普及了肉鸡玉米、豆粕减量替代技术，提供了豌豆、椰子粕、芝麻粕、向日葵粕、花生仁粕、米糠等非粮饲料原料营养价值库，为进一步降低饲料成本奠定基础。同时对夏季高温季节阳山鸡的健康饲养提供了方案，编制了《鸡抗热应激管理手册》并发放到技术人员手中；同时线上线下为三家企业提供技术支持、交流等 20 余次，为本项目的顺利实施和高质量完成提供了良好的前期合作基础。



图 1 清远阳山县阳山鸡产业调研



图 2 阳山润翔养殖发展有限公司宣传照



图 3 阳山县天瑞然农业有限公司宣传照



图 4 配合清远市政府制定阳山鸡产业帮扶计划

四、项目考核指标

1. 技术指标

- (1) 示范推广新品种、新产品、新工艺、新技术等
示范推广标准化阳山鸡笼养和生物发酵床养殖模式 2 个，建立

阳山鸡高效低排饲料配制和粪污资源化利用新工艺 2 个，制定《阳山鸡标准化饲养规程》1 套。

（2）开展技术咨询和技术服务内容、次数、人数

服务 5 家以上新型农业经营主体，举行阳山鸡标准化饲养技术培训会 2 次以上，培训人员 50 人次以上；线上线下养或指导肉鸡养殖 20 次以上，培养乡村振兴人才数量 5 人以上。

2. 预期经济、社会、环境效益（产业贡献、推广规模、节本增效、带动就业、提高生活水平、保护环境等）

（1）经济效益

通过采用“高校+企业+农户”的合作模式，通过示范推广阳山鸡标准化养殖技术，与养殖户建立紧密利益连接机制，肉鸡成活率提高 5%以上，饲养成本降低 3 元/只，在龙头企业示范推广量超 10 万只/年，实现经济增收 30 万元以上/年。

（2）社会、环境效益

通过研发推广绿色生态阳山鸡高效技术，实现养殖废弃物无害化、减量化、资源化综合利用，减少碳氮及矿物元素排放 10%以上。

3. 凝练典型案例（获得地市以上政府部门表彰或奖励）

针对阳山县阳山鸡特色产业发展，在落实“双百行动”中凝练“阳山鸡产业科技帮扶转化为新质生产力”主题的典型案例 1 个，获得清远市以上政府部门表彰或报道 1 次。

4. 决策咨询报告（或省部级及以上政府部门采纳或获得省部级及以上领导肯定性批示）

通过产业调研和帮扶计划制定及其成效，为清远市农业农村局提供阳山鸡产业发展调研和帮扶计划咨询报告 1 份，并获得省级

以上部门批示。

5. 发布相关新闻报道（省级及以上主流媒体报道）

通过帮扶计划的实施，在提升阳山鸡产业的整体竞争力、促进地方经济的全面发展和农民增收与乡村振兴的目标等方面在省级及以上主流媒体报道 2 次以上。

五、经费预算

(一) 经费预算表

单位: 万元

序号	预算科目名称	专项经费	备注
1	设备费		
	(1) 购置设备费		
	(2) 试制设备费		
	(3) 设备改造与租赁费		
2	材料费	3	用于试剂耗材等购买
3	测试化验加工费	1	用于指标的委托检测
4	燃料动力费		
5	差旅费	2	用于赴阳山县调研等租车等费用
6	会议费		
7	培训费	1	用于技术培训等费用
8	资料费		
9	劳务费	2	用于研究生等费用
10	专家咨询费		
11	其他支出		
合计		9	

说明:

- 1.不得预算购买仪器设备费用等与科技服务无关的研究性开支。
- 2.项目负责人差旅费报销额度不得超过差旅费预算的 50%。

六、申请单位推荐意见

同意

负责人（签章）：朱第1

2024年9月13日

单位（公章）：动物科学学院

2024年9月15日



七、乡村振兴与社会服务处意见

单位（公章）：

年 月 日





编 号： 210708176893238

专题编号： 2021020103-2

技术领域： 畜牧(畜禽繁育、养殖方法、饲料等) 专题名称： 3-2.科技特派员专项（企业科技特派员）

2021年广东省科技专项资金项目任务书

项 目 名 称:	夏季狮头鹅种鹅抗热应激饲料配制技术示范与应用				
专项资金类别:	2021年广东省科技专项资金（“大专项+任务清单”）				
业务类型:	四、农业农村创新能力提升				
申请单位:	华南农业大学				
通 讯 地 址:	广东省广州市天河区五山路华南农业大学动物科学学院				
项目起止时间:	2021-07-08至2021-07-08				
邮 政 编 码:	510642	单位电话:	020-38632819	传 真:	020-85281885
项 目 负 责 人:	朱勇文	联系电话:	18818912892	手 机:	18818912892
项 目 联 系 人:	朱勇文	联系电话:	020-38882017	手 机:	18818912892
电 子 邮 箱:	kjcgxk@scau.edu.cn				
主 管 部 门:	汕头市科学技术局				
申 报 日 期:	2021-07-08				

汕头市科学技术局

二零二一年制

一、项目基本情况表

项目名称	夏季狮头鹅种鹅抗热应激饲料配制技术示范与应用			
项目起止时间	2021-07-08至2021-07-08			
项目总经费预算	2万元			
课题研究方向	无			
社会经济目标	0903.畜牧业			
项目摘要（200字以内）：				
围绕夏季热应激下狮头鹅种鹅繁殖性能、采食量和养分消化率的变化规律及基于抗氧化和肠道健康提高其采食量和养分利用率2个方面的产学研合作与技术指导，并提供抗热应激饲养管理及粪污无害化处理等技术支持，最终形成夏季热应激下蛋种鸡抗热应激饲料配制及高效健康养殖技术一套，通过公司+合作社+养殖户的模式进行示范与推广应用，最终辐射带动其他相关技术需求涉农区。				
关键字（用“；”隔开）	狮头鹅；热应激；饲料营养；生产性能			
项目技术情况	技术领域	畜牧(畜禽繁育、养殖方法、饲料等)		
	研究活动类型	应用研究	项目研究阶段	前期研究
	技术来源	自有技术	学科	家禽营养学
预期成果形式	新工艺,论文论著			
项目已受财政资金资助情况	无			
简要说明：				
本项目基于前期合作基础及狮头鹅种鹅养殖企业技术需求提出的核心问题：夏季热应激狮头鹅种鹅生产效率低下，亟待改善热应激引起的狮头鹅种鹅采食量和繁殖性能下降等生产技术难题，开展科技特派员技术对接与服务。				
其它需要说明的问题（限100字）：				
无				

二、项目内容

<p>(一) 项目建设背景和意义 (200字以内)</p> <p>肉鹅养殖是农民增收主要来源之一。夏季热应激引起肉鹅生产性能和繁殖力等下降，特别在种鹅尤为突出且越受重视。热应激一般会引起5-10%的受精率和孵化率下降，造成难以估量经济损失。热应激首先降低采食量和营养物质吸收，进而影响机体生化代谢水平；此外，产生过多自由基引起机体氧化损伤，导致生产效率下降。因此，采用饲料营养调控手段缓解提高繁殖力低狮头鹅种鹅的抗热应激能力，对于狮头鹅产业良性发展具有重要意义。</p>
<p>(二) 项目研发内容和所采用的关键技术 (1000字以内)</p> <p>(参考提纲: ①主要研究内容②已解决的关键问题及技术路线③创新点)</p> <p>1、主要研究内容</p> <p>针对夏季热应激狮头鹅种鹅生产效率低下的技术需求问题，通过以下4步系统有层次地开展工作：</p> <p>(1) 研究夏季热应激下狮头鹅种鹅繁殖性能、采食量、消化道发育、消化酶分泌及养分消化率变化情况，明确夏季热应激种鹅繁殖性能与采食量和养分利用率的关系，初步确定夏季热应激种鹅生产效率低下是否与采食量和营养代谢机能下降有关。</p> <p>(2) 从提高饲料原料营养利用角度出发，分析饲料原料常规养分、抗营养因子和代谢能，建立能值预测模型；然后，通过添加复合酶处理，进一步评价能量和粗蛋白利用率改善情况，为夏季热应激下种鹅饲料原料的选择及营养价值判断提供试验依据。</p> <p>(3) 从改善肠道健康和提高抗氧化性能角度出发，研究抗氧化剂及酶制剂、益生菌和酸化剂组合使用对夏季热应激下狮头鹅种鹅繁殖性能的改善作用，为夏季热应激下种鹅饲料配制添加剂选择使用提供试验依据。</p> <p>(4) 结合饲养管理、生物防控和粪污处理等技术，最终形成夏季热应激下狮头鹅种鹅高效健康养殖与抗热应激饲料配制技术集成技术一套，通过公司+合作社+养殖户的模式进行示范推广应用，最终辐射带动其他相关技术需求涉农区。</p> <p>2、已解决的关键问题及技术路线</p> <p>在明确夏季热应激种鹅生产效率低下与采食量和营养代谢下降相关的基础上，基于抗氧化和肠道健康提高其采食量和养分利用率的饲料营养策略，结合饲养管理、生物防控和粪污处理等技术，形成夏季热应激下狮头鹅种鹅高效健康养殖与抗热应激饲料配制技术集成技术1套，通过公司+合作社+养殖户的模式进行示范推广与应用，最终辐射带动其他相关狮头鹅种鹅养殖技术需求涉农区。</p> <p>3、创新点</p> <p>基于抗氧化和肠道健康提高其采食量和养分利用率解决夏季热应激狮头鹅种鹅生产效率低下的技术需求问题，形成狮头鹅种鹅抗热应激的精准饲料配制技术。</p>

(三) 项目所获得成果 (单位: 项数)					
1.主要成果指标					
成果形式		成果数量	成果形式		成果数量
发明专利	申请	0	引进人才 (人)		0
	授权	0	培养人才 (人)		0
实用新型专利	申请	1	科技人才奖励 (人)		0
	授权	0	科技报告 (篇)		0
外观设计专利	申请	0	技术标准制定	牵头 (个)	0
	授权	0		参与 (个)	0
国际专利	PCT受理	0	软件著作权 (项)		0
	授权	0	论文论著 (篇)		2
获国家级奖项 (项)		0	被收录论文数 (篇))	SCI	1
获省级奖项 (项)		0		EI	0
新服务 (项)		0		ISTP	0
新产品 (或新材料、新装备、新品种 (系))		0	新工艺 (或新方法、新模式、新技术)		1
其他将提供的研究开发成果及形式(创新特色、成果宣传推介措施等, 限200字):					
形成夏季热应激下狮头鹅种鹅高效健康养殖技术规程1套 (新工艺)。					
项目所获得成果应用情况 (限300字)					
无					

四、项目承担单位与参与单位基本情况表

(一) 项目承担单位				
单位基本情况表				
单位名称及盖章	华南农业大学	组织机构代码	124400004554165634	
注册资金（万元）	311733	银行信用等级	AAA	
单位性质	高等院校	单位级别	省级	
所在地区	广州市-天河区	所属行业	农、林、牧、渔业	
职工总人数(人)	3401	研究人员数(人)	2283	
上年度研发经费(万元)	63000	技工贸总收入(万元)	0	
是否科技特派员派出单位	是			
近3年承担科技计划项目数量				
	省级	市级	其他	
项目数（项）	750	0	1498	
项目总经费（万元）	62000	0	100300	
建立的科研平台				
工程技术研究中心（个）	国家级	博士后科研工作站（个）	国家级	
企业重点实验室（个）	国家级	其它		
项目承担单位工作分工及经费分配情况				
工作分工	负责、协同和统筹整个项目实施、开展和验收			
经费预算分配情况	总经费分摊（万元）	1.6	后补助经费分配	1.6

(二) 项目参与单位

单位基本信息

单位名称及盖章	汕头市正治禽业有限公司			组织机构代码	91440515698191856D
注册资金（万元）	300	所在地区	汕头市-澄海区	银行信用等级	AA+
单位性质	有限责任公司	所属行业	A 农、林、牧、渔业	单位国别	中国大陆
单位级别	市级				
单位法人	徐伟汉	证件类型	身份证	证件号码	440521196708303815
职工总人数(人)	60			研究人员数(人)	30
上年度销售额(万元)	1200			年利税	100
上年度研发经费总额(万元)	100			研发经费占销售额比例(%)	8.33
企业认定情况	双软认证企业				
工作分工	协助开展狮头鹅饲养试验和样品采集				
经费预算分配情况	总经费分摊（万元）	0.4		后补助经费分配	0.4

五、项目组人员情况

项目负责人									
序号	姓名	职称	职务	最高学位	现从事专业	所在单位	身份证件号码	在本项目中承担的工作任务	签名
1	朱勇文	副教授	无	博士	家禽饲料配制技术、家禽营养代谢与调控	华南农业大学	431122198607243414	负责项目设计、实施和验收及对接企业其他技术需求，开展相关技术培训等。	
主要参与人员									
序号	姓名	职称	职务	最高学位	现从事专业	所在单位	身份证件号码	在本项目中承担的工作任务	签名
1	林渤	未取得	总经理	其他	种鹅场的管理和生产	汕头市正治禽业有限公司	440583198406293816	负责种鹅场的管理和生产	
2	叶慧	高级实验师	无	硕士	动物营养与饲料	华南农业大学	440106198010011849	负责样品收集与指标分析	
3	董泽敏	讲师	无	硕士	家禽营养与饲料配制	华南农业大学	420111197208045027	项目调研与养殖技术指导	
4	高威	未取得	无	学士	家禽营养与代谢	华南农业大学	500228199707206878	狮头鹅饲养试验与样品采集	

八、承担单位与本项目相关的研究开发能力与服务水平

近三年开展研究开发项目情况					
项目名称	立项部门、计划类型	项目经费	起止时间		
饲料资源开发利用	农业农村部-国家水禽产业体系岗位专家项目	350	2020-07-09 至 2025-07-09		
鹅的标准化饲养技术及南方地区经济作物副产物对鹅的饲用价值研究	科技部-公益性科研行业专项	210	2013-10-11 至 2017-09-22		
开展产学研合作情况					
项目名称	合作单位	项目经费	起止时间		
狮头鹅现代化网上立体养殖技术研发	汕头市滨裕养殖有限公司	50	2020-07-01 至 2025-07-01		
获得省部级以上科学技术奖励情况					
成果名称		奖励类别及等级	获奖年度		
近三年新产品清单					
产品名称		批准部门	批准年度		
获得自主知识产权情况					
序号	专利名称	类别	专利号或批准文号	授权单位	授权时间
1	一种禽类代谢笼	实用新型发明	CN201220277618.9	华南农业大学	2012-06-13
2	一种禽类动物代谢与育雏笼	实用新型发明	CN201720316952.3	华南农业大学	2017-12-14

十、本合同签约各方

管理单位（甲方）：汕头市科学技术局			(盖章)
单位地址：汕头市海滨路12号汕头科技馆九、十楼			
法定代表人（或授权代表）：			(签章)
联系人（经办人）姓名：			(签章)
E-mail: stkjzypzk@163.com			
电话：0754-88426648			
			年 月 日
承担单位（乙方）：华南农业大学			(盖章)
单位地址：五山路483号			
法定代表人（或法人代理）：			刘雅红 (签章)
联系人（项目主管）姓名：			朱勇文 (签章)
E-mail: kjcgxk@scau.edu.cn			
电话：020-38882017			
开户单位名称：华南农业大学			
开户银行：中国工商银行			
开户帐号：3602002609000310520			
			年 月 日
乙方主管部门（丙方）：汕头市科学技术局			(盖章)
单位地址：汕头市海滨路12号汕头科技馆九、十楼			
法定代表人（或法人代理）：			(签章)
联系人（项目主管）姓名：			(签章)
E-mail: stkjzypzk@163.com			
电话：0754-88426648			
开户单位名称：			
开户银行：			
开户帐号：			
			年 月 日

任务书编号：2024E04J0254

广州市科技计划项目 任务书

项目名称：天然活性物质在蛋鸡日粮中高效利用及其提升
蛋品质的关键技术应用

承担单位：华南农业大学

特派员：朱勇文

计划类别：创新环境计划

专题名称：2024年度农村科技特派员专题

支持方向：市外帮扶方向

组织单位：华南农业大学

起止时间：2024-01-01 至 2025-12-31

主管处室：农村和社会发展科技处

广州市科学技术局制

二〇二四年

一、项目基本信息

项目名称	天然活性物质在蛋鸡日粮中高效利用及其提升蛋品质的关键技术应用			
计划类别	创新环境计划		专题名称	2024年度农村科技特派员专题
支持方向	市外帮扶方向			
指南发布日	2023-04-15			
项目开始时间	2024-01-01		项目结束时间	2025-12-31
资助方式	竞争性前资助		申请市财政科技经费(万元)	10
广东省技术领域	农业技术畜牧(畜禽繁育、养殖方法、饲料等);			
承担单位	华南农业大学			
服务单位	纳雍县新华牧业有限公司			
组织单位	华南农业大学			
	姓名	办公电话	手机号码	电子邮箱
特派员	朱勇文	0203-8882017	18818912892	zhuyw0724@scau.edu.cn
项目联系人	朱勇文	020-38882017	18818912892	zhuyw0724@scau.edu.cn
承担单位法人	薛红卫	020-85280001	13701841036	kycjkh@scau.edu.cn
项目摘要	本项目围绕蛋鸡饲料玉米减量替代及其鸡蛋营养品质的产业焦点和需求，选择贵州省毕节地区消费市场潜力大的蛋鸡为研究对象，探究天然活性物质在蛋鸡玉米替代多元化和功能保健蛋生产日粮中的高效利用技术，聚焦质量稳定控制、抗氧化保护增效及肠道保健促吸收等关键环节，实现构建“原料稳定—饲料增效—蛋品提质”营养调控技术体系与示范推广，促进鸡蛋消费市场扩大及其产业的可持续发展。项目拟实现绩效目标：（1）形成基于玉米替代多元化日粮下功能保健蛋生产的饲料配制技术方法1套。（2）构建基于“原料稳定—饲料增效—蛋品提质”三位一体的天然活性物质整体应用的模式1套。（3）打造如“富硒低胆固醇鸡蛋”、“灵芝人参鸡蛋”等保健蛋产品1个以上。			

二、承担单位基本情况

单位名称	华南农业大学	统一社会信用代码	124400004554165634
注册时间	1952-01-01	单位类型	高等院校
注册地址	广东省广州市天河区五山路483号		
办公地址	广东省广州市天河区五山路483号		
联系人	姓名	倪慧群	
	手机号码	13711345768	
	电子邮箱	kjcgxk@scau.edu.cn	
开户银行	广东广州工行五山支行		
开户户名	华南农业大学		
银行账号	3602002609000310520		

三、服务单位基本情况

单位名称	纳雍县新华牧业有限公司		单位类型	其他
是否为独立法人	其他		统一社会信用代码	91520525MA6E34MR9U
注册地址	贵州省毕节市纳雍县贵州省毕节市纳雍县董地乡新华村岩上组			
服务单位法人	姓名	刘吕		
	手机号码	18748538888		
联系人	姓名	陈小静		
	办公电话	18212783660		
	手机号码	18212783660		
基本情况	纳雍县新华牧业有限公司创立于2017年7月， 注册资本1000万元，公司地址：贵州省毕节市纳雍县董地乡新华村岩头上组。公司主营业务含盖了蛋鸡、青年鸡、成品鸡的培育和喂养，全自动化生产加工。公司占地面积为85.32亩，标准化鸡舍5栋（其中4栋为产蛋鸡、1栋为育雏鸡，内含自动化设备），蛋库房2个，饲料加工车间（仓库）2个，饲料加工机组2套，员工宿舍1栋，食堂1栋，有机肥加工厂1个（含无害化处理设备2套），综合办公楼1栋，鸡舍喷雾消毒，自动清粪系统等相应设施设备配备完。目前饲养蛋鸡40万羽，其中产蛋鸡30万羽，青年鸡10万羽，日产蛋量约28万枚，日产值为18.75万元，年产值6750万元。2020年年产值为5400万，2021年年产值为6500万，2022年年产值为7000万，2023年年产值将突破8200元。			
技术需求	随着生活水平的提高，人们对鸡蛋营养品质和活性物质保健功能的要求郑逐步提高。但在集约化养殖条件下仅靠饲料原料中的少量活性物质（如玉米黄质、斑蝥黄等），其效果已不能满足鸡蛋消费市场的需求。因此，天然活性物质（如叶黄素、茶多酚、人参多糖等）广泛用于提高鸡蛋营养品质，也符合消费者对食品健康安全的需要。但多数天然活性物质在光、氧和热中不稳定，易在贮存和饲料生产过中被氧化破坏；且生物学效价受到原料品质、配方营养水平（如玉米减量替代、脂肪氧化酸败、霉菌毒素含量等）和宿主肠道健康（氧化应激、坏死性肠炎、球虫病等）等诸多因素的影响。因此，天然活性物质的高效利用在玉米替代多元化和功能保健鸡蛋生产日粮中应用越显重要。大量研究表明，植物提取物中功能活性物质不仅具有良好抗氧化活性，而且表现出较强的抑菌抗菌作用。因此，生产实践中如何实现通过从产品稳定性、抗氧化保护及肠			

	道促吸收等的关键环节确保天然活性物质效果，是实现鸡蛋品质提质增效的关键营养技术措施。
前期合作基础	<p>项目双方长期围绕家禽营养代谢与产品品质调控、天然色素及活性物质开发与利用、植物提取物等技术方面的研究和推广工作开展合作。项目双方合作单位近三年均承担或参加与本项目有关的多项自然科学基金项目、国家农业产业体系项目、十三五国家重点研发计划子课题10余项，在玉米减量替代与饲料资源开发利用、家禽产品品质与营养调控、植物提取物开发利用与肠道保健技术等研究领域已有探索性的积累和单项技术成果的储备。其中，项目申请团队前期围绕玉米豆粕减量替代和肠道营养保健的饲料配制技术方面，体内外系统评价了20多种减量替代玉米豆粕的非常规饲料原料（小麦和稻谷及其加工副产物、农产品加工副产物糟粕类、南方特色经济作物加工副产物）的养分利用效率与饲用价值。此外，探究了饲粮低蛋白水平、脂肪酸比例、植物提取物（姜黄素、黄梁木黄酮、酚酸等）、益生菌、酶制剂和抗营养因子含量（游离棉酚、硫葡萄糖苷、霉菌毒素等）对家禽生长发育、肉品质和肠道健康的影响，在饲料营养高效利用及产品品质营养调控等方面拥有较为深入的技术基础。以上研究基础为本项目的天然色素及活性物质组合在替代玉米型多元化日粮和肠道保健替抗日粮中高效利用技术和饲料配制优化等方面提供了技术支撑和方法保障。此外，本项目合作双方均与多家农牧养殖与饲料企业（温氏水禽公司、海大集团、金钱饲料公司、广东新安大农牧有限公司）合作，为天然色素及活性物质高效利用技术的示范与推广提供了应用落地平台。</p>

四、特派员及团队成员信息

特派员	姓名	朱勇文	证件类型	身份证	证件号码	431122198607243414	性别	男		
	出生日期	1986-07-24	民族	汉族	国籍	中国	学历	博士研究生		
	学位	博士	学位授予国家（或地区）	中国	职务	无	职称	副高级		
	所学专业	动物营养与饲料科学	手机号码	18818912892	办公电话	0203-8882017	电子邮箱	zhuyw0724@scau.edu.cn		
特派员团队成员（含特派员）										
序号	姓名	证件类型	证件号码	年龄	职务	职称	学位	项目分工	所在单位	手机号码
1	朱勇文	身份证	431122198607243414	36	无	副高级	博士	项目负责人	华南农业大学	18818912892
2	杨琳	身份证	230106196309292114	59	无	正高级	博士	项目指导与咨询	华南农业大学	13903075253
3	叶慧	身份证	440106198010011849	42	无	副高级	硕士	试验样品收集与分析	华南农业大学	15989221997
4	汪珩	身份证	42900619940111091X	29	无	无	硕士	试验样品收集与分析	华南农业大学	18520477161

五、特派员工作、服务经历

1. 工作经历					
序号	所在城市	起止时间	工作单位	行政职务	专业技术职务（职称）
1	广东省广州市天河区五山路483号华南农业大学动物科学学院	2016-07-01 至 2022-09-19	华南农业大学	无	副高级
特派员近3年技术领域和成果情况		近三年来作为国家科技特派员和广东驻镇帮镇扶村农村科技特派员，积极对接国家乡村振兴重点帮扶县贵州省毕节市纳雍县和遵义市务川仡佬族苗族自治县及广东省汕头市澄海区莲下镇和梅州市五华县棉洋镇，针对帮扶对象提出的畜禽（蛋鸡、肉牛、黄羽肉鸡和狮头鹅）健康高效养殖技术需求，开展调查研究、技术指导、成果转化、技能培训等服务。1、资源推广：技术推广转化行动，助力实现畜禽产业提质增效。因地制宜提出并推广地“保种选育+饲料营养+生态饲养”三位一体的纳雍优质鸡健康高效养殖新技术，重点打造“纳雍滚山鸡”品牌。2、研发创新：技术创新攻关行动，助力解决畜禽养殖难点问题。开发利用地源性工农业副产物（白酒糟粕、刺梨渣等）等非常规饲料资源，解决务川当地肉牛饲草资源不足的问题，实现养户增收。3、成果转化：成果推广转化行动，培育乡村振兴产业动能。联合创建一县（镇）一校一品。比如联合企业打造“富硒高卵磷脂DHA”多功能保健鸡蛋品牌。4、技术服务：打造高校特色的帮扶模式，夯实乡村振兴发展基础。“高校+研究所+驻镇队+农户”四位一体帮扶模式，学习强国等平台报道3次。3次。			
2. 服务经历					
序号	服务对象	服务起止时间	技术服务内容		

1	梅州市五华县棉洋镇绿水村、平安村、竹坑村和荣华村	2018-06-01 至 2022-12-31	采用“贫困户+基地+合作社+公司+引进技术和市场”的模式，组建家禽养殖脱贫合作社；总体上帮助解决夏季高温条件下蛋鸡蛋品质不佳、产蛋后期骨骼质量下降和死淘率高和夏季热应激中鸡生产效率低下、肉品质欠优、肠道健康等相关技术难题，并提供了相应缓解热应激的饲养管理和饲料营养措施；联动以“公司+农户”形式提供开展技术咨询/服务等30余人次，成功打造当地五黑鸡和富硒蛋等有机农产品品牌和销售渠道。
2	广东省汕头市澄海区莲下镇	2021-07-01 至 2025-12-31	作为广东省第三批驻镇帮镇扶村农村科技特派员，以“高校+研究所+驻镇队+农户”四位一体形式，调研了汕头市澄海区莲下镇等狮头鹅养殖产业状况，实地调研发现了解决狮头鹅痛风死亡率高和夏季热应激肉鹅生产效率低下的问题，协助提供了相应缓解痛风和热应激措施，以改善狮头鹅育雏期成活率；推广稻—草—鹅生态种养结合模式，可节约饲料成本5元/只以上。
3	贵州省毕节市纳雍县和六盘水水城区	2022-01-01 至 2025-12-31	作为国家科技特派员积极对接国家乡村振兴重点帮扶县贵州省毕节市纳雍县和六盘水水城区，因地制宜提出并推广地“保种选育+饲料营养+生态饲养”三位一体的纳雍优质鸡健康高效养殖新技术，重点打造“纳雍滚山鸡”品牌。成果推广转化行动，培育乡村振兴产业动能。联合创建帮扶去一县（镇）一校一品。正在联合企业打造“富硒高卵磷脂DHA”多功能保健鸡蛋品牌。
特派员开展科技推广服务工作基础简介			2018年被评为广东省农村科技特派员，2018年6月至今主持“广东地区夏季三黄鸡高效健康养殖与示范”广东省农村科技特派员项目，与梅州市五华县棉洋镇绿水村、平安村、竹坑村和荣华村对接帮扶，采用“贫困户+基地+合作社+公司+引进技术和市场”的模式，组建家禽养殖脱贫合作社；总体上帮助解决夏季高温条件下蛋鸡蛋品质不佳、产蛋后期骨骼质量下降和死淘率高和夏季热应激中鸡生产效率低下、肉品质欠优、肠道健康等相关技术难题，并提供了相应缓解热应激的饲养管理和饲料营养措施；联动以“公司+农户”形式提供开展技术咨询/服务等30余人次，成功打造当地五黑鸡和富硒蛋等有机农产品品牌和销售渠道；2019年与广州市番禺区南宝饲料有限公司就生长后期肉鹅采食量和养分利用率提高技术研究开展了相关技术需求合作，技术成果覆盖从化饲料经销商及其对接贫困养殖户；2020年对接广州市从化广东三天鲜畜牧有限公司，针对其提出的夏季蛋鸡高效健康养殖的技术需求，双方开展了夏季蛋鸡采食量和养分利用率提高技术研究

任务书签署

甲乙丙三方根据《广州市科技计划项目管理办法》《广州市科技计划项目经费管理办法》《广州市科技计划科技报告管理办法》等有关文件规定，以及有关法律、政策和管理要求，签署本任务书。

签订地点：广州市越秀区

广州市科学技术局（甲方）：广州市科学技术局
局项目经办人：刘晓辉 联系电话：83124045
责任处室负责人：陈洁

2024年01月09日

项目承担单位(乙方): 华南农业大学
二级部门: 华南农业大学动物科学学院
项目负责人: 朱勇文
项目经费汇入账号
账户名: 华南农业大学 账号: 3602002609000310520
开户银行: 广东广州工行五山支行
财务负责人: 肖斐

2023年12月18日

组织单位（丙方）：华南农业大学
项目经办人：倪慧群

2023年12月18日

编 号: 210723106900762

专题编号: 2021020101



专题名称: 1.现代农业重点项目

2021年广东省科技专项资金项目任务书

项 目 名 称:	狮头鹅绿色高效养殖技术创新与示范				
专项资金类别:	2021年广东省科技专项资金 (“大专项+任务清单”)				
业务类型:	四、农业农村创新能力提升				
申请单位:	汕头市正治禽业有限公司				
通 讯 地 址:	广东省汕头市澄海区盐鸿镇港头村				
邮 政 编 码:	515828	单位电话:	0754-85779601	传 真:	0754-85779601
项目 负责人:	徐伟汉	联系电话:	0754-85779823	手 机:	13509872658
项目 联系人:	朱勇文	联系电话:	020-3882017	手 机:	18818912892
电 子 邮 箱:	408034085@qq.com				
主 管 部 门:	澄海区科技局				
推 荐 单 位:	澄海区科技局				
申 报 日 期:	2021-07-01				
汕头市科学技术局					
二零二一年制					

一、项目基本情况表

项目名称	狮头鹅绿色高效养殖技术创新与示范		
项目起止时间	2021-07-03至2024-06-30		
项目总经费预算	80万元	申请经费资助	80万元
项目摘要（200字以内）：			
在当前高质量畜牧业发展理念的倡导下，绿色、健康、高效养殖技术已经成为水禽产业转型升级和提质增效的关键。本项目针对目前狮头鹅生产效率低、水体污染大及规模化程度低等现状，创新集成种鹅反季节高效饲养技术、肉鹅网上及生物床饲养技术、狮头鹅高效低排饲料配制及废弃物资源化利用技术，形成狮头鹅绿色高效养殖技术创新与示范，促进狮头鹅产业绿色高效和可持续循环生产，推动其产业向规模化、集约化和标准化升级发展。			
项目实施必要性（200字以内）：			
肉鹅产业是我国农业中十分重要的支柱产业之一；鹅肉是我国沿海地区民众的重要蛋白质营养来源。狮头鹅作为我国最大型的鹅种，已有300多年的养殖历史，是广东省农业主推品种之一。但狮头鹅繁殖性能低、饲料营养研究滞后、饲养方式落后和节能减排环保观念薄弱等因素，极大限制了狮头鹅产业的高质量发展。特别是近年来狮头鹅消费群体和消费量增长迅猛，有必要推动狮头鹅现代化养殖技术创新新和升级，满足市场多元化需求。			

二、项目内容

(一)、项目实施绩效(单位:项数)

1.主要成果指标

成果形式	成果数量	成果形式		成果数量
专利申请（件）	2	引进人才（人）		0
其中：发明	1	培养人才（人）		3
实用新型	1	科技人才奖励（人）		0
其中：国际专利	0	技术标准制定	牵头（个）	0
软件著作权（项）	0		参与(个)	0
获国家级奖项（项）	0	论文论著（篇）		5
获省级奖项（项）	0	被收录论文数（篇）	SCI	2
新产品（个）	0		IE	0
新材料（种）	0		CA	0
新装备（台/套）	0	新服务（项）		0
新工艺（或新方法、新模式）（个）	1			

其他将提供的研究开发成果及形式(创新特色、成果宣传推介措施等,限200字):

- 1、种鹅反季节生产中父母代种鹅产蛋数提高15%,商品代110日龄体重达到6.5千克。
- 2、建立狮头鹅网上和发酵床养殖示范基地1个。
- 3、年粪便有机物排放量降低10%以上,粪污资源化率达到80%以上。
- 4、举行肉鹅饲养技术培训会2次以上,培训人员50人次以上。

2.本项目完成后预计社会效益情况(限300字):

经济和社会效益:通过采用“企业+基地+农户”等合作模式,与农户建立紧密利益连接机制,将进一步拓宽农民增收致富渠道,显著提升农民收入。月可孵出雏鹅10.9万只,月可获毛利28万元。以每户种鹅饲养户存栏种鹅0.1万只计,可解决30户种鹅户孵蛋难问题。出雏率从75%提高到80%,月可多出雏鹅0.68万只,以每年10个生产月计算,年可多产雏鹅6.8万只,以平均每只鹅苗40元计,可增产272万元。

生态效益:通过研发推广绿色生态狮头鹅生产技术,应用生态环保饲养技术、粪便发酵后直接还田、废水循环再利用等先进实用技术,实现养殖废弃物无害化、减量化、资源化综合利用,减少碳氮及矿物元素排放10%以上。

3.其他主要技术经济指标及社会效益说明(限500字):

通过规模化、标准化养殖模式的推广应用，不断强化与科研院校的合作，全力推动现代化加工及仓储物流体系建设，探索发展农旅融合，在原来单一产销模式基础上逐步实现产加销一条龙、贸工农一体化，不断延伸产业链条，促进三产融合，推进狮头鹅产业化向纵深发展。

(二) 组织实施和保障措施 (400字以内) :

- 1、从技术层面，龙头企业、研究所和高校强强联合和产学研合作，保障狮头鹅技术创新平台的创建和升级。联合参与单位的水禽产业体系研发平台和技术优势，开展产学研合作及技术转化和创新，因地制宜发展规模化、标准化、生态化养殖，实行科学养殖。
- 2、从研究团队层面，项目（任务）参与人有主持省部级科技项目的经验，项目骨干多年从事狮头鹅养殖技术领域的工作，具有丰富的科研经验和知识能力储备，国际学术联系广泛，对国内外相关领域的发展动态十分熟悉。研究人员技术水平可以保障项目的顺利进行。
- 3、从研究平台层面，依托国家水禽产业体系岗站技术和资源优势，深化狮头鹅良种保育和扩繁工作，进一步加大研发和设施装备投入，突出良种保育的核心作用和示范效应，不断提升狮头鹅良种繁育体系的技术水平和竞争优势，并探索推广规模化、标准化、产业化的饲养模式，打造狮头鹅标准化养殖示范区。

(三)、项目计划进度

起止时间	主要工作内容及阶段目标
2021-07-03 至 2024-06-30	<p>1、种鹅高效反季节生产技术示范：确定棚舍环境、光照程序、等参数；确定光源、风机和湿帘等配套设备的安装方案；测定反季节种鹅生产性能、血清炎症因子、内毒素和激素水平；优化棚舍内环境控制、有害因子浓度、光照程序和抗热应激技术参数。</p> <p>2、网上及生物床饲养技术集成与示范：优化网上养殖舍内、外设施、网片结构等参数；优化不同季节和不同生长阶段网上养殖饲养密度、光照、通风、供水、给料等饲养管理关键技术参数；开展生态养殖发酵床设计、发酵菌种组合、垫料选择、制备及配比、营养素添加量与翻堆频率等生物发酵床养殖关键技术。</p> <p>3、高效低排饲料配制技术开发与应用：常用饲料原料营养与饲用价值评定；优化基于酶制剂的稻谷黑小麦为主体能量饲料的肉鹅日粮配合；肉鹅牧草-饲料协同饲喂高效利用技术。</p> <p>4、养殖废弃物资源化处理技术集成与示范：评价狮头鹅粪污特征参数及粪污产生量；优化创新废水处理系统、鹅粪堆肥发酵技术、鹅粪+农作物桔杆混合发酵生产有机肥技术；开展草-鹅生态种养模式。</p>

三、经费使用情况表

经费筹集情况：（单位：万元）						
总投入经费：80万元（自动生成）						
	市科技局 资金	其他资金				合计
		自有资金	贷款	其他政府 部门投入	其它	
已投入经 费	0	0	0	0	0	0
新增经费	80	0	0	0	0	80
其它政府部门、境外资金及其他资金投入情况说明：						
无						
新增经费预算(单位：万元)						
新增经费总额			其中：市科技局经费			
支出经费	经费额	用途说明	经费额	用途说明		
1、基建费	0		——	——		
(一)直接费用	70		70			
1,设备费	10	用于部分 养殖设备 采购	10	用于部分养殖设备采购		
2,材料费	30	用于饲料 营养和环 境应激相 关指标分 析	30	用于饲料营养和环境应激 相关指标分析		
3,测试化验加工外协费	10	用于组织 切片、血 液炎症因 子等指标 分析	10	用于组织切片、血液炎症 因子等指标分析		
4,燃料动力费	3	用于产业 调研、采 样等产生 的燃油费	3	用于产业调研、采样等产生 的燃油费		

5,差旅费/会议费/国际合作与交流费	4	用于参加水禽产业相关会议	4	用于参加水禽产业相关会议
6,出版/文献/信息传播/知识产权事务费	4	用于文章发表的版面费支出	4	用于文章发表的版面费支出
7,劳务费	9	用于参加本项目劳务人员和研究生的劳务支出	9	用于参加本项目劳务人员和研究生的劳务支出
8,人员费	0		0	
9,专家咨询费	0		0	
10,直接费其他支出	0		0	
11,科技金融服务体系其他费用	0		0	
(1)信用评级补贴	0		0	
(2)大赛场租	0		0	
(3)特派员奖励与补贴	0		0	
(二) 间接费用	10		10	
(1)间接成本	0		0	
(2)管理成本	4	用于科研单位经费管理费支出	4	用于科研单位经费管理费支出
(3)绩效支出	6	用于主要参加人员的绩效支出	6	用于主要参加人员的绩效支出
合计	80		80	

四、项目承担单位与参与单位基本情况表

(一) 项目承担单位基本情况表

单位名称		汕头市正治禽业有限公司		组织机构代码		698191856	
联系人	林渤	联系电话	13923913239	电子邮箱	408034085@qq.com		
单位性质	有限责任公司	所在地区	汕头市-澄海区	所属行业	A 农、林、牧、渔业		
单位法人	徐伟汉	证件类型	身份证	证件号码	440521196708303815		
单位级别		市级					
职工总人数(人)		47		研究人员数(人)		8	
单位R&D入情况(万元)				54.31			
其它情况说明		无					

(二) 参与单位基本情况表

单位名称	汕头市白沙禽畜良种繁育中心		组织机构代码	124405004559513047	
联系人	陈俊鹏	联系电话	13480222791	电子邮箱	chenjunpeng02@163.com
单位性质	科研机构	所在地区	汕头市-澄海区	所属行业	A 农、林、牧、渔业
单位法人	林祯平	证件类型	身份证	证件号码	440106197007071838
单位级别	市级				
职工总人数(人)	91		研究人员数(人)	56	
单位R&D入情况(万元)			3078		
其它情况说明			无		

(二) 参与单位基本情况表

单位名称	华南农业大学		组织机构代码	124400004554165634	
联系人	朱勇文	联系电话	18818912892	电子邮箱	408034085@qq.com
单位性质	高等院校	所在地区	广州市-天河区	所属行业	A 农、林、牧、渔业
单位法人	刘耀红	证件类型	身份证	证件号码	431122196605243414
单位级别	省级				
职工总人数(人)	2000		研究人员数(人)	1000	
单位R&D入情况(万元)			311733		
其它情况说明			无		

五、项目组人员情况

项目负责人							
序号	姓名	职称	职务	最高学位	现从事专业	所在单位	身份证件号码
1	徐伟汉	未取得	总经理	其他	狮头鹅良种培育	汕头市正治禽业有限公司	440521196708303815
在本项目中承担的工作任务							
负责统筹协调整个项目的实施、考核和总结。							徐伟汉
主要参与人员							
序号	姓名	职称	职务	最高学位	现从事专业	所在单位	身份证件号码
1	朱勇文	副教授	无	博士	家禽营养与饲料科学	华南农业大学	431122198607243414
在本项目中承担的工作任务							负责狮头鹅常用饲料原料营养与饲用价值评定和营养素需要量数据库的构建,并开展产学研合作,将已有技术成果转化落地。
2	林祯平	高级兽医师	所长	学士	家禽繁育与疫病防控	汕头市白沙禽畜原种研究所	440106197007071838
在本项目中承担的工作任务							饲养试验、数据采集、示范推广
3	林渤	未取得	总经理	其他	种鹅管理和生产	汕头市正治禽业有限公司	440583198406293816
在本项目中承担的工作任务							负责种鹅的合理和生产
4	陈俊鹏	高级畜牧师	股长	硕士	家禽繁育与饲养	汕头市白沙禽畜原种研究所	445121198312203617
在本项目中承担的工作任务							饲养试验、数据采集、示范推广
5	杨琳	教授	无	博士	家禽营养与饲料配制	华南农业大学	230106196309292114
在本项目中承担的工作任务							负责项目指导和专家咨询
6	汪珩	未取得	无	硕士	家禽营养与代谢	华南农业大学	42900619940111091X
在本项目中承担的工作任务							负责饲养试验跟踪与样品采集。

7	马渭青	未取得	无	硕士	水禽环境与管理	华南农业大学	370785199410236515	负责样品分析和数据统计。	马渭青
8	林树欣	助理兽医师	无	学士	水禽饲养与管理	汕头市白沙禽畜原种研究所	44050919900414363X	饲养试验、数据采集。	林树欣
9	潘育璇	助理兽医师	无	学士	水禽饲养与管理	汕头市白沙禽畜原种研究所	440583199104153521	饲养试验、数据采集。	潘育璇

六、本合同签约各方

管理单位（甲方）：汕头市科学技术局

(盖章)

单位地址：汕头市海滨路12号汕头科技馆九、十楼

法定代表人（或授权代表）：



(签章)

联系人（经办人）姓名：



(签章)

E-mail: stkjzypzk@163.com

电话：

2021年11月08日

承担单位（乙方）：汕头市正治禽业有限公司

单位地址：汕头市澄海区盐鸿镇港头村

法定代表人（或法人代理）：

徐伟汉

联系人（项目主管）姓名：

徐伟汉

E-mail: 408034085@qq.com

电话：0754-85779601

开户单位名称：汕头市正治禽业有限公司

开户银行：农村商业银行

开户帐号：80020000002906412



2021年11月18日

乙方主管部门（丙方）：澄海区科技局

单位地址：汕头市澄海区国道324线岭亭路段外经大楼7楼

法定代表人（或法人代理）：

温爽豪

联系人（项目主管）姓名：

林伟如

温书伟

E-mail: 565137568@qq.com

电话：

开户单位名称：汕头市澄海区工业和信息化局

开户银行：中国建设银行

开户帐号：44001650101050423760



2021年11月23日

国家民委中兽药重点开放实验室&民族 中兽药分离纯化技术国家地方联合工程 研究中心项目任务书

编号： 国家民委中兽药重点开放实验室[2022]09 号

项 目 名 称：中草药提取物 5-羟甲基糠醛在肉鸭

饲料中替抗效果的研究

依 托 单 位：华南农业大学

项目负责人：朱勇文

联 系 电 话：18818912892

合 作 单 位：铜仁职业技术学院

起 止 年 限：2023. 1. -2024. 12

填 报 日 期：2022. 9. 2

国家民委中兽药重点开放实验室&
民族中兽药分离纯化技术国家地方联合工程研究中心制

二零二二年七月

一、项目简表

项目主持人信息	姓 名	朱勇文	性 别	男	民 族	汉	出生年月	1986.07
	学 位	农 学 博 士	职 称	副教授		主要研究领域	动物营养与饲料科学	
	手 机	18818912892		电子邮件		zhuyw0724@scau.edu.cn		
	工作单位	华南农业大学						
项目主持单位信息	名 称	华南农业大学		组织机构代码		124400004554165634		
	联 系 人	朱勇文		电子邮件		zhuyw0724@scau.edu.cn		
	电 话	18818912892		网站地址		https://www.scau.edu.cn/main.htm		
	手 机	18818912892		主管部门		广东省教育部		
合作单位信息	名 称 (1)	铜仁职业技术学院		组织机构代码		12522200736649646Q		
	联 系 人	张华琦		联系电话		15185810919		
	名 称 (2)			组织机构代码				
	联 系 人			联系电话				
项目基本信息	项目名称	中草药提取物 5-羟甲基糠醛在肉鸭饲料中替抗效果的研究						
	项目类别	应用基础研究		所属学科		动物营养与饲料科学		
	执行年限	2 年		研究属性		2		
	资助经费 (万元)	5						
项目摘要	<p>本项目在家禽营养代谢与产品调控及活性物质开发与利用技术等前期研究基础上，结合目前饲料替抗与肠道健康的产业需求问题，选择南方消费市场潜力大的肉鸭为研究对象，聚焦 5-羟甲基糠醛功能稳定性、抗氧化保护及肠道保健关键环节，在体外评价 5-羟甲基糠醛（地黄等中药提取物主要成分）的抗氧化和抑菌效果的基础上，体内探究非玉米型多元化日粮及肠道疾病挑战条件下添加 5-羟甲基糠醛对肉鸭生产性能、抗氧化性能和肠道健康的影响，构建 5-羟甲基糠醛在肉鸭生产中科学利用与替抗饲料配制技术，有利于无抗养殖条件下肉鸭饲料产业的提质增效。项目拟实现绩效目标：（1）获得 5-羟甲基糠醛在肉鸭非玉米型多元化日粮中替抗应用技术方案 1 套；（2）发表 SCI 论文 1 篇以上，项目技术标准 1 项，科技报告 1 篇。</p>							
关键词(用分号分开，最多 5 个)： 中草药提取物；5-羟甲基糠醛；肉鸭；替抗								

二、项目组成员

项目 负责 人	姓名	朱勇文			性别	男		民族	汉					
	身份证号	431122198607243414			年龄	36		项目分工	负责人					
	职称	副教授			从事专业	动物营养与饲料科学								
	最高学历	博士研究生			职务	无		传真	020-38820121					
	手机	18818912892			联系电话	18818912892								
	E-mail	zhuyw0724@scau.edu.cn												
项目 组 人 数	7人		高级	5人		中级	0人		初级	0人		其他	2人	
项目组主要研究人员														
姓名	性别	年龄	职称	学历	研究领域	任务分工	所在单位	签名						
张华琦	男	48	教授	博士研究生	反刍动物营养	中草药饲料添加剂开发	铜仁职业技术学院							
张庆	男	36	副教授	博士研究生	植物源活性物质提取	体外效果评价	华南农业大学							
叶慧	女	42	高级实验师	硕士研究生	动物营养与饲料科学	项目指标分析	华南农业大学							
杨琳	男	59	教授	博士研究生	动物营养与饲料科学	项目咨询与指导	华南农业大学							
陈杨	男	21	无	硕士生	动物营养与饲料科学	开展肉鸭饲养试验	华南农业大学							
辛硕	男	22	无	硕士生	动物营养与饲料科学	样品收集与分析	华南农业大学							

三、经费预算表

预算科目名称	经费（万元）	专项经费	自筹经费	备注(计算依据与说明)
一、研究经费				
1.科研业务费	1	1		
(1) 测试/计算/分析费	0.5	0.5		用于肉鸭肠道健康相关指标检测
(2) 能源/动力费				
(3) 会议费/差旅费	0.5	0.5		用于与合作单位交流相关差旅支出
(4)出版/文献/信息传播				
(5) 其他				
2.实验材料费	2.7	2.7		
(1)原材料/试剂/药品购置费	2.7	2.7		样品采集和指标分析相关试剂耗材购买
(2)其它				
3.仪器设备费	0	0		
(1)购置				
(2)试制				
4.实验室改装费				
5.协作费				
6.国内学术交流				
二、劳务费	1	1		用于完成项目研究生的劳务支出
三、管理费	0.3	0.3		
合计	5	5		
经费预算其他需要说明的情况：				


四、研究计划

1、课题目标

本项目在家禽营养代谢与产品调控及活性物质开发与利用技术等前期研究基础上，结合目前饲料替抗与肠道健康的产业需求问题，选择南方消费市场潜力大的肉鸭为研究对象，聚焦 5-羟甲基糠醛功能稳定性、抗氧化保护及肠道保健关键环节，在体外评价 5-羟甲基糠醛(地黄等中药提取物主要成分)的抗氧化和抑菌效果的基础上，体内探究非玉米型多元化日粮及肠道疾病挑战条件下添加 5-羟甲基糠醛对肉鸭生产性能、抗氧化性能和肠道健康的影响，构建 5-羟甲基糠醛在肉鸭饲料中高效利用与配制技术，有利于无抗养殖条件下肉鸭饲料产业的提质增效。



八、依托单位意见

领导签字:  单位(公章)
2022 年 9 月 6 日

九、合作单位意见

领导签字: 单位(公章)
年 月 日

十、重点实验室意见

领导签字: 单位(公章)
年 月 日

十一、支撑附件材料

(职称、学历学位及研究成果证明材料、合作协议等)

合同编号:

技术开发（合作）合同

项目名称: 番鸭全消化道体外仿生消化法的建立与应用

项目类型: 华农温氏科创中心成果转化项目

甲 方: 温氏食品集团股份有限公司

乙 方: 华南农业大学

丙 方: 温氏食品集团股份有限公司研究院

签订时间: 2023 年 12 月 22 日

签订地点: 广东省云浮市新兴县

有效期限: 2023 年 12 月 1 日至 2025 年 11 月 30 日

技术开发（合作）合同

甲方： 温氏食品集团股份有限公司

住 所 地： 广东省云浮市新兴县新城镇东堤北路 9 号

法定代表人： 温志芬

项目联系人： 王凯

联系方式： 18819492878

通讯地址： 广东省云浮市新兴县

电 话： 0766-2986008 传 真： 0766-2986008

电子信箱： 373490238@qq.com

乙方： 华南农业大学

住 所 地： 广州市天河区五山路 483 号

法定代表人： 薛红卫

项目联系人： 朱勇文

联系方式： 18818912892

通讯地址： 广州市天河区五山华南农业大学动物科学学院

电 话： 18818912892 传 真： 020-38882017

电子信箱： zhuyw0724@scau.edu.cn

丙方： 温氏食品集团股份有限公司研究院

住 所 地： 广东省云浮市新兴县新城镇惠能北路温氏研究院

法定代表人： 陈丽

项目联系人： 魏师

联系方式： 13480362586

通讯地址： 广东省云浮市新兴县新城镇惠能北路温氏研究院

电 话： 0766-2986008 传 真： 0766-2986003

电子信箱： wskygl@163.com

本合同合作各方就共同参与研究开发 番鸭全消化道体外仿生消化法的建立与应用 项目事项，经过平等协商，在真实、充分地表达各自意愿的基础上，根据《中华人民共和国民法典》的规定，达成如下协议，并由合作各方共同恪守。

第一条 本合同合作研究开发项目的要求如下：

1. 技术目标：(1) 预期研究成果及达到的水平：获得不同饲料类型的番鸭仿生消化法消化酶谱、离子谱和 pH 的精准参数，精准度达到国内领先水平；建立与番鸭相配套的胃-小肠-盲肠三段式全消化道体外仿生消化方法，填补该领域空白点，达到国内领先水平；形成番鸭专属的饲料原料酶解能数据库 1 套。(2) 技术考核指标：获得不同饲料类型的番鸭仿生消化法消化酶谱、离子谱和 pH 的精准参数 1 套；建立与番鸭相配套的胃-小肠-盲肠三段式全消化道体外仿生消化方法 1 套；完成 18 种常用饲料原料体外仿生酶解能测定，建立新数据库 1 套，其中相比旧数据库，新数据库的配方能值梯度与料重比变化的相关系数提高 0.05 以上。(3) 经济考核指标：建立与番鸭相配套的胃-小肠-盲肠三段式全消化道体外仿生消化方法，结合传统生物学法，精准评价常用饲料原料有效能值，为节能增效营养配方设计提供技术支持，配方成本降低 10 元/吨。

2. 技术内容：针对番鸭饲料有效能评定欠精准的瓶颈问题，在获得番鸭仿生消化法消化酶谱、离子谱和 pH 精准参数的基础上验证和推广胃-小肠-盲肠三段式全消化道体外仿生消化方法的精准性。

3. 技术方法和路线：在建立适用于不同饲料类型的番鸭仿生消化法消化酶谱、离子谱和 pH 的精准参数的基础上，模拟后肠段微生物的消化作用，构建与番鸭相配套的胃-小肠-盲肠三段式全消化道体外仿生消化方法。接着，结合传统生物学法，在确定其精准性和专属性基础上，精准评价常用饲料原料有效能值，验证和推广番鸭专属饲料原料有效能数据库，提升其饲料有效能评定的快速化和精准化，实现番鸭能量精准供给和饲料

降本增效。

第二条 本合同合作各方在研究开发项目中，分工承担如下工作：

甲方：

1. 研究开发内容： 无。
2. 工作进度： 同乙方。
3. 研究开发期限： 同乙方。
4. 研究开发地点： 同乙方。

乙方：

1. 研究开发内容： 建立适用于不同饲料类型的番鸭仿生消化法消化酶谱、离子谱和 pH 的精准参数；模拟后肠段微生物的消化作用，验证和推广番鸭相配套的胃-小肠-盲肠三段式全消化道体外仿生消化方法，形成番鸭专属的饲料原料酶解能数据库 1 套，提升番鸭饲料营养价值评定技术的快速化、精准化和节能化。

2. 工作进度： 2023.12-2024.12：验证与番鸭相配套的胃-小肠-盲肠三段式全消化道体外仿生消化方法，确定其精准性和专属性。
2025.1-2025.11：与温氏合作进行番鸭相配套的胃-小肠-盲肠三段式全消化道体外仿生消化方法推广与应用；准备项目验收。

3. 研究开发期限： 2023 年 12 月 1 日-2025 年 11 月 30 日。
4. 研究开发地点： 华南农业大学。

丙方：

1. 研究开发内容： 提供经费支撑，协助构建和验证与番鸭相配套的胃-小肠-盲肠三段式全消化道体外仿生消化方法。

2. 工作进度： 同乙方。
3. 研究开发期限： 同乙方。
4. 研究开发地点： 同乙方。

第三条 为确保本合同的全面履行，合作各方确定，采取以下方式对

研究开发工作进行组织管理和协调：由乙方和丙方各派出一位负责人进行相关研究工作的协调和组织管理；甲方、丙方负责协调研发成果落地；乙方负责技术研究，形成可落地实施研成果。

第四条 合作各方确定，各自为本合同项目的研究开发工作提供以下技术资料和条件：

甲方：负责协调研发成果落地。

乙方：负责技术研究，形成可落地实施研成果。

丙方：负责协调研发成果落地。

本合同履行完毕后，上述技术资料和条件按以下方式处理：交付甲方和丙方使用。

第五条 合作各方确定，按如下方式提供或支付本合同项目的研究开发经费及其他投资：

甲方：

1. 提供或支付方式：无。

2. 支付或折算为技术投资的金额：无。

3. 使用方式：无。

乙方：

1. 提供或支付方式：无。

2. 支付或折算为技术投资的金额：无。

3. 使用方式：项目经费预算支出 40 万元，其中材料费 10 万元，测试化验加工费 5 万元，差旅费/会议费 5 万元，出版/文献/信息传播/知识产权事务费 5 万元，劳务费 5 万元，管理费 8 万元，绩效 2 万元。

丙方：

1. 提供或支付方式：提供项目总经费 50 万元，拨付华南农业大学的经费 40 万元，拨付温氏温氏食品集团股份有限公司研究院饲料营养中心科研基地非雷试验场的经费 10 万元。华南农业大学研究经费分两次拨

付, 每次拨付 20 万元, 第一笔经费在合同签定后一个月内拨付;第二笔经费, 获得不同饲料类型的番鸭仿生消化法消化酶谱、离子谱和 pH 的精准参数 1 套, 并完成建立与番鸭相配套的胃-小肠-盲肠三段式全消化道体外仿生消化方法 1 套, 结合传统生物学法, 精准评价 8 种常用饲料原料体外仿生酶解能测定的目标后, 经会议认定阶段性考核目标后, 拨付第二笔经费_____。

2. 支付或折算为技术投资的金额: 无_____。

3. 使用方式: 项目经费预算支出 10 万元, 其中材料费 5 万元, 测试化验加工费 5 万元。

第六条 以提供技术为投资的合作方应保证其所提供技术不侵犯任何第三人的合法权益。如发生第三人指控合作一方或多方因实施该项技术而侵权的, 提供技术方应当承担相应经济责任, 但乙方承担的金额不超过其已收取的金额。

第七条 本合同的变更必须由合作各方协商一致, 并以书面形式确定。但有下列情形之一的, 合作一方或多方可以向其他合作方提出变更合同权利与义务的请求, 其他合作方应当在7日内予以答复; 逾期未予答复的, 视为同意。

第八条 未经其他合作方同意, 合作一方或多方不得将本合同项目部分或全部研究开发工作转让给第三人承担。但有下列情况之一的, 合作一方或多方可以不经其他合作方同意, 将本合同项目部分或全部研究开发工作转让给第三人承担:

1. 甲方、丙方书面同意乙方转让的研发工作。

第九条 在本合同履行中, 因出现在现有技术水平和条件下难以克服的技术困难, 导致研究开发失败或部分失败, 并造成合作一方或多方损失的, 合作各方约定按以下方式承担风险损失:

1. 已支付的乙方不再返还, 还未支付的甲、丙方不再支付;

合作各方确定，本合同项目的技术风险按三方协商方式认定。认定技术风险的基本内容应当包括技术风险的存在、范围、程度及损失大小等。认定技术风险的基本条件是：

1. 本合同项目在现有技术水平条件下具有足够的难度；
2. 乙方在主观上无过错且经认定研究开发失败为合理的失败。

一方发现技术风险存在并有可能致使研究开发失败或部分失败的情形时，应当在10日内通知其他合作方并采取适当措施减少损失。逾期未通知并未采取适当措施而致使损失扩大的，应当就扩大的损失承担赔偿责任。

第十条 在本合同履行过程中，因作为研究开发标的技术已经由他人公开（包括以专利权方式公开），合作一方或多方应在7日内通知其他合作方解除合同。逾期未通知并致使其他合作方产生损失的，其他合作方有权要求予以赔偿。

第十一条 合作各方确定按以下方式交付研究开发成果：

甲方：

1. 研究开发成果交付的形式及数量：无。
2. 研究开发成果交付的时间及地点：无。

乙方：

1. 研究开发成果交付的形式及数量：建立与番鸭相配套的胃-小肠-盲肠三段式全消化道体外仿生消化方法1套；完成18种常用饲料原料体外仿生酶解能测定，建立新数据库1套，其中相比旧数据库，新数据库的配方能值梯度与料重比变化的相关系数提高0.05以上。

2. 研究开发成果交付的时间及地点：2025年11月前温氏食品集团股份有限公司研究院。

丙方：

1. 研究开发成果交付的形式及数量：无。

2. 研究开发成果交付的时间及地点： 无。

第十二条 合作各方确定，按以下标准及方法对合作一方完成的研究开发工作成果进行验收：

甲方： 组织会议验收。

乙方： 获得不同饲料类型的番鸭仿生消化法消化酶谱、离子谱和 pH 的精准参数 1 套；建立与番鸭相配套的胃-小肠-盲肠三段式全消化道体外仿生消化方法 1 套；完成 18 种常用饲料原料体外仿生酶解能测定，建立新数据库 1 套，其中相比旧数据库，新数据库的配方能值梯度与料重比变化的相关系数提高 0.05 以上。

丙方： 协助甲方组织会议验收。

第十三条 履行本合同前各方独有的成果及其相关知识产权权利，归各方所有；履行本合同新产生的、与原成果有本质区别的成果及其相关知识产权权利归甲乙丙三方共同所有；经验证有效且符合甲方需求的，甲方有优先转让使用权，成果转让费用另行协商，转让费用应考虑甲方本次的项目投入；或按温氏食品集团股份有限公司相关文件（温氏〔2022〕80 号，温氏股份科技成果转化评价及激励办法），依据成果实际创造的利润进行奖励。

第十四条 合作各方分别独立完成并与履行本合同有关的阶段性技术成果的研究开发人员，享有在有关此阶段性技术成果文件上写明技术成果完成者的权利和取得有关荣誉证书、奖励的权利。

合作各方应以协商方式确定最终研究成果的完成人员名单。此完成人员享有在有关最终技术成果文件上写明技术成果完成者的权利和取得有关荣誉证书、奖励的权利。

第十五条 合作各方确定，任何一方有权利利用本合同项目研究开发所完成的技术成果，进行后续改进。由此产生的具有实质性或创造性技术进步特征的新的技术成果，归 完成方（完成方、合作各方）方所有。

具体相关利益的分配办法如下：各方协商确定。

第十六条 为有效履行本合同，合作各方确定，在本合同有效期内，甲方指定王凯为甲方项目联系人，乙方指定朱勇文为乙方项目联系人，丙方指定魏师为丙方项目联系人。项目联系人承担以下责任：

一方变更项目联系人的，应当及时以书面形式通知其他各方。未及时通知并影响本合同履行或造成损失的，应承担相应的责任。

第十七条 合作各方确定，出现下列情形，致使本合同的履行成为不必要或不可能的，可以解除本合同：

1. 因发生不可抗力和技术风险；

第十八条 合作各方因履行本合同而发生的争议，应协商、调解解决。协商、调解不成的，确定按以下第 1 种方式处理：

1. 提交云浮仲裁委员会仲裁；
2. 依法向人民法院起诉。

第十九条 本合同一式四份，具有同等法律效力。

第二十条 本合同经合作各方签字盖章后生效。

以下无正文，转签章页

甲方：温氏食品集团股份有限公司 (盖章)

法定代表人/委托代理人：陈 强 (签名)

2023年12月22日

乙方：华南农业大学 (技术合同专用章)

法定代表人/委托代理人：薛红 (签名)

2023年12月22日

项目负责人：朱毅 (签字)

丙方：温氏食品集团股份有限公司研究院 (盖章)

法定代表人/委托代理人： (签名)

2023年12月22日

项目负责人：魏师 (签字)

2023年12月22日

合同编号：

技术服务合同

项目名称：不同营养水平饲粮添加乳化剂和酶制剂对快速型黄羽肉鸡生产性能和养分利用率的影响

委 托 方：建明（中国）科技有限公司
(甲 方) _____

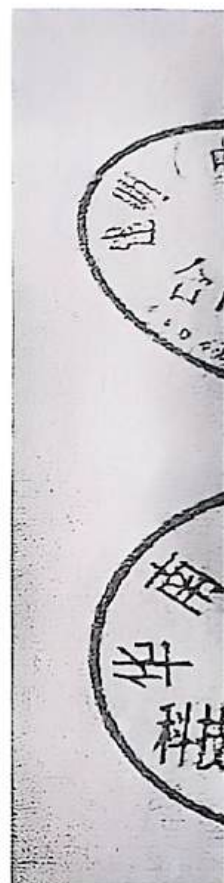
受 托 方：华南农业大学
(乙 方) _____

签订时间：2020.7

签订地点：广州市天河区

有效期限：2021.7

中华人民共和国科学技术部印制



技术服务合同

委托方（甲方）：建明（中国）科技有限公司

住 所 地：广东省珠海市金湾区三灶镇琴石路 25 号

法定代表人：Christopher E Nelson

项目联系人：王立志

联系方式：13600386100

通讯地址：广东省珠海市金湾区三灶镇琴石路 25 号

电 话：0756-3989000 传真：0756-3989523

电子信箱：lizhi.wang@kemin.com

受托方（乙方）：华南农业大学

住 所 地：广州市天河区五山路 483 号

法定代表人：刘雅红

项目联系人：朱勇文

联系方式：18818912892

通讯地址：广州市天河区五山华南农业大学动物科学学院

电 话：18818912892 传真：020-38882017

电子信箱：zhuyw0724@scau.edu.cn

本合同甲方委托乙方就不同营养水平饲粮添加乳化剂和酶制剂对快速型黄羽肉鸡生产性能和养分利用率的影响技术服务，并支付相应的技术服务报酬。双方经过平等协商，在真实、充分地表达各自意愿的基础上，根据《中华人民共和国合同法》的规定，达成如下协议，并由双方共同恪守。

第一条：甲方委托乙方进行技术服务的内容如下：

1. 技术服务的目标：明确不同营养水平饲粮添加乳化剂和酶制剂对快速型黄羽肉鸡生产性能和养分利用率的积极影响。

2. 技术服务的内容：开展 1-56 日龄快速型黄羽肉鸡饲养试验和消化代谢试验，结合评价不同营养水平饲粮添加乳化剂和酶制剂（蛋白酶和葡萄糖氧化酶）对快速型黄羽肉鸡生产性能和养分利用率的影响。

3. 技术服务的方式：按照合同约定的试验方案的要求和步骤进行，完成饲养试验和样品分析，出具标准的试验研究报告作为验收标准。

第二条：乙方应按下列要求完成技术服务工作：

1. 技术服务地点：广东省广州市

2. 技术服务期限：甲方项目实施期间

3. 技术服务进度：2020.8 -2020.9 完成原料的采购和饲料配制等工作；2020.10 完成试验场地消毒、快速型黄羽肉鸡购买与分组饲养

等工作；2020.11-2020.12 开展 1-56 日龄快速型黄羽肉鸡饲养试验和消化代谢试验，获得快速型黄羽肉鸡生长性能和营养物质消化率数据 1 套；2020.5-2022.12 完成样品氨基酸、肠道微生物等指标分析，出具试验研究报告一份。

4. 技术服务质量要求：乙方按照试验方案规定的时间完成试验项目，包括动物试验、实验室分析、统计分析，并出具一个最终试验报告。

5. 技术服务质量期限要求：不拖延项目进度为期限

第三条：为保证乙方有效进行技术服务工作，甲方应当向乙方提供下列工作条件和协作事项：

1. 提供技术资料：

(1) 不同营养水平饲粮添加乳化剂和酶制剂对快速型黄羽肉鸡生产性能和养分利用率的影响高低档快速型黄羽肉鸡饲料配方 1 套。

(2) 不同营养水平饲粮添加乳化剂和酶制剂对快速型黄羽肉鸡生产性能和养分利用率的影响试验方案中乳化剂和酶制剂添加剂产品各 1 个。

(3) 不同营养水平饲粮添加乳化剂和酶制剂对快速型黄羽肉鸡生产性能和养分利用率的影响试验方案中乳化剂和酶制剂添加剂使用推荐量。

2. 提供工作条件：

(1) 共同讨论和分析不同营养水平饲粮添加乳化剂和酶制剂对快速型黄羽肉鸡生产性能和养分利用率的影响技术方案和试验数据。

3. 其他： 无。

4. 甲方提供上述工作条件和协作事项的时间及方式： 由甲方安排，提前 1 个月通知乙方。

第四条： 甲方向乙方支付技术服务报酬及支付方式为：

1. 技术服务费总额为： 拾贰万元整（¥120,000.00）

2. 技术服务费由甲方 分期（一次或分期）支付乙方。

具体支付方式和时间如下：

（1） 付款方式：转账

（2） 第一期：签订合同后 15 天内，付款 6 万；第二期：试验结束乙方提交实验报告验收通过后 15 天内付款 6 万。

乙方开户银行名称、地址和帐号为：

开户银行： 中国工商银行广州五山支行

地址： 广州市天河区五山路 483 号

帐号： 3602002609000310520-000000001

第五条： 双方确定因履行本合同应遵守的保密义务如下：

甲方：

1. 保密内容（包括技术信息和经营信息）： /。

2. 涉密人员范围： /；

3. 保密期限： /。

4. 泄密责任： /。

乙方：

1. 保密内容（包括技术信息和经营信息）： 乙方不得向其他任何

机构和个人泄露和发表试验得出的任何关于建明（中国）科技有限公司的产品或解决方案在生产和机理层面负效果的数据和言论。

2. 涉密人员范围：参与项目组所有人员。

3. 保密期限：永久。

4. 泄密责任：支付违约金并追究法律责任。

第六条：本合同的变更必须由双方协商一致，并以书面形式确定。但有下列情形之一的，一方可以向另一方提出变更合同权利与义务的请求，另一方应当在7日内予以答复；逾期未予答复的，视为同意：

1. 有一方违反合同条款，使合同不能履行的

2. 服务过程中因技术原因需要对合同进行调整的

第七条：双方确定以下列标准和方式对乙方的技术服务工作成果进行验收：

1. 乙方完成技术服务工作的形式：按照试验方案规定的时间完成试验项目，包括动物试验、实验室分析、统计分析，并出具一个最终试验报告。

2. 技术服务工作成果的验收标准：本合同约束的各项指标。

3. 技术服务工作成果的验收方法：由甲方组织相关专业技术人员验收，写出验收报告。

4. 验收的时间和地点：验收的时间和地点由甲方安排，提前 1 个月通知乙方。

第八条：双方确定：

1. 在本合同有效期内，甲方利用乙方提交的技术服务工作成果

所完成的新的技术成果，归甲（甲、双）方所有。

2. 在本合同有效期内，乙方利用甲方提供的技术资料和工作条件所完成的新的技术成果，归乙（乙、双）方所有。

第九条：双方确定，按以下约定承担各自的违约责任：

1. 甲方违反本合同第3-8条约定，应当支付合同总金额的 50%作为违约金（支付违约金或损失赔偿额的计算方法）。

2. 乙方违反本合同第二和第五至八条约定，应当支付合同总金额的 50%作为违约金（支付违约金或损失赔偿额的计算方法）。

第十条：双方确定，在本合同有效期内，甲方指定王立志为甲方项目联系人，乙方指定朱勇文为乙方项目联系人。项目联系人承担以下责任：

1. 开展 1-56 日龄快速型黄羽肉鸡饲养试验和消化代谢试验，结合评价不同营养水平饲粮添加乳化剂和酶制剂（蛋白酶和葡萄糖氧化酶）对快速型黄羽肉鸡生产性能和养分利用率的影响。

2. 负责项目中期检查和结题验收。

3. 负责项目经费计划的制定、监督项目经费的使用。

一方变更项目联系人的，应当及时以书面形式通知另一方，未及时通知并影响本合同履行或造成损失的，应承担相应的责任。

第十一条：双方确定，出现下列情形，致使本合同的履行成为不必要或不可能的，可以解除本合同：

1. 发生不可抗力；

2. 双方协商解除合同的。

3. 乙方违反合同的，经协商无效的

第十二条：双方因履行本合同而发生的争议，应协商、调解解决。

协商、调解不成的，确定按以下第1种方式处理：

1. 提交广州市仲裁委员会仲裁；

2. 依法向人民法院起诉。

第十三条：双方确定：本合同及相关附件中所涉及的有关名词和技术术语，其定义和解释如下：

1. /

2. /

3. /

4. /

5. /

第十四条：与履行本合同有关的下列技术文件，经双方确认后，为本合同的组成部分：

1. 技术背景资料：/；

2. 可行性论证报告：/；

3. 技术评价报告：/；

4. 技术标准和规范：/；

5. 原始设计和工艺文件：/；

6. 其他：/；

第十五条：双方约定本合同其他相关事项为：如果该研究成

甲方：_____ (盖章)

法定代表人 / 委托代理人：_____ (签名)



乙方：_____ (盖章)

法定代表人 / 委托代理人：_____ (签名)



2020年12月25日

印花税票粘贴处：



合同编号：

技术开发（合作）合同家禽

项目名称：中药渣在家禽饲料中的应用

甲 方：广东昊天环保科技有限公司

乙 方：华南农业大学

签订时间：2023 年 9 月 20 日

签订地点：广东省广州市增城区

有效期限：2023 年 9 月 20 日-2024 年 9 月 19 日

中华人民共和国科学技术部印制

技术开发（合作）合同

甲 方: 广东昊天环保科技有限公司

住 所 地：广州市增城区新城大道 400 号新城创新中心

法定代表人：张誉山

项目联系人: 张维丽

联系方式: 13926409080

通讯地址: 广州市增城区新城大道 400 号新城创新中心 28 号楼 708

自编 02

电 话: _____ 传真: _____

电子信箱: _____

乙 方：华南农业大学

住 所 地: 广州市天河区五山路 483 号

法定代表人： 刘雅红

项目联系人: 朱勇文

联系方式: 18818912892

通讯地址: 广州市天河区五山华南农业大学动物科学学院

电 话: 18818912892 传真: 020-38882017

电子信箱: zhuyw0724@scau.edu.cn

本合同合作双方就共同参与研究开发中药渣在家禽饲料中的应用项目项目事项，经过平等协商，在真实、充分地表达各自意愿的基础上，根据《中华人民共和国民法典》的规定，达成如下协议，供合作双方共同恪守。

第一条 本合同合作技术成果转化应用项目的要求如下：

1. 项目目标：项目方向是中药渣在家禽饲料领域的应用内容，包括(但不限于)中药渣转化/萃取功能饲料/酸化水等无公害家禽养殖方面的实验成果、养殖技术等方面所涉及的一切技术研究、试验和推广领域。

2. 项目内容：(1) 研发中药渣在家禽饲料中的应用和技术输出；(2) 家禽饲料在喂养实验中的成果。

3. 技术方法和路线：以广东昊天环保科技有限公司技术平台为依托，联合实验基于中药渣在家禽饲料中的应用和技术输出；

第二条 本合同合作各方在研究开发项目中，分工承担如下工作：

甲方：

1. 购买实验所需家禽（鸡、鸭等）以及相关物料。
2. 提供所需的活动和检测经费给乙方。
3. 项目总费用 5 万元。

乙方：

1. 实验内容：基于中药渣在家禽饲料中的技术成果转化应用实验成果、养殖技术。

2. 工作进度：2023.9-2024.9：基于中药渣在家禽饲料中的技术成果转化在（鸡、鸭等）实验成果、养殖技术。

3. 研究开发地点：华南农业大学动物科学学院。

第三条 为确保本合同的全面履行，合作双方确定，采取以下方式对研究开发工作进行组织管理和协调：甲方监督乙方的工作进展

和资金使用情况，乙方根据甲方要求制定本项目的研发计划、及时汇报工作进展和资金使用情况。

第四条 合作双方确定，各自为本合同项目的研究开发工作提供以下技术资料和条件：

甲方：购买实验所需家禽（鸡、鸭等）以及相关物料、提供所需的经费给乙方作为研究实验，总项目费用5万元。

乙方：基于中药渣在家禽饲料中的技术成果转化应用实验成果、养殖技术。

本合同履行完毕后，上述技术资料和条件按以下方式处理：甲、乙双方对共同研制的产品均有知情权，依照本合同双方共同研制产品在取得权威机构检测认定且具备生产资格后，经双方同意，联合申请专利，以示对专有知识产权的尊重；乙方研究获得的与本项目相关技术资料甲方有知情权和使用权、但未公开资料甲方需征得乙方同意后才能透露给第三方，在甲方支付完本项目研发经费后，本合同项下的全部研发项目及成果的知识产权完全归甲方持有。

第五条 合作双方确定，按费用标准及支付比例提供或支付本合同项目的研究开发经费及其他投资：

甲方：

1. 提供研发经费：合同签订5日内向乙方支付合同全款作为本合同项目运作资金；本合同项目研发产品成果、技术及关键参数须取得权威机构检测认定且乙方需出具相关检测报告并将本项目产品所有研发资料及检测报告交付甲方。

2. 使用方式：按预算拨付给乙方科研账户，可要求和监督乙方合理使用所拨经费。

乙方：

1. 提供研究有关资料：提供甲方拨付经费所需的科研账户、票

据等相关财务资料。

2. 使用方式：按年度预算和有关财务制度开支使用。

第六条 乙方提供的研发产品成果、技术及关键参数等应保证不侵犯任何第三人的合法权益。如发生第三人主张权利的，乙方应当负责处理。

第七条 本合同的变更必须由合作双方协商一致，并以书面形式确定。但有下列情形之一的，合作一方可以向另一方提出变更、解除或终止合同的请求，另一方应当在15日内予以答复；逾期未予答复的，视为同意：

1. 甲方未按计划拨付项目研发经费；
2. 乙方未计划开展研发工作；
3. 乙方违规使用经费；
4. 涉及国家和地方政府相关政策、有关法规条例、自然灾害及天气气候、疫情和疫病因素使得项目无法正常开展。

第八条 未经甲方同意，乙方不得将本合同项目部分或全部研究开发工作转让给第三人承担。

第九条 在本合同履行中，因出现在现有技术水平和条件下难以克服的技术困难，导致研究开发失败或部分失败，并造成合作双方损失的，合作双方约定按以下方式承担风险损失：

1. 双方共同协商解决，第三方专家风险评估认定；
2. /；
3. /。

合作双方确定，本合同项目的技术风险按甲乙双方共同协商，第三方专家风险评估的方式认定。认定技术风险的基本内容应当包括技术风险的存在、范围、程度及损失大小等。认定技术风险的基本条件是：

1. 本合同项目在现有技术水平条件下具有足够的难度；

2. 乙方在主观上无过错且经认定研究开发失败为合理的失败。

一方发现技术风险存在并有可能致使研究开发失败或部分失败的情形时，应当在30日内通知另一方并采取适当措施减少损失。逾期未通知并未采取适当措施而致使损失扩大的，应当就扩大的损失承担赔偿责任。

第十条 在本合同履行过程中，因作为研究开发标的的技术已经由他人公开（包括以专利权方式公开），合作一方应在30日内通知其他合作方解除合同。逾期未通知并致使其他合作方产生损失的，其他合作方有权要求予以赔偿。

第十一条 合作双方确定因履行本合同应遵守的保密义务如下：

甲方：

1.保密内容（包括技术信息和经营信息）：企业经营经济信息、企业财务信息、企业内部加工技术资料。

2.涉密人员范围：项目组所有开发成员。

3.保密期限：按公司有关保密制度约定。

4.泄密责任：按公司有关保密制度处理。

乙方：

1.保密内容（包括技术信息和经营信息）：实验获得的技术参数。

2.涉密人员范围：项目组所有人员。

3.保密期限：发表文章、出版书籍、学术报告、专利等需获得课题负责人许可，在获得许可公开之日前均属于保密期。

4.泄密责任：按学校有关保密制度处理。

第十二条：合作双方确定按以下方式交付研究开发成果：

乙方：

1.实验成果交付的形式：基于中药渣在家禽饲料中的技术成果

转化应用实验成果、养殖技术。

2. 研究开发成果交付的时间及地点：2024 年 9 月前，交付基于中药渣在家禽饲料中的技术成果转化应用实验成果、养殖技术。

交付地点：广东昊天环保科技有限公司。

第十三条：合作双方确定，按以下标准及方法对乙方完成的研究开发工作成果进行验收：

1、由第三方科技评价与专家组验收，线上或线下双方商定。

第十四条：合作双方确定，因履行本合同所产生完成的全部技术成果及其相关知识产权权利归属归甲方所有。

第十五条：合作双方分别独立完成并与履行本合同有关的阶段性技术成果的研究开发人员，享有在有关此阶段性技术成果文件上写明技术成果完成者的权利和取得有关荣誉证书、奖励的权利。

合作双方应以协商方式确定最终研究成果的完成人员名单。此完成人员享有在有关最终技术成果文件上写明技术成果完成者的权利和取得有关荣誉证书、奖励的权利。

第十六条：合作双方利用共同投资的研究开发经费所购置与研究开发工作有关的设备、器材、资料等财产，归购买方所有。

第十七条：合作双方确定：任何一方违反本合同约定义务，造成另一方研究开发工作停滞、延误或失败的，应当按以下约定承担违约责任：

1、违约方应向守约方承担违约责任，同时承担守约方因主张权利产生的律师费、诉讼费、差旅费等一切损失。

2、甲方违反本合同第四条约定，应当向乙方支付合同总金额的 50%作为违约金（支付违约金或损失赔偿额的计算方法）。

3、乙方违反本合同第四条约定，应当向甲方支付合同总金额的 50%作为违约金（支付违约金或损失赔偿额的计算方法）。

4、乙方违反本合同第第十二条约定，应当向甲方支付合同总金额的 50%作为违约金（支付违约金或损失赔偿额的计算方法）。

第十八条：合作双方确定，任何一方有权利用本合同项目研究开发所完成的技术成果，进行后续改进。由此产生的具有实质性或创造性技术进步特征的新的技术成果，归完成方（完成方、合作各方）方所有。具体相关利益的分配办法如下：无。

第十九条：为有效履行本合同，合作各方确定，在本合同有效期内，甲方指定张维丽为甲方项目联系人，乙方指定朱勇文为乙方项目联系人。项目联系人承担以下责任：

1. 负责项目涉及产品的联合研发、实验。

2. 负责项目经费计划的制定、购买研究所需物料、监督项目经费的使用。

一方变更项目联系人的，应当及时并以书面形式通知另一方。未及时通知并影响本合同履行或造成损失的，应承担相应的责任。

第二十条：合作双方确定，出现下列情形，致使本合同的履行成为不必要或不可能的，可以解除本合同：

1. 因发生不可抗力和技术风险；

2. 双方协商解除合同的。

3. _____ / _____

第二十一条：合作双方因履行本合同而发生的争议，应协商、调解解决。协商、调解不成的，确定按以下第 2 种方式处理：

1. 提交 广州 仲裁委员会仲裁；
2. 依法向原告所在地人民法院起诉。

第二十二条：与履行本合同有关的下列技术文件，经合作双方确认后， 项目实施方案与技术标准和规范 为本合同的组成部分：

1. 技术背景资料： / ；
2. 可行性论证报告： / ；
3. 技术评价报告： / ；
4. 技术标准和规范： 基于中药渣在家禽饲料中的技术成果转化应用实验成果、养殖技术 。
5. 原始设计和工艺文件： / ；
6. 其他： / 。

第二十三条：本合同一式 4 份，经甲乙双方盖章签名后立即生效，双方各持 2 份，具有同等法律效力。

（以下无正文）

甲方：广东昊天环保科技有限公司（盖章）

法定代表人 / 委托代理人：

2023 年 10 月 5 日



乙方：华南农业大学（盖章）

法定代表人 / 委托代理人

2023 年 10 月 5 日



合同编号:

技术服务合同

项目名称: 不同营养水平饲料添加乳化剂和酶制剂对肉鸭生产性能和养分利用率的影响

委托方: 建明(中国)科技有限公司

(甲方)

受托方: 华南农业大学

(乙方)

签订时间: 2020.2

签订地点: 广州市天河区

有效期限: 2021.2

中华人民共和国科学技术部印制

技术服务合同

委托方（甲方）：建明（中国）科技有限公司

住 所 地：广东省珠海市金湾区三灶镇琴石路 25 号

法定代表人：Christopher E Nelson

项目联系人：王立志

联系方式：13600386100

通讯地址：广东省珠海市金湾区三灶镇琴石路 25 号

电 话：0756-3989000 传真：0756-3989523

电子信箱：lizhi.wang@kemin.com

受托方（乙方）：华南农业大学

住 所 地：广州市天河区五山路 483 号

法定代表人：刘雅红

项目联系人：朱勇文

联系方式：18818912892

通讯地址：广州市天河区五山华南农业大学动物科学学院

电 话：18818912892 传真：020-38882017

电子信箱：zhuyw0724@scau.edu.cn

本合同甲方委托乙方就 不同营养水平饲粮添加乳化剂和酶制剂对肉鸭生产性能和养分利用率的影响 技术服务，并支付相应的技术服务报酬。双方经过平等协商，在真实、充分地表达各自意愿的基础上，根据《中华人民共和国合同法》的规定，达成如下协议，并由双方共同恪守。

第一条： 甲方委托乙方进行技术服务的内容如下：

1. 技术服务的目标：明确不同营养水平饲粮添加乳化剂和酶制剂对肉鸭生产性能和养分利用率的积极影响。

2. 技术服务的内容：开展 1-42 日龄肉鸭饲养试验和消化代谢试验，结合评价不同营养水平饲粮添加乳化剂和酶制剂对肉鸭生产性能和养分利用率的影响。

3. 技术服务的方式：按照合同约定的试验方案的要求和步骤进行，完成饲养试验和样品分析，出具标准的试验研究报告作为验收标准。

第二条： 乙方应按下列要求完成技术服务工作：

1. 技术服务地点：广东省广州市

2. 技术服务期限：甲方项目实施期间

3. 技术服务进度：2020.2 -2020.3 完成原料的采购和饲料配制等工作；2020.3-2020.4 完成试验场地消毒、肉鸭购买与分组饲养等工作；2020.4-2020.5 开展 1-42 日龄肉鸭饲养试验和消化代谢试验，获得肉

鸭生长性能和营养物质消化率数据 1 套；2020.5-2022.12 完成样品氨基酸、肠道屏障等指标分析，出具试验研究报告一份。

4. 技术服务质量要求：乙方按照试验方案规定的时间完成试验项目，包括动物试验、实验室分析、统计分析，并出具一个最终试验报告。

5. 技术服务质量期限要求：不拖延项目进度为期限

第三条：为保证乙方有效进行技术服务工作，甲方应当向乙方提供下列工作条件和协作事项：

1. 提供技术资料：

(1) 不同营养水平饲粮添加乳化剂和酶制剂对肉鸭生产性能和养分利用率的影响高低档肉鸭饲料配方 1 套。

(2) 不同营养水平饲粮添加乳化剂和酶制剂对肉鸭生产性能和养分利用率的影响试验方案中乳化剂和酶制剂添加剂产品各 1 个。

(3) 不同营养水平饲粮添加乳化剂和酶制剂对肉鸭生产性能和养分利用率的影响试验方案中乳化剂和酶制剂添加剂使用推荐量。

2. 提供工作条件：

(1) 共同讨论和分析不同营养水平饲粮添加乳化剂和酶制剂对肉鸭生产性能和养分利用率的影响技术方案和试验数据。

3. 其他：无。

4. 甲方提供上述工作条件和协作事项的时间及方式：由甲方安排，提前 1 个月通知乙方。

第四条：甲方向乙方支付技术服务报酬及支付方式为：

1. 技术服务费总额为： 拾万元整（¥100,000.00 万）

2. 技术服务费由甲方 分期 （一次或分期）支付乙方。

具体支付方式和时间如下：

(1) 付款方式：转账

(2) 第一期：签订合同后 15 天内，付款 5 万；第二期：试验结束乙方提交实验报告验收通过后 3 天内付款 5 万。

乙方开户银行名称、地址和帐号为：

开户银行： 中国工商银行广州五山支行

地址： 广州市天河区五山路 483 号

帐号： 3602002609000310520-000000001

第五条：双方确定因履行本合同应遵守的保密义务如下：

甲方：

1. 保密内容（包括技术信息和经营信息）： /。

2. 涉密人员范围： /；

3. 保密期限： /。

4. 泄密责任： /。

乙方：

1. 保密内容（包括技术信息和经营信息）：乙方不得向其他任何机构和个人泄露和发表试验得出的任何关于建明（中国）科技有限公司的产品或解决方案在生产和机理层面负效果的数据和言论。

2. 涉密人员范围：参与项目组所有人员。

3. 保密期限：永久。

4. 泄密责任：支付违约金并追究法律责任。

第六条：本合同的变更必须由双方协商一致，并以书面形式确定。但有下列情形之一的，一方可以向另一方提出变更合同权利与义务的请求，另一方应当在7日内予以答复；逾期未予答复的，视为同意：

1. 有一方违反合同条款，使合同不能履行的
2. 服务过程中因技术原因需要对合同进行调整的

第七条：双方确定以下列标准和方式对乙方的技术服务工作成果进行验收：

1. 乙方完成技术服务工作的形式：按照试验方案规定的时间完成试验项目，包括动物试验、实验室分析、统计分析，并出具一个最终试验报告。

2. 技术服务工作成果的验收标准：本合同约束的各项指标。

3. 技术服务工作成果的验收方法：由甲方组织相关专业技术人员验收，写出验收报告。

4. 验收的时间和地点：验收的时间和地点由甲方安排，提前 1 个月通知乙方。

第八条：双方确定：

1. 在本合同有效期内，甲方利用乙方提交的技术服务工作成果所完成的新的技术成果，归甲（甲、双）方所有。

2. 在本合同有效期内，乙方利用甲方提供的技术资料和工作条件所完成的新的技术成果，归乙（乙、双）方所有。

第九条：双方确定，按以下约定承担各自的违约责任：

1. 甲方违反本合同第3-8条约定,应当支付合同总金额的 50%作为违约金 (支付违约金或损失赔偿额的计算方法)。

2. 乙方违反本合同第二和第五至八条约定,应当支付合同总金额的 50%作为违约金 (支付违约金或损失赔偿额的计算方法)。

第十条: 双方确定,在本合同有效期内,甲方指定王立志为甲方项目联系人,乙方指定朱勇文为乙方项目联系人。项目联系人承担以下责任:

1. 开展 1-42 日龄肉鸭饲养试验和消化代谢试验,结合评价不同营养水平饲粮添加乳化剂和酶制剂对肉鸭生产性能和养分利用率的影响。

2. 负责项目中期检查和结题验收。

3. 负责项目经费计划的制定、监督项目经费的使用。

一方变更项目联系人的,应当及时以书面形式通知另一方,未及时通知并影响本合同履行或造成损失的,应承担相应的责任。

第十一条: 双方确定,出现下列情形,致使本合同的履行成为不必要或不可能的,可以解除本合同:

1. 发生不可抗力;
2. 双方协商解除合同的。
3. 乙方违反合同的,经协商无效的

第十二条: 双方因履行本合同而发生的争议,应协商、调解解决。协商、调解不成的,确定按以下第1种方式处理:

甲方：_____ (盖章)

法定代表人 / 委托代理人：_____ (签名)

2020年3月02日



乙方：_____ (盖章)

法定代表人 / 委托代理人：_____ (签名)

2020年2月25日



印花税票粘贴处：

合同编号:

技术服务合同

项目名称: 夏季高温条件下丙酸铬和蛋白酶及甘露聚糖酶对快速黄鸡生产性能、肉品质和养分利用率的影响

委托方(甲方): 建明(中国)科技有限公司

受托方(乙方): 华南农业大学

签订时间: 2023.05

签订地点: 广州

有效期限: 1 年

中华人民共和国科学技术部印制

填写说明

一、本合同为中华人民共和国科学技术部印制的技术服务合同示范文本,各技术合同认定登记机构可推介技术合同当事人参照使用。

二、本合同书适用于一方当事人（受托方）以技术知识为另一方（委托方）解决特定技术问题所订立的合同。

三、签约一方为多个当事人的，可按各自在合同关系中的作用等，在“委托方”、“受托方”项下（增页）分别排列为共同委托人或共同受托人。

四、本合同书未尽事项，可由当事人附页另行约定，并作为本合同的组成部分。

五、当事人使用本合同书时约定无需填写的条款，应在该条款处注明“无”等字样。

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技术服务合同

委托方（甲方）：建明（中国）科技有限公司

住 所 地：广东省珠海市三灶琴石路 25 号

法定代表人：Christopher E Nelson

项目联系人：张瑛

联系方式：15210318613

通讯地址：广东省珠海市三灶琴石路 25 号 建明（中国）科技
有限公司

电 话：0756-3989000 传 真：0756-3989001

电子信箱：Johnny.zhang@kemin.com

受托方（乙方）华南农业大学

住 所 地：广东省广州市天河区五山路 483 号

法定代表人：

项目联系人：朱勇文

联系方式：18818912892

通讯地址：广东省广州市天河区五山路 483 号

电 话： 传 真：

手 机：18818912892

电子信箱：zhuyw0724@scau.edu.cn

本合同甲方委托乙方进行夏季高温条件下丙酸铬和蛋白酶及甘露聚糖酶对快速黄鸡生产性能、肉品质和养分利用率的影响试验，并支付研究开发经费和报酬，乙方接受委托并进行此项研究开发工作。双方经过平等协商，在真实、充分地表达各自意愿的基础上，根据《中华人民共和国合同法》的规定，达成如下协议，并由双方共同恪守。

第一条 本合同研究开发项目的要求如下：

1. 技术目标 本试验旨在考查黄鸡料中添加丙酸铬，或添加不同酶制剂（蛋白酶、甘露聚糖酶）对夏季热应激条件下快大黄鸡生长性能、营养物质消化吸收率、肠道菌群、血液指标以及肠道健康等的影响，为夏季黄鸡的热应激提供最佳的解决方案。

2. 技术内容：饲养及评价指标测定

3. 技术方法和路线：详见附件

第二条 乙方应在本合同生效后5日内向甲方提交研究开发计划。研究开发计划应包括以下主要内容：

1. 根据试验要求，设计详细的试验方案、试验进度时间表及相关负责人

第三条 乙方应按下列进度完成研究开发工作：

1. 2023年7月1日前正式开展

2. 2023年9月30日提供生产数据试验报告，2023年12月20日提供其他的试验数据报告。

第四条 甲方应向乙方提供的技术资料及协作事项如下：

1. 技术资料清单：添加剂注册信息、使用方法和注意事项

2. 提供时间和方式：试验开始前一周 快递

3. 其他协作事项：提供试验所需样品

本合同履行完毕后，上述技术资料按以下方式处理：对甲方所提供的技术资料保密

第五条 甲方应按以下方式支付研究开发经费和报酬：

1. 研究开发经费和报酬总额为壹拾陆万元整人民币（RMB 160000元）。

2. 研究开发经费由甲方 分期 支付乙方及其协作单位。具体支付方式和时间如下：

(1) 合同生效时，实验开始前甲方支付总实验经费的 50% 捌万元整人民币（80000 RMB）给乙方，乙方收到预付款后 14 天内必须给甲方提供增值税专用发票；

(2) 甲方收到试验报告后，14 个工作日内向乙方支付剩余 50% 实验经费，捌万元整人民币（80000 RMB），乙方收到款后 14 天内必须给甲方提供增值税专用发票。

乙方开户银行名称、地址和帐号为：

开户银行：广州工行五山之行

户名：华南农业大学

行号：102581000546

帐号：3602002609000310520

第六条 本合同变更必须由双方协商一致，并以书面形式确定。但有下列情形之一的，一方可以向另一方提出变更合同权利与义务的请求，另一方应当在 10 日内予以答复；逾期未予以答复的，视为同意。

1. 甲方变更技术内容；

第七条 未经甲方同意，乙方不得将本合同项目部分或全部研究开发工作转让给第三人承担。

第八条 在本合同履行中，认定技术风险的基本条件是：

1. 本合同项目在现有技术水平条件下具有足够的难度；
2. 乙方在主观上无过错且经认定研究开发失败为合理的失败。

因饲养管理不当发生烈性传染病或者生长指标大幅偏离所在日龄生长曲线，造成试验失败，乙方需要重新进行试验。一方发现技术风险存在并有可能致使研究开发失败或部分失败的情形时，应当在 10 日内通知另一方并采取适当措施减少损失。逾期未通知并未采取适当措施而致使损失扩大的，应当就扩大的损失承担赔偿责任。

第九条 双方确定因履行本合同应遵守的保密义务如下：

甲方：

1. 保密内容（包括技术信息和经营信息）：所有试验原始记录及试验内容。

2. 涉密人员范围：该项目的技术人员和管理人员。

3. 涉密期限：2025 年 07 月 30 日。

4. 涉密责任：负泄密责任。

乙方：

1. 保密内容（包括技术信息和经营信息）：所有试验数据及试验内容

2. 涉密人员范围：课题的负责人和研究人员

3. 涉密期限：2025 年 07 月 30 日

4. 涉密责任：负泄密责任

第十条 乙方应当按以下方式向甲方交付研究开发成果：

1. 研究开发成果交付的形式及数量：试验报告

2. 研究开发成果交付的时间及地点：试验报告提交日期为实验室工作结束后 15 个工作日内。

第十一条 乙方应当保证其交付给甲方的研究开发成果不侵犯任何第三人的合法权益。如发生第三人指控甲方实施的技术侵权的，乙方应当负经济责任。

第十二条 双方确定，因履行本合同所产生的研究开发成果及其相关知识产权权利归属，按下列方式处理：

甲方享有申请专利的权利。专利权取得后的使用和有关利益分配方式如下：专利权取得后使用权和有关利益归甲方享有。

甲方享有文章发表署名权利，乙方发表该研究成果发表文章需要经过甲方同意，并有一名甲方人员为文章作者之一，在文章中必须写明所用产品来源于建明（中国）科技有限公司。

第十三条 乙方不得自行将研究开发成果转让给第三人。

第十四条 乙方完成本合同项目的研究开发人员享有在有关技术成果文件上写明技术成果完成者的权利和取得有关荣誉证书、奖励的权利。

第十五条 乙方利用研究开发经费所购置与研究开发工作有关的设备、器材、资料等财产，归乙方所有。

第十六条 双方确定,乙方应在向甲方交付研究开发成果后,根据甲方的请求,为甲方指定的人员提供技术指导和培训,或提供与使用该研究开发成果相关的技术服务。

1. 技术服务和指导内容: 无。
2. 地点和方式: 无。
3. 费用及支付方式: 无。

第十七条 双方确定:任何一方违反本合同约定,造成研究开发工作停滞、延误或失败的,按以下约定承担违约责任:

1. 甲方违反本合同第四条约定,应当赔偿乙方损失 RMB 16,000 元(支付违约金或损失赔偿额的计算方法)。
2. 乙方违反本合同第三或第十条约定,应当赔偿甲方损失 RMB 32,000 元(支付违约金或损失赔偿额的计算方法)。
3. 乙方违反本合同第十二条约定,应当赔偿甲方损失 RMB 48,000 元。(支付违约金或损失赔偿额的计算方法)。

第十八条 双方确定,甲方有权利用乙方按照本合同约定提供的研究开发成果,进行后续改进。由此产生的具有实质性或创造性技术进步特征的新的技术成果及其权利归属,由甲方享有。

乙方无权在完成本合同约定的研究开发工作后,利用该项研究开发成果进行后续改进,经甲方许可,乙方可以在国内外刊物发表研究内容及作为毕业论文材料。

第十九条 双方确定,在本合同有效期内,甲方指定张瑛为甲方项目联系人,乙方指定朱勇文为乙方项目联系人和试验负责人,以便与甲方联系人及时沟通试验进展。项目联系人承担以下责任:

1. 及时通报第九条合同规定内容;
2. 乙方联系人及时通报试验结果给甲方联系人。

一方变更项目联系人的,应当及时以书面形式通知另一方。未及时通知并影响本合同履行或造成损失的,应承担相应的责任。

第二十条 双方确定,出现下列情形,致使本合同的履行成为不必要或不可能的,一方可以通知另一方解除本合同;

1. 因发生不可抗力或技术风险。

第二十一条 双方因履行本合同而发生的争议,应协商、调解解决。协商、调解不成的,确定按以下第 2 种方式处理:

1. 提交珠海市劳动仲裁委员会仲裁;

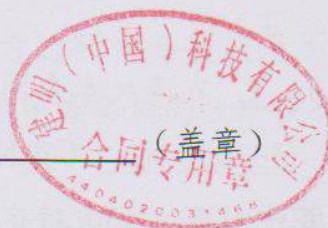
2. 依法向人民法院起诉。

第二十二条 双方约定本合同其他相关事项为:甲方支付研究开发经费和报酬时,乙方需提供正规发票给甲方。

第二十三条 本合同一式 3 份,具有同等法律效力。

第二十四条 本合同经双方签字盖章后生效。

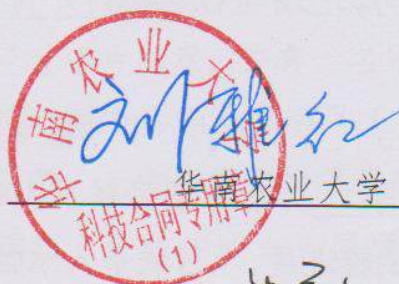
甲方: 建明(中国)科技有限公司



项目负责人: 张强 (签名)

2023年06月07日

乙方: 华南农业大学 (盖章)



项目负责人: 朱毅文 (签名)

2023年6月7日

成。

3.9 粪便和食糜中抗营养因子含量

采用 ELISA 法测定大豆球蛋白、胰蛋白酶抑制因子、甘露聚糖含量。

3.10 肠道免疫相关基因表达

采用实时荧光定量和 WB 方法分别测定空肠主要先天性免疫力 (TLR3、TLR4、IL-1 β 、IL-6、AvBD12 等) 和屏障功能 (Occludin 蛋白及微管蛋白等) 功能基因的表达水平。

3.10 统计分析

采用 SPAA 19.0 统计软件进行单因素方差分析, 并对差异显著者进行 Duncan 多重比较, 显著水平为 $P < 0.05$ 。

4 经费预算

表 5 经费预算表 (单位: 万元)

支出内容		预算金额
项目	试验用鸡苗、疫苗、消毒液等购买和运输等费用	2.0
	饲料原料购买、运输和处理组饲料配制、运输等费用	4.0
	肉鸡饲养试验场地、水电等费用	4.0
	人工劳务费	1.0
	养分利用率、基因表达、氨基酸含量等指标检测费用	4.0
	学校项目管理费	1.0
合计		16

合同编号: 22Y-JS-20230513-v11

技术服务合同

项目名称: 三丁酸甘油酯对肉鸡生长性能与肠道健康的影响

委 托 方: 湖南植之源农牧有限公司

(甲 方)

受 托 方: 华南农业大学

(乙 方)

签订时间: 2022. 10

签订地点: 广州市天河区

有效期限: 2023. 10

中华人民共和国科学技术部印制

填写说明

一、本合同为中华人民共和国科学技术部印制的技术服务合同示范文本，各技术合同认定登记机构可推介技术合同当事人参照使用。

二、本合同书适用于一方当事人（受托方）以技术知识为另一方（委托方）解决特定技术问题所订立的合同。

三、签约一方为多个当事人的，可按各自在合同关系中的作用等，在“委托方”、“受托方”项下（增页）分别排列为共同委托人或共同受托人。

四、本合同书未尽事项，可由当事人附页另行约定，并作为本合同的组成部分。

五、当事人使用本合同书时约定无需填写的条款，应在该条款处注明“无”等字样。

技术服务合同

委托方（甲方）：湖南植之源农牧有限公司

住 所 地：湖南省泸溪县武溪镇高新区创新创业园标准厂房

法定代表人：赵敏

项目联系人：赵敏

联系方式：19974375921

通讯地址：湖南省泸溪县武溪镇高新区创新创业园标准厂房

电 话： 传真：

电子信箱：459162585@qq.com

受托方（乙方）：华南农业大学

住 所 地：广州市天河区五山路 483 号

法定代表人：刘雅红

项目联系人：朱勇文

联系方式：18818912892

通讯地址：广州市天河区五山华南农业大学动物科学学院

电 话：18818912892 传真：020-38882017

电子信箱：zhuyw0724@scau.edu.cn

本合同甲方委托乙方就 三丁酸甘油酯对肉鸡生长性能与肠道健康的影响 提供技术服务，并支付相应的技术服务报酬。双方经过平等协商，在真实、充分地表达各自意愿的基础上，根据《中华人民共和国民法典》的规定，达成如下协议，并由双方共同恪守。

第一条：甲方委托乙方进行技术服务的内容如下：

1. 技术服务的目标：明确三丁酸甘油酯对肉鸡生长性能与肠道健康的积极影响。

2. 技术服务的内容：本试验以新型饲料添加剂三丁酸甘油酯为研究对象，评价其对肉鸡生长性能、血清免疫指标、血清抗氧化指标、肠道形态及胃食糜 pH 和蛋白酶活性、肠道微生物的影响，为三丁酸甘油酯在畜牧行业中的推广应用提供客观、真实、有效的参考依据。

3. 技术服务的方式：按照合同签订的试验方案的要求和步骤进行，完成饲养试验和样品分析，出具法人单位盖章的试验研究报告作为验收标准。

第二条：乙方应按下列要求完成技术服务工作：

1. 技术服务地点：广东省广州市

2. 技术服务期限：2022.12.1~2023.6.30

3. 技术服务方案：(1) 2022.12.1~2022.12.31：完成鸡舍温控、水线、光照及通风方面设备的调试和消毒等工作。(2) 2023.1.1~2023.1.5 完成生长前期饲料配制和进鸡。(3) 2023.1.6~2023.4.4 正式饲养试

验和样品采集：2023.4.5~2023.6.30 完成肠道健康相关指标分析，出具盖章试验研究报告一份。

4. 技术服务质量要求：乙方按照试验方案规定的时间完成试验项目，包括动物试验、实验室分析、统计分析，并出具一个最终试验报告。

5. 技术服务质量期限要求：不拖延项目进度、保质保量完成

第三条：为保证乙方有效进行技术服务工作，甲方应当向乙方提供下列工作条件和协作事项：

1. 提供技术资料：

(1) 三丁酸甘油酯对肉鸡生长性能与肠道健康的影响试验方案中三丁酸甘油酯产品 1 个。

(2) 三丁酸甘油酯对肉鸡生长性能与肠道健康的影响试验方案中三丁酸甘油酯产品推荐使用量。

2. 甲方提供上述工作条件和协作事项的时间及方式：由甲方安排，提前 1 个月通知乙方。

第四条：甲方向乙方支付技术服务报酬及支付方式为：

1. 技术服务费总额为：贰万元整（¥20,000.00）

2. 技术服务费由甲方一次（一次或分期）支付乙方。

具体支付方式和时间如下：

(1) 付款方式：银行账户转账

(2) 付款时间：试验结束乙方提交实验报告，甲方自验收通过后 15 天内付款 2 万元。

乙方开户银行名称、地址和帐号为：

开户银行： 中国工商银行广州五山支行

地址： 广州市天河区五山路 483 号

帐号： 3602002609000310520

第五条：双方确定因履行本合同应遵守的保密义务如下：

甲方：

1. 保密内容（包括技术信息和经营信息）： / 。

2. 涉密人员范围： / ；

3. 保密期限： / 。

4. 泄密责任： / 。

乙方：

1. 保密内容（包括技术信息和经营信息）：乙方不得向其他任何机构和个人泄露和发表：试验过程中涉及湖南植之源农牧有限公司的在生产和机理层面负效果的任何数据和言论。

2. 涉密人员范围： 参与项目组所有人员。

3. 保密期限： 永久。

4. 泄密责任： 支付合同款项 20%的违约金并赔偿甲方因泄密遭受的全部经济或名誉损失。

第六条：本合同的变更必须由双方协商一致，并以书面形式确定。但有下列情形之一的，一方可以向另一方提出变更合同权利与义务的请求，另一方应当在 7 日内予以答复；逾期未予答复的，视为同意：

1. 有一方违反合同条款，使合同不能履行的

2. 服务过程中因技术原因需要对合同进行调整的

第七条：双方确定以下列标准和方式对乙方的技术服务工作成果进行验收：

1. 乙方完成技术服务工作的形式：按照试验方案规定的时间完成试验项目，包括动物试验、实验室分析、统计分析，并出具一个法人单位盖章的试验报告。

2. 技术服务工作成果的验收标准：包含甲方完成项目所必需的的各项指标，以相关专业技术人员验收标准为准。

3. 技术服务工作成果的验收方法：由甲方组织相关专业技术人员验收，出示验收报告。

4. 验收的时间和地点：由甲方安排，提前半个月通知乙方。

第八条：双方确定：

1. 在本合同有效期内，甲方利用乙方提交的技术服务工作成果所完成的新的技术成果，归甲（甲、双）方所有。

2. 在本合同有效期内，乙方利用甲方提供的技术资料和工作条件所完成的新的技术成果，归乙（乙、双）方所有。

第九条：双方确定，按以下约定承担各自的违约责任：

1. 甲方违反本合同约定致使合同根本目的不能实现的，应当支付合同总金额的 30%作为违约金（支付违约金或损失赔偿额的计算方法）。

2. 乙方违反本合同约定致使合同根本目的不能实现的，应当支付合同总金额的 30%作为违约金（支付违约金或损失赔偿

额的计算方法)。

第十条：双方确定，在本合同有效期内，甲方指定 赵敏 为甲方项目联系人，乙方指定 朱勇文 为乙方项目联系人。项目联系人承担以下责任：

1. 开展三丁酸甘油酯对肉鸡生长性能与肠道健康的影响试验。
2. 负责项目中期检查和结题验收。
3. 负责项目经费计划的制定、监督项目经费的使用。

一方变更项目联系人的，应当及时以书面形式通知另一方，未及时通知并影响本合同履行或造成损失的，应承担相应的责任。

第十一条：双方确定，出现下列情形，致使本合同的履行成为不必要或不可能的，可以解除本合同：

1. 发生不可抗力；
2. 双方协商解除合同的；
3. 乙方违反合同约定，经协商无法达成一致意见的。

第十二条：双方因履行本合同而发生的争议，应协商、调解解决。协商、调解不成的，确定按以下第 1 种方式处理：

1. 提交 广州市 仲裁委员会仲裁；
2. 依法向人民法院起诉。

第十三条：双方确定：本合同及相关附件中所涉及的有关名词和技术术语，其定义和解释如下：

1. /
2. /

3. _____ / _____

第十四条：与履行本合同有关的下列技术文件，经双方确认后，
为本合同的组成部分：

1. 技术背景资料： _____ / _____；
2. 可行性论证报告： _____ / _____；
3. 技术评价报告： _____ / _____；
4. 技术标准和规范： _____ / _____；
5. 原始设计和工艺文件： _____ / _____；
6. 其他： _____ / _____；

第十五条：双方约定本合同其他相关事项为： 以该研究成果发表文章，甲方有权在文章中署名一位作者。

第十六条：本合同一式 四 份，双方各持两份，具有同等法律效力。

第十七条：本合同经双方签字或盖章后生效。

甲方：_____ (盖章)

法定代表人 / 委托代理人：_____ (签名)



年 月 日

乙方：_____ (盖章)

法定代表人 / 委托代理人：_____ (签名)



年 月 日

合同编号:

技术开发（合作）合同

项目名称: 不同剂型多功能宠物食品的技术研发

甲 方: 广东尚嘉宠物用品有限公司

乙 方: 华南农业大学

丙 方: _____

签订时间: 2021年6月7日

签订地点: 广东省广州市天河区

有效期限: 2021年7月1日-2023年7月1日

中华人民共和国科学技术部印制

技术开发（合作）合同

甲 方：广东尚嘉宠物用品有限公司

住 所 地：广东省汕头市金平区潮汕路金平工业城

法定代表人：余斐

项目联系人：都小华

联系方式：0754-88223047

通讯地址：广东省汕头市金平区潮汕路金平工业城十一片区 M3 片

厂房

电 话：13005225345 传真：0754-88223047

电子信箱：308656320@qq.com

乙 方：华南农业大学

住 所 地：广州市天河区五山路 483 号

法定代表人：刘雅红

项目联系人：朱勇文

联系方式：18818912892

通讯地址：广州市天河区五山华南农业大学动物科学学院

电 话：18818912892 传真：020-38882017

电子信箱：zhuyw0724@scau.edu.cn

本合同合作各方就共同参与研究开发不同剂型多功能宠物食品的技术研发项目事项，经过平等协商，在真实、充分地表达各自意愿的基础上，根据《中华人民共和国合同法》的规定，达成如下协议，并由合作各方共同恪守。

第一条 本合同合作研究开发项目的要求如下：

1. 技术目标：联合研制不同剂型护毛、护骨、护关节、护肠道等多功能性宠物食品 25 个系列，联合共建华南农业大学动物科学学院-广东尚嘉宠物用品有限公司院实践教学基地 1 个，开展宠物毛发防脱剂及原籍益生菌的开发。

2. 技术内容：(1) 联合研制并优化不同剂型护毛、护骨、护关节、护肠道等多功能性宠物食品；(2) 共建宠物食品加工方向的院实践教学实习基地；(3) 宠物毛发防脱剂及原籍益生菌技术开发。

3. 技术方法和路线：以广东尚嘉宠物用品有限公司技术平台为依托，联合研制不同剂型护毛、护骨、护关节、护肠道等多功能宠物食品，优化部分产品配方参数和探索部分新品的开发；基于上述产学研合作基础，共建宠物食品加工方向的院实践教学基地 1 个。

第二条 本合同合作各方在研究开发项目中，分工承担如下工作：

甲方：

1. 研究开发内容：联合研制不同剂型护毛、护骨、护关节、护肠道等多功能宠物食品 25 个系列，联合共建院实践教学基地 1 个。

2. 工作进度：2021-2022 年度联合研制不同剂型多功能宠物食品 15 个以上，2022-2023 年度联合共建院实践教学基地，实现基地挂牌。

3. 研究开发期限：2021 年 7 月 1 日至 2023 年 7 月 1 日。

4. 研究开发地点：广东尚嘉宠物用品有限公司基地。

乙方：

1. 研究开发内容：联合研制不同剂型护毛、护骨、护关节、护肠道等多功能宠物食品 25 个系列，联合共建实践教学基地 1 个，研发新品 1-2 个。

2. 工作进度：2021-2022 年度联合研制不同剂型多功能宠物食品 15 个以上，2022-2023 年度联合共建院实践教学基地，实现基地挂牌；研发新品 1 个以上。

3. 研究开发期限：2021 年 7 月 1 日至 2023 年 7 月 1 日。

4. 研究开发地点：华南农业大学动物科学学院。

丙方：

1. 研究开发内容：/。

2. 工作进度：/。

3. 研究开发期限：/。

4. 研究开发地点：/。

第三条 为确保本合同的全面履行，合作各方确定，采取以下方式对研究开发工作进行组织管理和协调：甲方监督乙方的工作进展和资金使用情况，乙方制定本项目的研发计划、及时汇报工作进展和资金使用情况。

第四条 合作各方确定，各自为本合同项目的研究开发工作提供以下技术资料和条件：

甲方：提供联合研制不同剂型多功能宠物食品研发平台；提供所需的经费人民币 16 万元给乙方作为研发与实习基地建设，并配合建设院实践教学基地。

乙方：联合研制不同剂型多功能宠物食品研发，优化部分产品配方参数和探索部分新品的开发；协助共建院实践教学基地。

丙方：/。

本合同履行完毕后，上述技术资料和条件按以下方式处理：双方

研制的产品双方有知悉权，依照本合同双方共同研制产品在取得权威机构检测认定且具备生产资格后，经双方同意，其包装和宣传物料上可标注甲方和乙方项目合作单位联合研制等字样，以示对知识产权的尊重；乙方研究基地获得的与本项目相关技术资料甲方有知悉权和使用权、但未公开资料甲方需征得乙方同意后才能透露给第三方，乙方在自身研究基地取得的知识产权归乙方。

第五条 合作各方确定，按如下方式提供或支付本合同项目的研究开发经费及其他投资：

甲方：

1. 提供或支付方式：支付方式：转账；支付时间：签订合同后10天内。

2. 支付或折算为技术投资的金额：人民币壹拾陆万元整支付给乙方。

3. 使用方式：按预算拨付给乙方科研账户，可要求和监督乙方合理使用所拨经费。

乙方：

1. 提供或支付方式：提供甲方拨付经费所需的科研账户、票据等相关财务资料。

2. 支付或折算为技术投资的金额：/。

3. 使用方式：按年度预算和有关财务制度开支使用。

丙方：

1. 提供或支付方式：/。

2. 支付或折算为技术投资的金额：/。

3. 使用方式：/。

第六条 以提供技术为投资的合作方应保证其所提供技术不侵犯任何第三人的合法权益。如发生第三人指控合作一方或多方因实施

该项技术而侵权的，提供技术方应当承担侵权责任和相应损失。

第七条 本合同的变更必须由合作各方协商一致，并以书面形式确定。但有下列情形之一的，合作一方或多方可以向其他合作方提出变更合同权利与义务的请求，其他合作方应当在15日内予以答复；逾期未予答复的，视为同意：

1. 甲方未按计划拨付项目研发经费；
2. 乙方未计划开展研发工作；
3. 乙方违规使用经费；
4. 涉及国家和地方政府相关政策、有关法规条例、自然灾害及天气气候、疫情和疫病因素使得项目无法正常开展。

第八条 未经其他合作方同意，合作一方或多方不得将本合同项目部分或全部研究开发工作转让给第三人承担。但有下列情况之一的，合作一方或多方可以不经其他合作方同意，将本合同项目部分或全部研究开发工作转让给第三人承担：

1. 甲方和乙方均无能力独自完成的研发工作；
2. 乙方未按计划完成研发工作；
3. / 。

合作一方或多方可以转让的具体内容包括：研发新品的专利或技术方案等资料。

第九条 在本合同履行中，因出现在现有技术水平和条件下难以克服的技术困难，导致研究开发失败或部分失败，并造成合作一方或多方损失的，合作各方约定按以下方式承担风险损失：

1. 双方共同协商解决，第三方专家风险评估认定；
2. / ；
3. / 。

合作各方确定，本合同项目的技术风险按甲乙双方共同协商，

第三方专家风险评估的方式认定。认定技术风险的基本内容应当包括技术风险的存在、范围、程度及损失大小等。认定技术风险的基本条件是：

1. 本合同项目在现有技术水平条件下具有足够的难度；
2. 乙方在主观上无过错且经认定研究开发失败为合理的失败。

一方发现技术风险存在并有可能致使研究开发失败或部分失败的情形时，应当在30日内通知其他合作方并采取适当措施减少损失。逾期未通知并未采取适当措施而致使损失扩大的，应当就扩大的损失承担赔偿责任。

第十条 在本合同履行过程中，因作为研究开发标的的技术已经由他人公开（包括以专利权方式公开），合作一方或多方应在30日内通知其他合作方解除合同。逾期未通知并致使其他合作方产生损失的，其他合作方有权要求予以赔偿。

第十一条 合作各方确定因履行本合同应遵守的保密义务如下：

甲方：

1. 保密内容（包括技术信息和经营信息）：企业经营经济信息、企业财务信息、企业内部加工技术资料、。

2. 涉密人员范围：项目组所有开发成员。

3. 保密期限：按公司有关保密制度约定。

4. 泄密责任：按公司有关保密制度处理。

乙方：

1. 保密内容（包括技术信息和经营信息）：研发获得的技术参数。

2. 涉密人员范围：项目组所有人员。

3. 保密期限：发表文章、出版书籍、学术报告、专利等需获得课题负责人许可，在获得许可公开之日前均属于保密期。

4. 泄密责任: 按学校有关保密制度处理。

丙方:

1. 保密内容 (包括技术信息和经营信息): / 。

2. 涉密人员范围: / 。

3. 保密期限: / 。

4. 泄密责任: / 。

第十二条: 合作各方确定按以下方式交付研究开发成果:

甲方:

1. 研究开发成果交付的形式及数量: 联合研制不同剂型护毛、护骨、护关节、护肠道等多功能宠物食品 25 个系列, 联合共建院实践教学基地 1 个。

2. 研究开发成果交付的时间及地点: 2023 年 7 月 1 日前, 广东尚嘉宠物用品有限公司。

乙方:

1. 研究开发成果交付的形式及数量: 联合研制不同剂型护毛、护骨、护关节、护肠道等多功能宠物食品 25 个系列, 联合共建院实践教学基地 1 个, 研发新品 1 个。

2. 研究开发成果交付的时间及地点: 2023 年 7 月 1 日前, 广东尚嘉宠物用品有限公司。

丙方:

1. 研究开发成果交付的形式及数量: / 。

2. 研究开发成果交付的时间及地点: / 。

阶段性技术成果及其相关知识产权权利归属，特别约定如下：在甲方广东尚嘉宠物用品有限公司产生的成果归甲方，在乙方研究基地产生的成果和知识产权归乙方。

第十六条：合作各方确定，因履行本合同所产生的最终研究开发技术成果及其相关知识产权权利归属，按第1种方式处理：

1. 完成方享有申请专利的权利。

专利权取得后的使用和有关利益分配方式如下：无。

2. 按技术秘密方式处理。有关使用和转让的权利归属及由此产生的利益按以下约定处理：

(1) 技术秘密的使用权：/

(2) 技术秘密的转让权：/

(3) 相关利益的分配办法：/；

合作各方对因履行本合同所产生的最终研究开发技术成果及其相关知识产权权利归属，特别约定如下：/。

第十七条：合作各方分别独立完成并与履行本合同有关的阶段性技术成果的研究开发人员，享有在有关此阶段性技术成果文件上写明技术成果完成者的权利和取得有关荣誉证书、奖励的权利。

合作各方应以协商方式确定最终研究成果的完成人员名单。此完成人员享有在有关最终技术成果文件上写明技术成果完成者的权利和取得有关荣誉证书、奖励的权利。

第十八条：合作一方或多方利用共同投资的研究开发经费所购置与研究开发工作有关的设备、器材、资料等财产，归购买方所

约金或损失赔偿额的计算方法)。

3. 违反本合同第 / 条约定, 应当 / (支付违约金或损失赔偿额的计算方法)。

第二十条: 合作各方确定, 任何一方有权利用本合同项目研究开发所完成的技术成果, 进行后续改进。由此产生的具有实质性或创造性技术进步特征的新的技术成果, 归 完成方 (完成方、合作各方) 方所有。具体相关利益的分配办法如下: 无 。

第二十一条: 为有效履行本合同, 合作各方确定, 在本合同有效期内, 甲方指定 都小华 为甲方项目联系人, 乙方指定 朱勇文 为乙方项目联系人, 丙方指定 / 为丙方项目联系人。项目联系人承担以下责任:

1. 负责项目涉及产品的联合研发。
2. 负责项目中院实践教学实习基地的联合建设。
3. 负责项目经费计划的制定、监督项目经费的使用。

一方变更项目联系人的, 应当及时并以书面形式通知其他合作各方。未及时通知并影响本合同履行或造成损失的, 应承担相应的责任。

第二十二条: 合作各方确定, 出现下列情形, 致使本合同的履行成为不必要或不可能的, 可以解除本合同:

1. 因发生不可抗力和技术风险;
2. 双方协商解除合同的。
3. /

第二十三条: 合作各方因履行本合同而发生的争议, 应协商、

第二十六条：合作各方约定本合同其他相关事项为：无。

第二十七条：本合同一式 4 份，具有同等法律效力。

第二十八条：本合同经合作各方签字盖章后生效。

甲方：广东尚嘉宠物用品有限公司

(盖章)

法定代表人 / 委托代理人: 余斐 (签名)

年 月 日

乙 方： 华南农业大学

(盖章)

法定代表人 / 委托代理人: 刘雅红 (签名)

年 月 日

丙方: _____ (盖章)

法定代表人 / 委托代理人: _____ (签名)

年 月 日

印花税票粘贴处:

(以下由技术合同登记机构填写)

合同登记编号:

合同编号：

技术开发（合作）合同

项目名称：食品工业非标品在功能宠物食品中应
用的技术研发

甲 方：广东昊天环保科技有限公司

乙 方：华南农业大学

签订时间：2023 年 5 月 10 日

签订地点：广东省广州市增城区

有效期限：2023 年 5 月 10 日-2024 年 5 月 10 日

中华人民共和国科学技术部印制

填写说明

一、本合同为中华人民共和国科学技术部印制的技术开发（合作）合同示范文本，各技术合同认定登记机构可推介技术合同当事人参照使用。

二、本合同书适用于当事人各方就共同进行新技术、新产品、新工艺或者新材料及其系统的研究开发所订立的技术开发合同。

三、本合同书未尽事项，可由当事人附页另行约定，并可作为本合同的组成部分。

四、当事人使用本合同书时约定无需填写的条款，应在该条款处注明“无”等字样。

技术开发（合作）合同

甲 方：广东昊天环保科技有限公司

住 所 地：广州市增城区新城大道 400 号新城创新中心

法定代表人：张誉山

项目联系人：张维丽

联系方式：13926409080

通讯地址：广州市增城区新城大道 400 号新城创新中心 28 号楼 708

自编 02

电 话： 传真：

电子信箱：

乙 方：华南农业大学

住 所 地：广州市天河区五山路 483 号

法定代表人：刘雅红

项目联系人：朱勇文

联系方式：18818912892

通讯地址：广州市天河区五山华南农业大学动物科学学院

电 话：18818912892 传真：020-38882017

电子信箱：zhuyw0724@scau.edu.cn

本合同合作双方就共同参与研究开发食品工业非标品在功能宠物食品中的技术应用项目事项，经过平等协商，在真实、充分地表达各自意愿的基础上，根据《中华人民共和国民法典》的规定，达成如下协议，供合作双方共同恪守。

第一条 本合同合作研究开发项目的要求如下：

1. 技术目标：联合研制基于不同食品工业非标品的环保宠物食品 3~4 个；联合共建华南农业大学动物科学学院-广东昊天环保科技有限公司院实践教学基地 1 个。

2. 技术内容：(1) 联合研制基于不同食品工业非标品的环保宠物食品；(2) 共建宠物食品加工方向的院实践教学实习基地。

3. 技术方法和路线：以广东昊天环保科技有限公司技术平台为依托，联合研制基于不同食品工业非标品的环保宠物食品；基于上述产学研合作基础，共建宠物食品加工方向的院实践教学基地 1 个。

第二条 本合同合作各方在研究开发项目中，分工承担如下工作：

甲方：

1. 提供联合研制 3~4 个基于不同食品工业非标品的环保宠物食品研发平台。

2. 提供所需的经费给乙方作为研发与实习基地建设，费用按人民币 1.5 万元/个。

3. 并配合建设院实践教学基地。

乙方：

1. 研究开发内容：研制基于不同食品工业非标品的环保宠物食品 3~4 个，自行落实实验对象和场地，联合共建院实践教学基地 1 个。

2. 工作进度：2023.5-2023.11：研制基于不同食品工业非标品的环保宠物食品 2~3 个；2023.12-2024.5：完成项目技术研发内容，联合共建院实践教学基地，实现基地挂牌。

3. 研究开发期限：2023年5月10日至2024年5月10日。

4. 研究开发地点：华南农业大学动物科学学院。

第三条 为确保本合同的全面履行，合作双方确定，采取以下方式对研究开发工作进行组织管理和协调：甲方监督乙方的工作进展和资金使用情况，乙方根据甲方要求制定本项目的研发计划、及时汇报工作进展和资金使用情况。

第四条 合作双方确定，各自为本合同项目的研究开发工作提供以下技术资料和条件：

甲方：提供联合研制 3~4 个基于不同食品工业非标品的环保宠物食品研发平台，按 1.5 万元/个作为技术研发费用支付给乙方，并配合建设院实践教学基地。

乙方：提供联合研制 3~4 个基于不同食品工业非标品的环保宠物食品研发产品成果、技术及关键参数；联合共建院实践教学基地。

本合同履行完毕后，上述技术资料和条件按以下方式处理：甲、乙双方对共同研制的产品均有知情权，依照本合同双方共同研制产品在取得权威机构检测认定且具备生产资格后，经双方同意，其包装和宣传物料上可标注甲方和乙方项目合作单位联合研制等字样，以示对专有技术的尊重；乙方研究获得的与本项目相关技术资料甲方有知情权和使用权、但未公开资料甲方需征得乙方同意后才能透露给第三方，在甲方支付完本项目研发经费后，本合同项下的全部研发项目及成果的知识产权完全归甲方持有。

第五条 合作双方确定，按下表中费用标准及支付比例提供或支付本合同项目的研究开发经费及其他投资：

编号	研发产品	费用（元/个）	启动定金	交付尾款	备注
1	产品 1	15000	10500	4500	该费用包含乙方自行提
2	产品 2	15000	10500	4500	

3	产品 3	15000	10500	4500	供研发所需
4	产品 4	15000	10500	4500	实验场所
合计:		60000	42000	18000	

甲方:

1. 提供研发经费: 合同签订 10 日内向乙方支付 70% 作为每个项目启动定金; 研发产品成果、技术及关键参数取得权威机构检测认定且具备生产资格, 乙方完成每个产品所有研发资料交付后支付剩余 30%。

2. 使用方式: 按预算拨付给乙方科研账户, 可要求和监督乙方合理使用所拨经费。

乙方:

1. 提供研究有关资料: 提供甲方拨付经费所需的科研账户、票据等相关财务资料。

2. 使用方式: 按年度预算和有关财务制度开支使用。

第六条 乙方提供的研发产品成果、技术及关键参数等应保证不侵犯任何第三人的合法权益。如发生第三人主张权利的, 乙方应当负责处理。

第七条 本合同的变更必须由合作双方协商一致, 并以书面形式确定。但有下列情形之一的, 合作一方可以向另一方提出变更、解除或终止合同的请求, 另一方应当在15 日内予以答复; 逾期未予答复的, 视为同意:

1. 甲方未按计划拨付项目研发经费;
2. 乙方未计划开展研发工作;
3. 乙方违规使用经费;
4. 涉及国家和地方政府相关政策、有关法规条例、自然灾害及天气气候、疫情和疫病因素使得项目无法正常开展。

第八条 未经甲方同意,乙方不得将本合同项目部分或全部研究开发工作转让给第三人承担。

第九条 在本合同履行中,因出现在现有技术水平和条件下难以克服的技术困难,导致研究开发失败或部分失败,并造成合作双方损失的,合作双方约定按以下方式承担风险损失:

1. 双方共同协商解决,第三方专家风险评估认定;
2. /;
3. /。

合作双方确定,本合同项目的技术风险按甲乙双方共同协商,第三方专家风险评估的方式认定。认定技术风险的基本内容应当包括技术风险的存在、范围、程度及损失大小等。认定技术风险的基本条件是:

1. 本合同项目在现有技术水平条件下具有足够的难度;
2. 乙方在主观上无过错且经认定研究开发失败为合理的失败。

一方发现技术风险存在并有可能致使研究开发失败或部分失败的情形时,应当在30日内通知另一方并采取适当措施减少损失。逾期未通知并未采取适当措施而致使损失扩大的,应当就扩大的损失承担赔偿责任。

第十条 在本合同履行过程中,因作为研究开发标的的技术已经由他人公开(包括以专利权方式公开),合作一方应在30日内通知其他合作方解除合同。逾期未通知并致使其他合作方产生损失的,其他合作方有权要求予以赔偿。

第十一条 合作双方确定因履行本合同应遵守的保密义务如下:
甲方:

1. 保密内容(包括技术信息和经营信息):企业经营经济信息、企业财务信息、企业内部加工技术资料。
2. 涉密人员范围:项目组所有开发成员。

3.保密期限：按公司有关保密制度约定。

4.泄密责任：按公司有关保密制度处理。

乙方：

1.保密内容（包括技术信息和经营信息）：研发获得的技术参数。

2.涉密人员范围：项目组所有人员。

3.保密期限：发表文章、出版书籍、学术报告、专利等需获得课题负责人许可，在获得许可公开之日前均属于保密期。

4.泄密责任：按学校有关保密制度处理。

第十二条：合作双方确定按以下方式交付研究开发成果：

乙方：

1.研究开发成果交付的形式及数量：基于不同食品工业非标品的环保宠物食品 3~4 个。

2.研究开发成果交付的时间及地点：2023 年 11 月前，交付基于不同食品工业非标品的环保宠物食品 2~3 个；2024 年 5 月前交付剩余成果，联合共建院实践教学基地，实现基地挂牌。

交付地点：广东昊天环保科技有限公司。

第十三条：合作双方确定，按以下标准及方法对乙方完成的研究开发工作成果进行验收：

1、由第三方科技评价与专家组验收，线上或线下双方商定。

第十四条：合作双方确定，因履行本合同所产生完成的全部技术成果及其相关知识产权权利归属归甲方所有。

第十五条：合作双方分别独立完成并与履行本合同有关的阶段性技术成果的研究开发人员，享有在有关此阶段性技术成果文件上写明

技术成果完成者的权利和取得有关荣誉证书、奖励的权利。

合作双方应以协商方式确定最终研究成果的完成人员名单。此完成人员享有在有关最终技术成果文件上写明技术成果完成者的权利和取得有关荣誉证书、奖励的权利。

第十六条：合作双方利用共同投资的研究开发经费所购置与研究开发工作有关的设备、器材、资料等财产，归购买方所有。

第十七条：合作双方确定：任何一方违反本合同约定义务，造成另一方研究开发工作停滞、延误或失败的，应当按以下约定承担违约责任：

1、违约方应向守约方承担违约责任，同时承担守约方因主张权利产生的律师费、诉讼费、差旅费等一切损失。

2、甲方违反本合同第四条约定，应当向乙方支付合同总金额的 50%作为违约金（支付违约金或损失赔偿额的计算方法）。

3、乙方违反本合同第四条约定，应当向甲方支付合同总金额的 50%作为违约金（支付违约金或损失赔偿额的计算方法）。

4、乙方违反本合同第第十二条约定，应当向甲方支付合同总金额的 50%作为违约金（支付违约金或损失赔偿额的计算方法）。

第十八条：合作双方确定，任何一方有权利用本合同项目研究开发所完成的技术成果，进行后续改进。由此产生的具有实质性或创造性技术进步特征的新的技术成果，归完成方（完成方、合作各方）方所有。具体相关利益的分配办法如下：无。

第十九条：为有效履行本合同，合作各方确定，在本合同有效期

内，甲方指定张维丽为甲方项目联系人，乙方指定朱勇文为乙方项目联系人。项目联系人承担以下责任：

1. 负责项目涉及产品的联合研发。
2. 负责项目中院实践教学实习基地的联合建设。
3. 负责项目经费计划的制定、监督项目经费的使用。

一方变更项目联系人的，应当及时并以书面形式通知另一方。未及时通知并影响本合同履行或造成损失的，应承担相应的责任。

第二十条：合作双方确定，出现下列情形，致使本合同的履行成为不必要或不可能的，可以解除本合同：

1. 因发生不可抗力和技术风险；
2. 双方协商解除劳动合同的。
3. /

第二十一条：合作双方因履行本合同而发生的争议，应协商、调解解决。协商、调解不成的，确定按以下第2种方式处理：

1. 提交广州仲裁委员会仲裁；
2. 依法向原告所在地人民法院起诉。

第二十二条：与履行本合同有关的下列技术文件，经合作双方确认后，项目实施方案与技术标准和规范为本合同的组成部分：

1. 技术背景资料：/；
2. 可行性论证报告：/；
3. 技术评价报告：/；
4. 技术标准和规范：联合研制基于不同食品工业非标品的环保

宠物食品 3~4 个，联合共建院实践教学基地 1 个。

5. 原始设计和工艺文件： / ；

6. 其他： / 。

第二十三条： 本合同一式 4 份，经甲乙双方盖章签名后立即生效，双方各持 2 份，具有同等法律效力。

(以下无正文)

甲方： 广东昊天环保科技有限公司 (盖章)

法定代表人 / 委托代理人： 叶维的

2023 年 5 月 日

乙方： 华南农业大学 (盖章)

法定代表人 / 委托代理人： 朱毅

202 年 5 月 日

11X211/20231699

合同编号：

技术开发（合作）合同

项目名称：食品工业非标品在功能钓鱼饵料中应
用的技术研发

甲 方：广东海鲸环保科技有限公司

乙 方：华南农业大学

签订时间：2023 年 5 月 20 日

签订地点：广东省广州市

有效期限：2023 年 5 月 20 日-2026 年 5 月 19 日

中华人民共和国科学技术部印制

技术开发（合作）合同

甲 方：广东海鲸环保科技有限责任公司

住 所 地：广州市花都区狮岭镇联合村利达路工业横巷 1 号

法定代表人：马银兰

项目联系人：曾绍辉

联系方式：13642698286

通讯地址：广州市花都区狮岭镇联合村利达路工业横巷 1 号

电 话： 传真：

电子信箱：

乙 方：华南农业大学

住 所 地：广州市天河区五山路 483 号

法定代表人：刘雅红

项目联系人：朱勇文

联系方式：18818912892

通讯地址：广州市天河区五山华南农业大学动物科学学院

电 话：18818912892 传真：020-38882017

电子信箱：zhuyw0724@scau.edu.cn

本合同合作双方就共同参与研究开发食品工业非标品在功能钓鱼饵料中的技术应用项目事项，经过平等协商，在真实、充分地表达各自意愿的基础上，根据《中华人民共和国民法典》的规定，达成如下协议，供合作双方共同恪守。

第一条 本合同合作研究开发项目的要求如下：

1. 技术目标：联合研制基于不同食品工业非标品的环保钓鱼饵料，首批项目开发为鲢鳙鱼类饵料，草鱼和罗非鱼类饵料。

2. 技术内容：(1) 联合研制基于不同食品工业非标品的环保钓鱼饵料。

3. 技术方法和路线：以广东海鲸环保科技有限公司技术平台为依托，联合研制基于不同食品工业非标品的环保钓鱼饵料。

第二条 本合同合作各方在研究开发项目中，分工承担如下工作：

甲方：

1. 提供联合研制基于不同食品工业非标品的环保钓鱼饵料研发平台。

2. 提供所需的经费给乙方作为研发，费用按人民币 1.5 万元/项。

乙方：

1. 研究开发内容：研制基于不同食品工业非标品的环保钓鱼饵料，自行落实实验对象和场地。

2. 工作进度：2023 年 6 月完成研制基于不同食品工业非标品草鱼罗非鱼类饵料和研制基于不同食品工业非标品鲢鳙鱼类饵料 2 个课题；后期继续优化饵料配方，完成项目技术研发内容。

3. 研究开发期限：2023 年 5 月 20 日至 2026 年 5 月 19 日。

4. 研究开发地点：华南农业大学动物科学学院。

第三条 为确保本合同的全面履行，合作双方确定，采取以下方式对研究开发工作进行组织管理和协调：甲方监督乙方的工作进展

和资金使用情况，乙方根据甲方要求制定本项目的研发计划、及时汇报工作进展和资金使用情况。

第四条 合作双方确定，各自为本合同项目的研究开发工作提供以下技术资料和条件：

甲方：提供联合研制基于不同食品工业非标品的环保钓鱼饵料研发平台，按 1.5 万元/项作为技术研发费用支付给乙方。

乙方：提供联合研制基于不同食品工业非标品的环保钓鱼饵料研发产品成果、技术及关键参数。

本合同履行完毕后，上述技术资料和条件按以下方式处理：甲、乙双方对共同研制的产品均有知情权，依照本合同双方共同研制产品在取得权威机构检测认定且具备生产资格后，经双方同意，其包装和宣传物料上可标注甲方和乙方项目合作单位联合研制等字样，以示对专有技术的尊重；乙方研究获得的与本项目相关技术资料甲方有知情权和使用权、但未公开资料甲方需征得乙方同意后才能透露给第三方，在甲方支付完本项目研发经费后，本合同项下的全部研发项目及成果的知识产权完全归甲方持有。

第五条 合作双方确定，按以下费用标准及支付比例提供或支付本合同项目的研究开发经费及其他投资：

甲方：

1. 提供研发经费：合同签订 5 个工作日内向乙方支付研发经费；研发产品成果、技术及关键参数取得权威机构检测认定且具备生产资格，乙方把每个产品所有研发资料交付甲方。

2. 使用方式：按预算拨付给乙方科研账户，可要求和监督乙方合理使用所拨经费。

乙方:

1. 提供研究有关资料: 提供甲方拨付经费所需的科研账户、票据等相关财务资料。

2. 使用方式: 按年度预算和有关财务制度开支使用。

第六条 乙方提供的研发产品成果、技术及关键参数等应保证不侵犯任何第三人的合法权益。如发生第三人主张权利的,乙方应当全责处理。

第七条 本合同的变更必须由合作双方协商一致,并以书面形式确定。但有下列情形之一的,合作一方可以向另一方提出变更、解除或终止合同的请求,另一方应当在15日内予以答复;逾期未予答复的,视为同意:

1. 甲方未按计划拨付项目研发经费;
2. 乙方未计划开展研发工作;
3. 乙方违规使用经费;
4. 涉及国家和地方政府相关政策、有关法规条例、自然灾害及天气气候、疫情和疫病因素使得项目无法正常开展。

第八条 未经甲方同意,乙方不得将本合同项目部分或全部研究开发工作转让给第三人承担。

第九条 在本合同履行中,因出现在现有技术水平和条件下难以克服的技术困难,导致研究开发失败或部分失败,并造成合作双方损失的,合作双方约定按以下方式承担风险损失:

1. 双方共同协商解决,第三方专家风险评估认定;
2. /;
3. /。

合作双方确定,本合同项目的技术风险按甲乙双方共同协商,第三方专家风险评估的方式认定。认定技术风险的基本内容应当包括技术风险的存在、范围、程度及损失大小等。认定技术风险的基本条件

是：

1. 本合同项目在现有技术水平条件下具有足够的难度；
2. 乙方在主观上无过错且经认定研究开发失败为合理的失败。

一方发现技术风险存在并有可能致使研究开发失败或部分失败的情形时，应当在30日内通知另一方并采取适当措施减少损失。逾期未通知并未采取适当措施而致使损失扩大的，应当就扩大的损失承担赔偿责任。

第十条 在本合同履行过程中，因作为研究开发标的的技术已经由他人公开（包括以专利权方式公开），合作一方应在30日内通知其他合作方解除合同。逾期未通知并致使其他合作方产生损失的，其他合作方有权要求予以赔偿。

第十一条 合作双方确定因履行本合同应遵守的保密义务如下：

甲方：

1. 保密内容（包括技术信息和经营信息）：企业经营经济信息、企业财务信息、企业内部加工技术资料。

2. 涉密人员范围：项目组所有开发成员。

3. 保密期限：按公司有关保密制度约定。

4. 泄密责任：按公司有关保密制度处理。

乙方：

1. 保密内容（包括技术信息和经营信息）：研发获得的技术参数。

2. 涉密人员范围：项目组所有人员。

3. 保密期限：发表文章、出版书籍、学术报告、专利等需获得课题负责人许可，在获得许可公开之日前均属于保密期。

4. 泄密责任：按学校有关保密制度处理。

第十二条：合作双方确定项目课题按以下方式交付研究开发成果：

责任:

1、违约方应向守约方承担违约责任,同时承担守约方因主张权利产生的律师费、诉讼费、差旅费等一切损失。

2、甲方违反本合同第四条约定,应当向乙方支付合同总金额的 50%作为违约金 (支付违约金或损失赔偿额的计算方法)。

3、乙方违反本合同第四条约定,应当向甲方支付合同总金额的 50%作为违约金 (支付违约金或损失赔偿额的计算方法)。

4、乙方违反本合同第第十二条约定,应当向甲方支付合同总金额的 50%作为违约金 (支付违约金或损失赔偿额的计算方法)。

第十八条: 合作双方确定,任何一方有权利用本合同项目研究开发所完成的技术成果,进行后续改进。由此产生的具有实质性或创造性技术进步特征的新的技术成果,归合作各方 (完成方、合作各方)所有。具体相关利益的分配办法如下: 完成方为主。

第十九条: 为有效履行本合同,合作各方确定,在本合同有效期内,甲方指定曾绍辉为甲方项目联系人,乙方指定朱勇文为乙方项目联系人。项目联系人承担以下责任:

1. 负责项目涉及产品的联合研发。
2. 负责项目经费计划的制定、监督项目经费的使用。

一方变更项目联系人的,应当及时并以书面形式通知另一方。未及时通知并影响本合同履行或造成损失的,应承担相应的责任。

第二十条: 合作双方确定,出现下列情形,致使本合同的履行成为不必要或不可能的,可以解除本合同:

甲方：广东海鲸环保科技有限公司（盖章）

法定代表人 / 委托代理人：

2023 年 5 月 日



乙方：华南农业大学（盖章）

法定代表人 / 委托代理人：

202 年 5 月 日 (1)



印花税票粘贴处：

合同编号：

技术服务合同

项目名称： 猫健康评估与营养方案开发

委 托 方： 上海汇澄恩生物科技有限公司

(甲 方) _____

受 托 方： 华南农业大学

(乙 方) _____

签订时间： 2021. 4. 30

签订地点： 广州市天河区

有效期限： 2022. 4. 30

中华人民共和国科学技术部印制

技术服务合同

委托方（甲方）：上海汇澄恩生物科技有限公司

住 所 地：上海市普陀区梅川路 1247 号 4 幢 5 层 501 室-40

法定代表人：林芯慧

项目联系人：林芯慧

联系方式：

通讯地址：上海市普陀区梅川路 1247 号 4 幢 5 层 501 室-40

电 话： 传真：

电子信箱：

受托方（乙方）：华南农业大学

住 所 地：广州市天河区五山路 483 号

法定代表人：刘雅红

项目联系人：朱勇文

联系方式 18818912892

通讯地址：广州市天河区五山华南农业大学动物科学学院

电 话：18818912892 传真：020-38882017

电子信箱：zhuyw0724@scau.edu.cn

本合同甲方委托乙方就猫健康评估与营养方案开发技术
服务，并支付相应的技术服务报酬。双方经过平等协商，在真实、
充分地表达各自意愿的基础上，根据《中华人民共和国合同法》的规
定，达成如下协议，并由双方共同恪守。

第一条：甲方委托乙方进行技术服务的内容如下：

1. 技术服务的目标：形成猫健康评估整体方案，开发营养性技术解决方案1套。

2. 技术服务的内容：猫健康评估整体流程、猫局部健康剖析、问卷调查设计、猫营养保健功能包研发的选择与设计。

3. 技术服务的方式：市场调研、猫健康评估整体流程设计、保健功能包研发，协助上海汇澄恩生物科技有限公司构建猫健康评估系统。

第二条：乙方应按下列要求完成技术服务工作：

1. 技术服务地点：上海市普陀区

2. 技术服务期限：甲方项目实施期间

3. 技术服务进度：2021.4-2021.10 完成猫健康评估整体流程设计与保健功能包设计；2021.11-2022.4 完成猫健康评估系统构建和保健功能包研发；

4. 技术服务质量要求：自主研发猫营养保健功能包1-2个，协助构建新猫健康评估系统1个。

5. 技术服务质量期限要求：不拖延项目进度为期限

第三条：为保证乙方有效进行技术服务工作，甲方应当向乙方提

供下列工作条件和协作事项:

1. 提供技术资料:

(1) 产品调研: 了解目前猫健康问题, 包括全身性还是局部性的问题。

(2) 猫健康评估整体流程设计: 包括一般性资料、既往健康资料、目前健康资料的设计。

(3) 猫健康评估系统的构建: 完成猫健康剖析及问卷设计。

(4) 猫营养保健功能包研发的选择与设计: 根据全身性和局部性的健康问题, 针对性的开发 1-2 个通用型和肠道护理营养功能包。

2. 提供工作条件:

(1) 提供猫健康评估整体流程设计方案 1 个。

(2) 研发猫营养保健功能包 1-2 个。

3. 其他: 无。

4. 甲方提供上述工作条件和协作事项的时间及方式: 由甲方安排, 提前 1 个月通知乙方。

第四条: 甲方向乙方支付技术服务报酬及支付方式为:

1. 技术服务费总额为: 伍万 (¥5.00 万)

2. 技术服务费由甲方 一次 (一次或分期) 支付乙方。

具体支付方式和时间如下:

(1) 付款方式: 转账

(2) 签订合同后 10 天内

乙方开户银行名称、地址和帐号为:

1. 乙方完成技术服务工作的形式：提供猫健康评估整体流程设计方案 1 个，研发猫营养保健功能包 1-2 个。

2. 技术服务工作成果的验收标准：本合同约束的各项指标。

3. 技术服务工作成果的验收方法：由甲方组织相关专业技术人员验收，写出验收报告。

4. 验收的时间和地点：验收的时间和地点由甲方安排，提前 1 个月通知乙方。

第八条：双方确定：

1. 在本合同有效期内，甲方利用乙方提交的技术服务工作成果所完成的新的技术成果，归 甲（甲、双）方所有。

2. 在本合同有效期内，乙方利用甲方提供的技术资料和工作条件所完成的新的技术成果，归 乙（乙、双）方所有。

第九条：双方确定，按以下约定承担各自的违约责任：

1. 甲方违反本合同第 3-8 条约定，应当 支付合同总金额的 3% 作为违约金（支付违约金或损失赔偿额的计算方法）。

2. 乙方违反本合同第 二和第五至八 条约定，应当 支付合同总金额的 3% 作为违约金（支付违约金或损失赔偿额的计算方法）。

第十条：双方确定，在本合同有效期内，甲方指定 林芯慧 为甲方项目联系人，乙方指定 朱勇文 为乙方项目联系人。项目联系人承担以下责任：

1. 负责猫健康评估整体流程的设计和营养保健功能包研发。

1. 技术背景资料: _____/_____;
2. 可行性论证报告: _____/_____;
3. 技术评价报告: _____/_____;
4. 技术标准和规范: _____/_____;
5. 原始设计和工艺文件: _____/_____;
6. 其他: _____/_____;

第十五条: 双方约定本合同其他相关事项为: _____/_____。

第十六条: 本合同一式 4 份, 具有同等法律效力。

第十七条: 本合同经双方签字盖章后生效。

甲方: _____ (盖章)

法定代表人 / 委托代理人: _____ (签名)

年 月 日

乙方: _____ (盖章)

法定代表人 / 委托代理人: _____ (签名)

年 月 日

MASTER FIELD TRIAL AGREEMENT

THIS MASTER FIELD TRIAL AGREEMENT ("Agreement"), effective June 1, 2021 (the "Effective Date"), is made and entered into by and between and **Danisco Singapore Pte. Ltd.**, a corporation organized and existing under the laws of Singapore, having a place of business located at 21 Biopolis Road, #06-21 Nucleos, South Tower, Singapore 138567, Republic of Singapore (together with all Affiliates (defined below) herein referred to as "**Danisco**") and **South China Agricultural University** having a place of business located at 520 Animal Science Building, No. 483 Wushan Avenue, Tianhe District, Guangzhou, China ("**Provider**"). Provider and Danisco may be referred to individually as a "**Party**" or collectively as the "**Parties**."

The Parties hereby agree as follows:

1. Affiliates.

For purposes of this Agreement, the term "Affiliate" shall mean, with respect to a particular Party, any entity which Controls, is Controlled by or is under common Control with such Party as of the Effective Date, where "Control" (and derivations of such term) shall mean the legal, beneficial, or equitable ownership, directly or indirectly, of greater than 50% of the aggregate of all voting interests in an entity.

2. Series of Trials, Protocols, Trial Investigator and Provider Staff.

(a) Series of Trials. Danisco desires to engage Provider and Provider employees to initially engage in a series of field trials (the "**Trials**"), as such Trials are to be described in detail in the "Protocols" to be defined by Danisco throughout the term of this Agreement (with each approved protocol being herein referred to as a "**Protocol**" and with all Protocols collectively referred to herein as the "**Protocols**").

(b) Protocols.

(i) Danisco will approve each Protocol and there is no obligation to move forward with any or all of the Trials unless and until the relevant Protocol has been approved.

(ii) Each Protocol will be attached to this Master Field Trial Agreement and made a part hereof by reference thereto. Each Protocol is subject to the terms of this Agreement as if entered into at the time of execution of this Agreement.

(iii) Each Protocol is incorporated herein and made a part of this Agreement as if fully stated herein. Whenever possible, the terms of this Agreement and the terms of each Protocol shall be read and construed as being consistent. However, if notwithstanding the foregoing directive, any provision of this Agreement conflicts with a provision or various provisions of any Protocol, then the provisions of this Agreement shall govern and the conflicting provision or provisions in any applicable Protocol shall be disregarded.

(iv) The Parties agree that the terms of this Agreement and the specific Protocol shall together govern the Trials. Insofar as the Provider provides Danisco with a purchase order, invoice, offer, description of services, standard terms & conditions or any other documents of similar import governing the Trials described herein, all such documentation shall be null and void. Moreover, for avoidance of doubt all of the Trials will be conducted under the terms of this Agreement, and all other terms and conditions other than those specifically acknowledged by Danisco, will not be viewed as an amendment or modification to, or any way, otherwise supersede or replace any terms of this Agreement.

(v) Each Protocol will bear the signature and sign off of the Trial Investigator described in 1(c) below.

(c) Trial Investigator and Provider Staff. Each Trial will be performed solely by the applicable Provider employee responsible for ensuring the Trials are conducted in accordance with an approved Protocol ("Trial Investigator") and Provider employees under the appointed Trial Investigator's strict supervision (each Trial Investigator and supervised Provider employees referred to collectively as "**Provider Staff**") at Provider's facility located at 520 Animal Science Building, No. 483 Wushan Avenue, Tianhe District, Guangzhou, China. It is a condition of this Agreement that its terms and conditions be accepted by each appointed Trial Investigator. Provider is responsible for and ensures Provider Staff's compliance with the terms and conditions of this Agreement. The Trial Investigator shall sign each Protocol with the following declaration:

I hereby declare that I am familiar with and agree to be bound by all terms and conditions of the Master Field Trial Agreement. In addition, I hereby agree that the Trials described in the Protocols to be defined will be conducted under my supervision and in compliance with the Master Field Trial Agreement as well as with all Applicable Laws (as defined in the Master Field Trial Agreement).

3. Trial Start and Duration. Each approved Trial will commence on the approved date as described in the applicable Protocol. Unless earlier terminated in accordance with the terms of this Agreement, the term of this Agreement will expire on the later of: (a) the last expiration under any active Trial with an approved Protocol, or (b) May 31, 2023. The Provider and Danisco may agree in writing to extend the term of this Agreement or any Trial.

4. Trial Performance. Provider will perform each of the Trials in strict accordance with the applicable Protocols and in full compliance with all applicable laws, regulations, guidelines and codes of practice ("**Applicable Laws**"). Provider and Provider Staff will not deviate from the applicable Protocols without Danisco's prior written consent.

5. Final Report. Unless otherwise agreed under the approved Protocol, upon completion of each Trial and within thirty (30) days after delivery of the raw data, Provider will provide Danisco with a final detailed report that accurately states the results of the applicable Trial and include any further information required under the applicable Protocol (the "**Final Report**"). All Final Reports and all related data and results from the Trials are all owned exclusively by Danisco.

6. Payment for Performance. For performance of the Trials and delivery of the deliverables in accordance with this Agreement, Danisco will pay Provider in accordance to be agreed by both parties under the applicable Protocol (as reflected in the approved Protocol herein "Provider Fee"). Invoices may be paid by Danisco or any of its Affiliates, including but not limited to Danisco (UK) Limited and Danisco US Inc.

7. Trial Materials; Supply of Trial Materials. For purposes of this Agreement, "**Trial Materials**" means: (a) the materials for the Trials set forth in the approved Protocols; (b) any material incorporating the materials, or any component of the materials, set forth in the approved Protocols; (c) any material that could not have been made but for the materials set forth in (a) and (b) above, and (d) all documentation and descriptions of the Trial Materials. In order for Provider to perform each of the Trials, Danisco will supply Provider with the necessary Trial Materials set forth in the approved Protocol in quantities determined in the sole discretion of Danisco. Provider acknowledges that Danisco exclusively owns all Trial Materials provided in connection with each of the Trials.

8. Use of Trial Materials; Confidential Nature; Ownership & Use of Trial Materials, Reports and Trial Records.

(a) Provider and Provider Staff will not use the Trial Materials for any use or purpose other than performance of the applicable Trial in strict accordance with the Protocol; or provide the Trial

Materials to anyone other than Danisco and Provider Staff who need access to the Trial Materials for purposes of performance of the applicable Trial. Provider will collect any samples of the Trial Materials remaining after completion of the applicable Trial and promptly return them to Danisco and destroy all copies of documents containing any information related to the Trial Materials, Danisco Confidential Information (as defined herein), derivative works and products made from samples of Trial Materials; provided, however, that Danisco understands that the Provider may not be in a position to destroy all computer records or files that have been created pursuant to Provider's automatic archiving and back-up procedures. However, Danisco acknowledges and agrees that any such computer records or files created pursuant to Provider's automatic archiving and back-up procedures will be deleted in accordance with that Provider's standard retention policies. Notwithstanding the foregoing, with the exception of the Trial Materials that need to be returned in accordance with this Section 8, the Provider's Legal Department or its designee within the business may retain one copy of such Danisco Confidential Information for the sole purpose of monitoring its obligations under this Agreement. Provider acknowledges that the Trial Materials are experimental in nature and are not for human use.

(b) Unless specifically provided in the Protocol, Provider will not chemically analyze, disassemble or reverse engineer the composition or formulation of the Trial Materials, and shall not disclose, give or sell the Trial Materials to any third party.

(c) Without limiting the provisions of Sections 5 and 7 of this Agreement, Provider acknowledges that Danisco exclusively owns the Trial Materials, all Trial Records and reports (including, but not limited to the Final Report) and all data generated in connection with the Trials. Provider also acknowledges that all Trial Materials, all Trial Records and reports (including the Final Report) and all data generated in connection with the Trials shall be considered as "Danisco Confidential Information" (as defined in Section 13 below) and protected in accordance with the provisions of Section 13 below.

(d) Danisco and all Danisco Affiliates shall have the right to use and disclose all Trial Materials, Trial Records and reports (including, but not limited to the Final Report) and all data generated in connection with the Trials in any manner Danisco deems appropriate.

9. Handling and Storage of Trial Materials. Provider will keep all samples of Trial Materials in accordance with Danisco's handling instructions and in a secure location to which access will be limited to Provider Staff who need access to the Trial Materials for purposes of performance of the Trial.

10. Trial Materials Regulatory Status; Animal Owner Consent. Unless otherwise expressly stated in the approved Protocol, or unless required by Applicable Laws to conduct the applicable Trial, the Trial Materials are experimental in nature and do not have GRAS (Generally Recognized As Safe) status or any other regulatory status or approvals. Provider will obtain the prior consent of the owner of the animals. In addition, to the extent required to conduct the Trial, it is the responsibility of the Provider to obtain all regulatory approvals to conduct each Trial, but only to the extent such consents and approvals are required to comply with Applicable Laws.

11. Trial Records; Trial Reports and Information. Provider will maintain complete, accurate and orderly records for each of the Trials performed and results observed in accordance with the applicable Protocol ("Trial Records"). Provider Staff will date and sign Trial Records for each Trial to acknowledge their accuracy. All Trial Records are the property of Danisco and belong to Danisco. Provider will maintain all Trial Records in a secure place until Danisco requests them. Provider will not destroy any original documentation without Danisco's prior written consent. Provider will provide Danisco with all information and reports required to be delivered to Danisco in accordance with the approved Protocol for each Trial, and such other written or oral reports required by Danisco.

12. Danisco Involvement and Regulatory Inspections. Danisco is entitled to consult with any persons engaged in any of the Trials as and when Danisco deems it reasonably necessary, provided that such consult shall take place during regular business hours and upon reasonable notice. Provider will notify

Danisco immediately should any unexpected observations be made during any of the Trials. Provider will permit representatives of Danisco and regulatory authorities to inspect Provider facilities and Trial Records, during regular business hours, on request. Provider will use Provider best endeavours to enable representatives of Danisco to be present for any regulatory authority inspection relating to any of the Trials.

13. Confidentiality and Non-Use Obligations. Subject to the publication rights in Section 15 herein, Provider and Provider Staff will not disclose to any person or entity other than Danisco and its Affiliates, and will not use for any use or purpose other than performance of the applicable Trial in strict accordance with the applicable Protocol: (i) any information relating to the applicable Trial or the results obtained from the applicable Trial, including without limitation the Final Report and Trial Records; or (ii) any non-public information of any kind and in any format (tangible or intangible) provided to Provider or acquired from Danisco or its Affiliates during the term of this Agreement. Provider acknowledges that all information described in this Section 13 above shall be defined as "**Danisco Confidential Information**", and all Danisco Confidential Information is exclusively owned by Danisco. The obligations imposed on Provider by this Section 13 shall remain in effect and binding during the term of this Agreement, the term of all Trials and for ten (10) years after the termination or expiration of this Agreement and all Trials conducted under the terms of this Agreement.

14. Ownership and Protection of Danisco IP. Danisco owns: (a) any invention or discovery (whether patentable or not), (b) all Danisco Confidential Information (which includes all confidential know-how that Provider or Provider Staff makes or discovers during, or communicates to Danisco during, the course of any of the Trials); (c) results from or arising out of performance of any of the Trials; or results from or arising out of any research or development work conducted by Danisco or any Danisco Affiliates following any of the Trials, (d) all copyright and associated intellectual property rights in the Trial Records and in all data, reports including the Final Report (collectively, "**Danisco IP**"). Danisco will direct how the Danisco IP will be filed, which Danisco IP may be filed under the name of Danisco or any of Danisco's Affiliates. No royalty or other payment is due to Provider or Provider Staff in respect of Danisco IP. Provider and Provider Staff: (y) hereby assign to Danisco or its designee (which designee may be an Affiliate), all right, title and interest in and to Danisco IP, and any copyright, patent application or similar intellectual property rights claiming or covering Danisco IP; and (z) will execute all assignments and any other documents, and take any other actions reasonably necessary, at Danisco's or Danisco's designee's cost and expense, to enable Danisco or its designee to file, register, prosecute, maintain or enforce any intellectual property rights of any kind in any territory, claiming or covering Danisco IP. Provider represents and ensures that Provider Staff are legally required to assign Danisco IP and any intellectual property rights claiming or covering Danisco IP to Provider or directly to Danisco or Danisco's designee.

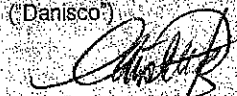
15. Publications. Provider will give Danisco prior written notice of any publications proposed by Provider or any Provider Staff relating to Danisco IP, Trial Materials, any information disclosed by Danisco, or any other information or material arising in the course of the Trials, and will not publish or otherwise disclose to third parties any such publication without Danisco's prior written consent (which consent may be withheld in Danisco's sole discretion to protect Danisco Confidential Information as well as to protect Danisco IP and any corresponding rights associated with Danisco IP). Notwithstanding the foregoing, prior to publication, Provider agrees to anonymize the results to prevent identification of Danisco or Danisco-branded products.

16. Use of Names. Danisco will not use Provider's name or the name of any Provider Staff for advertising or publicity purposes without Provider or their respective written consents, except that Danisco may include in its promotional material or otherwise make reference to quotations from publications. Provider will not use Danisco's name, logos, trademarks or identifying marks for advertising or publicity purposes without Danisco's prior written consent.

17. Termination. Danisco is entitled to terminate this Agreement by written notice to Provider at any time with or without cause. In the event of termination by Danisco, Danisco will reimburse Provider for all expenses Provider reasonably incurred in setting up and performing any of the active Trials to the date of termination and pay Provider a fair and reasonable proportion of the remainder of the agreed Provider Fees for the then active Trials as may in all the circumstances be appropriate. Termination of this Agreement

Upon the signature of the duly authorized representatives of the Parties below, this Master Field Trial Agreement becomes effective as of the Effective Date.

DANISCO SINGAPORE PTE. LTD.
("Danisco")




(Signature)

Christina Pang
(Printed Name)

Director
(Title)

SOUTH CHINA AGRICULTURAL UNIVERSITY
("Provider")



(Signature)

Qinghua Nie
(Printed Name)

Vice-president, college of Animal Science
(Title)

Master Field Trial Agreement effective June 1, 2021 by and between Danisco Singapore Pte. Ltd.
and South China Agricultural University

Protocol: ASPAC-CH-APXAP-2101

I hereby declare that I am familiar with and agree to be bound by all terms and conditions of the Master Field Trial Agreement. In addition, I hereby agree that the Trials described in the Protocols to be defined will be conducted under my supervision and in compliance with the Master Field Trial Agreement as well as with all Applicable Laws (as defined in the Master Field Trial Agreement).

Accepted and Agreed by the Trial Investigator

Dr. Yongwen Zhu
(Signature)

Dr. Yongwen Zhu
(Printed Name)

Associate professor, College of Animal Science
(Title)

编号：CARS-42-15

国家水禽产业技术体系 2024 年度任务书

岗位名称：水禽产业技术体系

岗位科学家：王文策

岗位科学家依托单位：华南农业大学

依托单位法定代表人：薛红卫

农业农村部科学技术司

二〇二四年六月

一、基本情况表

(一) 岗位科学家情况					
岗位名称	饲料资源开发利用				
岗位科学家	王文策	性别	女	出生年月	1982.10
职称	教授	学历	博士研究生	行政职务	副院长
工作单位	华南农业大学				
通讯地址/邮编	广州市天河区五山路 483 号华南农业大学动物科学学院/510642				
电话/电子信箱	15920117970/36084492@qq.com				
所属功能研究室	营养与饲料				
功能研究室主任	王文策				
(二) 团队成员情况					
姓名	学历/职称	出生年月	性别	工作单位	电话/邮箱
曹庆云	博士研究生/ 高级实验师	1978.06	女	华南农业大学	饲料分析检测
朱勇文	博士研究生/ 副教授	1986.07	男	华南农业大学	饲养试验
杨琳	博士研究生/ 教授	1963.09	男	华南农业大学	试验方案设计、 数据整理
叶慧	硕士研究生/ 高级实验师	1980.10	女	华南农业大学	饲料样品分析

二、重点任务

（一）体系产业重大关键技术攻关

CARS-42-01A：鸭遗传资源创新与新品种培育及高效养殖技术研究示范

1、研发任务		
<p>（1）研究不同品种肉鸭消化生理指标差异</p> <p>对比北京鸭、番鸭不同发育阶段消化道酶活组成、离子浓度等生理指标差异，探究不同品种鸭消化道酶谱变化规律，为后续番鸭饲料营养价值评定提供基础数据。</p> <p>（2）探究番鸭不同发育阶段体成分沉积规律</p> <p>以中速型地方品种肉鸭番鸭为研究对象，研究笼养条件下，研究不同生长发育阶段番鸭体成分变化，阐明番鸭不同发育阶段体成分沉积规律。</p> <p>（3）研究白羽番鸭生长期常用饲料原料净能值</p> <p>以中速型地方品种番鸭为研究对象，选取常用能量饲料原料玉米、碎米、小麦、高粱，以及常用蛋白饲料原料豆粕、菜籽粕和葵花粕等，采用呼吸测热法测定和记录番鸭生长情况和呼吸产热数据，获得番鸭饲料原料的净能值数据。</p>		
2、任务分工		
序号	专家/成员姓名	任务分工
1	王文策	探究番鸭不同发育阶段体成分沉积规律
2	曹庆云	研究白羽番鸭生长期常用饲料原料净能值
3	朱勇文	番鸭生长曲线拟合分析
4	杨琳	北京鸭消化道酶谱变化规律分析
5	叶慧	研究不同品种肉鸭消化生理指标差异
3、预期结果		
<p>（1）阐明不同品种肉鸭消化生理指标的差异，建立番鸭不同发育阶段消化道酶谱；</p> <p>（2）阐明番鸭不同发育阶段体成分沉积规律，针对体成分沉积变化为后期番鸭不同阶段饲料配制提供基础数据；</p> <p>（3）通过测定番鸭生长期常用饲料原料净能值数据，为生长期番鸭饲料配方精准配制提供基础数据。</p>		
4、考核指标		
<p>（1）获得不同品种肉鸭消化生理指标对比数据 1 套；</p> <p>（2）获得番鸭不同发育阶段体成分沉积规律 1 套；</p> <p>（3）获得番鸭生长期常用饲料原料净能数据 1 套；</p> <p>（4）技术服务与培训 8-10 次。</p>		

CARS-42-02A：肉鹅遗传资源创新与新品种培育及高效养殖技术研究示范

1、研发任务		
<p>(1) 研究辣椒粕在肉鹅生长发育的适宜使用量</p> <p>以南方特色品种肉鹅为研究对象，采用不同梯度辣椒粕使用量，结合生长性能、血清及肝脏生化指标、肠道发育等指标，获得辣椒粕做为饲料原料在肉鹅生长发育的最适添加量及添加限量。</p> <p>(2) 制定马冈鹅表观代谢能测定技术规范</p> <p>依据前期不同饲料原料在马冈鹅上代谢能数据差异及排空时间等参数，建立马冈鹅表观代谢能测定技术规范。</p> <p>(3) 研究维生素 B₂ 添加水平对肉鹅生长发育的影响</p> <p>以南方特色品种肉鹅马冈鹅为研究对象，采用不同添加梯度，研究维生素 B₂ 添加水平对肉鹅生长性能、器官发育、血清生化指标等的影响。</p> <p>(4) 研究植物提取物缓解霉变饲料对肉鹅发育损伤的作用</p> <p>以南方特色肉鹅为研究对象，研究姜黄素和紫锥菊提取物缓解霉变玉米皮对肉鹅生长发育损伤的作用，通过生长性能、肠道发育、血清生化及代谢等指标，阐明植物提取物缓解霉变饲料造成肉鹅损伤的作用及机制。</p>		
2、任务分工		
序号	专家/成员姓名	任务分工
1	王文策	研究植物提取物缓解霉变饲料对肉鹅发育损伤的作用
2	曹庆云	研究霉变饲料对肉鹅生长性能、肠道及肝脏的损伤作用
3	朱勇文	研究维生素 B ₂ 添加水平对肉鹅生长发育的影响
4	杨琳	辣椒粕作为饲料原料对肉鹅生长发育最适添加量研究
5	叶慧	辣椒粕作为饲料原料对肉鹅添加限量研究
3、预期结果		
<p>(1) 获得辣椒粕做为饲料原料在肉鹅生长发育的最适添加量及添加限量；</p> <p>(2) 阐明不同维生素 B₂ 添加水平对肉鹅生长性能及器官发育等的影响，获得维生素 B₂ 的适宜添加量；</p> <p>(3) 阐明植物提取物缓解霉变饲料造成肉鹅损伤的作用。</p>		
4、考核指标		
<p>(1) 获得辣椒粕在肉鹅生长发育的适宜使用量数据 1 套。</p> <p>(2) 获得维生素 B₂ 添加水平对肉鹅生长发育影响的数据 1 套；</p> <p>(3) 获得植物提取物缓解霉变饲料对肉鹅发育损伤的技术数据 1 套。</p>		

（二）岗位重点任务

CARS-42-03A：水禽饲料原料营养评价方法学研究

1、重要意义		
<p>（1）本产业本领域中存在的问题</p> <p>我国非粮型饲料资源的来源广、种类多、总量大，每年非常规饲料产量高达 40 多亿吨，但可真正被用于畜禽生产的不足 1/4，而限制非常规饲料使用的关键因素在于缺乏准确的饲料营养价值评定体系。</p> <p>（2）重要意义</p> <p>建立规范统一的饲料营养价值评价方法，开发非常规饲料原料，促进水禽产业中标准化养殖技术、饲料高效利用及绿色健康养殖技术的利用和推广。</p>		
2、参与本功能研究室重点任务		
<p>（1）二段式和三段式仿生消化试验的对比评定研究</p> <p>根据前期番鸭空肠、盲肠消化参数，设置不同番鸭盲肠段仿生消化时间，根据玉米、豆粕和麦麸的仿生消化能值以及干物质消化率结果确定适宜的盲肠段仿生消化时长。根据上述确定的消化时长等仿生消化参数，分别开展“胃-小肠”二段式和“胃-小肠-盲肠”三段式仿生消化试验对比评定常规饲料有效能值。</p> <p>（2）建立番鸭饲料营养价值评价技术规程</p> <p>依据前期番鸭饲料原料评价数据，以及番鸭消化道酶谱、酶解能方法及测定数据等，建立番鸭饲料营养价值评价技术规程，为后续番鸭饲料营养价值测定奠定方法学基础。</p>		
参与本研究室重点任务分工		
序号	专家/成员姓名	任务分工
1	王文策	番鸭饲料营养价值评价技术规程制定
2	曹庆云	番鸭饲料营养价值评价技术规程技术参数制定
3	朱勇文	二段式仿生消化试验评价常规饲料原料有效能值
4	杨琳	三段式仿生消化试验评价常规饲料原料有效能值
5	叶慧	盲肠仿生消化酶谱建立及优化
参与本研究室重点任务预期结果		
<p>（1）探索二段式和三段式酶水解能方法对番鸭饲料原料消化参数的影响，进一步优化酶水解能测定方法的准确性；</p> <p>（2）针对番鸭饲料营养价值评价技术建立标准化规程。</p>		
参与本研究室重点任务考核指标		
<p>（1）获得番鸭酶水解能方法的对比评定数据 1 套；</p> <p>（2）建立番鸭饲料营养价值评价技术规程 1 个。</p>		
3、本岗位重点研发任务		
<p>（1）研究不同饲料原料在马冈鹅上的强饲排空时间变化规律</p> <p>采集常用能量饲料玉米、高粱、小麦、麦麸，以及常用蛋白饲料豆饼、菜籽饼、棉粕、米糠等，</p>		

采用排空强饲法对空腹马冈鹅进行强饲，阐明不同类型饲料原料在马冈鹅上的强饲排空时间的变化规律； (2) 制定马冈鹅表观代谢能测定技术规范。		
本岗位重点研发任务分工		
序号	专家/成员姓名	任务分工
1	王文策	饲料原料排空强饲法技术规范制定
2	曹庆云	饲料原料代谢能指标测定
3	朱勇文	马冈鹅蛋白质饲料原料代谢能测定
4	杨琳	马冈鹅能量饲料原料代谢能测定
5	叶慧	常规及非常规原料饲用价值评定
本岗位重点研发任务预期结果		
(1) 阐明不同饲料原料在马冈鹅上的强饲排空时间变化规律，为排空强饲法在南方品种肉鹅上的规范化使用提供基础数据； (2) 制定马冈鹅表观代谢能测定技术规范。		
本岗位重点研发任务考核指标		
(1) 获得不同饲料原料在马冈鹅上的强饲排空时间变化规律数据 1 套； (2) 制定马冈鹅表观代谢能测定技术规范 1 套。		

(三) 服务县域经济发展

CARS-42×-01B：内蒙古自治区省赤峰市宁城县肉鸭产业

1、任务内容
以宁城县肉鸭产业的转型升级为核心、以结构调整为主线、精心谋划，科学布局，实现以肉鸭产业带动县域经济。加强肉鸭育种基地建设，升级商品肉鸭的养殖模式，建设规模化肉鸭养殖小区，年出栏肉鸭实现 10% 的增长。通过打造草原鸭品牌，提高养殖效益，并加大对鸭肉及其他鸭产品的深加工投入，延伸肉鸭产品的产业链，提高肉鸭的附加值。继续加强塞飞亚与全国最大的鸭肉产品熟食连锁企业湖南绝味公司合作，把行业内的新技术、新工艺、新机制等优势资源充分优化配置到塞飞亚肉鸭产业中，使企业又快又好持续稳步发展。采用“公司+农户”的发展模式，以技术、设备、生产加工和销售一站式方式，实现农业产业一体化，致力于发展肉鸭产业带动脱贫致富、乡村振兴，将宁城县打造成为中国肉鸭的育种、养殖和加工的基地。宁城县肉鸭产业的全面发展，带动了宁城县和赢种鸭养殖专业合作社、喀喇沁旗星辰农机种植养殖专业合作社等合作社的发展。农牧民通过合作社组织获得了较高的经济收入。为提高肉鸭养殖效率，示范推广肉鸭高效网上饲养、立体笼养和发酵床饲养技术，为养殖户提供技术支持。开展鸭抗甲肝病毒的育种工作，降低小鸭肝炎对肉鸭产业的危害。
2、工作机制
该项目成立由首席科学家牵头，由赤峰综合试验站依托单位内蒙古塞飞亚农业科技发展股份有限公司组织具体实施。项目组由首席科学家、12 位岗位科学家和 9 位试验站站长及其团队成员参加，建立岗位-试验站合作机制。

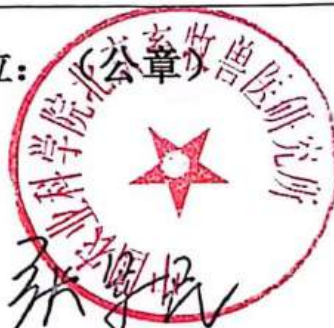
六、签约方

国家水禽产业技术体系首席科学家（签字）：

侯永志

2024年6月20日

国家水禽产业技术研发中心依托单位：



依托单位法定代表人（签字）：

张军民

2024年6月20日

功能研究室主任（签字）：

王军

2024年6月15日

岗位科学家（签字）：

王军

2024年6月15日

岗位依托单位：

（公章）

依托单位法定代表人（签字）：

薛红



2024年6月15日

三、论文、著作等

检索证明

根据委托人提供的论文材料, 委托人华南农业大学动物科学学院 朱勇文 19 篇论文收录情况如下表。

序号	论文名称	发表刊物及发表的年月卷期/页码等	作者排名	论文等级	作者文中单位	收录情况	影响因子	中科院大类分区
1	Notch mediates the glycolytic switch via PI3K/Akt signaling to support embryonic development	CELLULAR & MOLECULAR BIOLOGY LETTERS 出版年: 2023 出版日期: JUN 26 卷期: 28 1 页码: - 文献号: 50 文献类型: Article	共同通讯 (倒数第一)	T2 类	华南农业大学 动物科学学院	SCI	IF2-year=9.2 IF5-year=8.1 (2023)	生物学 1 区 Top 期刊: 否 (2023)
2	Developmental changes in lipid and fatty acid metabolism and the inhibition by in ovo feeding oleic acid in Muscovy duck embryogenesis	ANIMAL NUTRITION 出版年: 2023 出版日期: MAR 卷期: 12 页码: 321-333 文献类型: Article	共同通讯 (倒数第一)	T2 类	华南农业大学 动物科学学院	SCI	IF2-year=6.1 IF5-year=6.6 (2023)	农林科学 1 区 Top 期刊: 是 (2023)
3	Evidence from an Avian Embryo Model that Zinc-Inducible MT4 Expression Protects Mitochondrial Function Against Oxidative Stress	JOURNAL OF NUTRITION 出版年: 2024 出版日期: MAR 卷期: 154 3 页码: 896-907 文献类型: Article	共同通讯 (倒数第一)	B 类	华南农业大学 动物科学学院	SCI	IF2-year=3.8 IF5-year=4.2 (2024)	医学 3 区 Top 期刊: 否 (2025)

4	Effect of Maternal Marginal Zinc Deficiency on Development, Redox Status, and Gene Expression Related to Oxidation and Apoptosis in an Avian Embryo Model	OXIDATIVE MEDICINE AND CELLULAR LONGEVITY 出版年: 2021 出版日期: OCT 19 卷期: 2021 页码: - 文献号: 9013280 文献类型: Article	共同通讯 (倒数第一)	A 类	华南农业大学 动物科学学院	SCI	IF2-year=7.31 IF5-year=8.427 (2021)	生物学 2 区 Top 期刊: 否 (2021)
5	Improving the quality of Napier grass silage with pyroligneous acid: Fermentation, aerobic stability, and microbial communities	FRONTIERS IN MICROBIOLOGY 出版年: 2022 出版日期: NOV 14 卷期: 13 页码: - 文献号: 1034198 文献类型: Article	共同通讯 (倒数第一)	A 类	华南农业大学 动物科学学院	SCI	IF2-year=5.2 IF5-year=6.2 (2022)	生物学 2 区 Top 期刊: 是 (2022)
6	Change of zinc mobilization and gene expression of key zinc transport proteins between the yolk sac membrane and liver of duck embryonic developing	POULTRY SCIENCE 出版年: 2022 出版日期: JAN 卷期: 101 1 页码: - 文献号: 101562 文献类型: Article	通讯	A 类	华南农业大学 动物科学学院	SCI	IF2-year=4.4 IF5-year=4.4 (2022)	农林科学 2 区 Top 期刊: 是 (2022)
7	The developmental pattern related to fatty acid uptake and oxidation in the yolk sac membrane and jejunum during embryogenesis in Muscovy duck	POULTRY SCIENCE 出版年: 2024 出版日期: SEP	通讯	A 类	华南农业大学 动物科学学院	SCI	IF2-year=4.2 IF5-year=4.5 (2024)	农林科学 2 区 Top 期刊: 是 (2025)

		卷期: 103 9 页码: - 文献号: 103929 文献类型: Article						
8	Effects of fermented cottonseed meal inclusions on growth performance, serum biochemical parameters and hepatic lipid metabolism of geese during 28-70 d of age	POULTRY SCIENCE 出版年: 2024 出版日期: JUN 卷期: 103 6 页码: - 文献号: 103702 文献类型: Article	通讯	A 类	华南农业大学 动物科学学院	SCI	IF2-year=4.2 IF5-year=4.5 (2024)	农林科学 2 区 Top 期刊: 是 (2025)
9	Exogenous Linoleic Acid Intervention Alters Hepatic Glucose Metabolism in an Avian Embryo Model	FRONTIERS IN PHYSIOLOGY 出版年: 2022 出版日期: FEB 24 卷期: 13 页码: - 文献号: 844148 文献类型: Article	共同通讯	A 类	华南农业大学 动物科学学院	SCI	IF2-year=4.0 IF5-year=4.7 (2022)	医学 2 区 Top 期刊: 是 (2022)
10	Mineral requirements in ducks: an update	POULTRY SCIENCE 出版年: 2020 出版日期: DEC 卷期: 99 12 页码: 6764-6773 文献类型: Article	共同通讯 (倒数第一)	A 类	华南农业大学 动物科学学院	SCI	IF2-year=3.352 IF5-year=3.679 (2020)	农林科学 2 区 Top 期刊: 是 (2020)

11	Responses of Combined Non-starch Polysaccharide Enzymes and Protease on Growth Performance, Meat Quality, and Nutrient Digestibility of Yellow-Feathered Broilers Fed With Diets With Different Crude Protein Levels	FRONTIERS IN VETERINARY SCIENCE 出版年: 2022 出版日期: JUL 18 卷期: 9 页码: - 文献号: 946204 文献类型: Article	共同通讯 (倒数第一)	A 类	华南农业大学 动物科学学院	SCI	IF2-year=3.2 IF5-year=3.5 (2022)	农林科学 2 区 Top 期刊: 否 (2022)
12	Predicting the metabolizable energy and metabolizability of gross energy of conventional feedstuffs for Muscovy duck using in vitro digestion method	JOURNAL OF ANIMAL SCIENCE 出版年: 2023 出版日期: JAN 3 卷期: 101 页码: - 文献号: skad018 文献类型: Article	通讯	A 类	华南农业大学 动物科学学院	SCI	IF2-year=2.7 IF5-year=3.2 (2023)	农林科学 2 区 Top 期刊: 否 (2023)
13	In vitro evaluation of efficacy of nonstarch polysaccharides enzymes on wheat by simulating the avian digestive tract	JOURNAL OF ANIMAL SCIENCE 出版年: 2023 出版日期: JAN 3 卷期: 101 页码: - 文献类型: Article	共同通讯 (倒数第一)	A 类	华南农业大学 动物科学学院	SCI	IF2-year=2.7 IF5-year=3.2 (2023)	农林科学 2 区 Top 期刊: 否 (2023)
14	Effects of dietary iron sources on growth performance, iron status, Fe-containing enzyme activity and gene expression related to iron	FRONTIERS IN VETERINARY SCIENCE 出版年: 2023 出版日期: MAR 8	共同通讯 (倒数第一)	A 类	华南农业大学 动物科学学院	SCI	IF2-year=2.6 IF5-year=3.1 (2023)	农林科学 2 区 Top 期刊: 是 (2023)

	homeostasis in tissues of weaned pigs	卷期: 10 页码: - 文献号: 1111257 文献类型: Article						
15	Dietary vitamin D ₃ requirement of magang goslings from 1-21 days of age	ANIMAL FEED SCIENCE AND TECHNOLOGY 出版年: 2023 出版日期: OCT 卷期: 304 页码: - 文献号: 115757 文献类型: Article	共同通讯 (倒数第一)	A 类	华南农业大学 动物科学学院	SCI	IF2-year=2.5 IF5-year=2.9 (2023)	农林科学 2 区 Top 期刊: 否 (2023)
16	Effect of Dietary Zinc Level on Egg Production Performance and Eggshell Quality Characteristics in Laying Duck Breeders in Furnished Cage System	BIOLOGICAL TRACE ELEMENT RESEARCH 出版年: 2020 出版日期: AUG 卷期: 196 2 页码: 597-606 文献类型: Article	共同通讯 (倒数第一)	B 类	华南农业大学 动物科学学院	SCI	IF2-year=3.738 IF5-year=3.44 (2020)	生物学 3 区 Top 期刊: 否 (2020)
17	Effect of dietary Moringa stem meal level on growth performance, slaughter performance and serum biochemical parameters in geese	JOURNAL OF ANIMAL PHYSIOLOGY AND ANIMAL NUTRITION 出版年: 2020 出版日期: JAN 卷期: 104 1 页码: 126-	共同通讯	B 类	华南农业大学 动物科学学院	SCI	IF2-year=2.13 IF5-year=2.322 (2020)	农林科学 3 区 Top 期刊: 否 (2020)

		135 文献类型: Article						
18	The pattern of body growth and intestinal development of female Chinese native geese from 1 to 10 weeks of age	JOURNAL OF APPLIED ANIMAL RESEARCH 出版年: 2022 出版日期: DEC 31 卷期: 50 1 页码: 380-385 文献类型: Article	通讯	B 类	华南农业大学 动物科学学院	SCI	IF2-year=1.4 IF5-year=2.0 (2022)	农林科学 3 区 Top 期刊: 否 (2022)
19	肠道菌群及其代谢产物调节动物线粒体功能的研究进展	畜牧兽医学报 出版年: 2023 卷期: 页码: 文献号: 文献类型:	通讯	A 类	华南农业大学	北大核心	无	无

说明: 论文等级和中科院大类分区按《华南农业大学学位论文评价方案(试行)》划分。

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2025-07-17

检索证明

根据委托人提供的论文材料，委托人华南农业大学动物科学学院 朱勇文 3 篇论文收录情况如下表。

序号	论文名称	发表刊物及发表的年月卷期/页码等	作者排名	论文等级	作者文中单位	收录情况	影响因子	中科院大类分区
1	Effect of oral spray with Lactobacillus on growth performance, intestinal development and microflora population of ducklings	ASIAN-AUSTRALASIAN JOURNAL OF ANIMAL SCIENCES 出版年：2020 出版日期：MAR 卷期：33 3 页码：456-464 文献类型：Article	共同通讯 (倒数第一)	B 类	华南农业大学 动物科学学院	SCI	IF2-year=2.509 IF5-year=2.604 (2020)	农林科学 3 区 Top 期刊：否 (2020)
2	硒营养对种禽繁殖性能的影响研究进展	中国畜牧杂志 出版年：2022 卷期： 页码：- 文献号： 文献类型：	通讯	C 类	华南农业大学 动物科学学院	北大核心	无	无
3	家禽支链氨基酸营养需要研究进展	畜牧兽医学报 出版年：2024 卷期： 页码：- 文献号： 文献类型：	通讯	A 类	华南农业大学 动物科学学院	北大核心	无	无

说明：论文等级和中科院大类分区按《华南农业大学学术论文评价方案（试行）》划分。

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RESEARCH LETTER

Open Access



Notch mediates the glycolytic switch via PI3K/Akt signaling to support embryonic development

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[†]Heng Wang and Wenqi Liang contributed equally to this work.

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Abstract

Background: Energy metabolism disorder or insufficient energy supply during incubation will affect the development and survival of avian embryos. Especially, β -oxidation could not provide the continuous necessary energy for avian embryonic development due to the increasing energy demand under hypoxic conditions during the mid-late embryonic stages. The role and mechanism of hypoxic glycolysis replacing β -oxidation as the main source of energy supply for avian embryonic development in the mid-late stages is unclear.

Results: Here, we found that in ovo injection with glycolysis inhibitor or γ -secretase inhibitor both decreased the hepatic glycolysis level and impaired goose embryonic development. Intriguingly, the blockade of Notch signaling is also accompanied by the inhibition of PI3K/Akt signaling in the embryonic primary hepatocytes and embryonic liver. Notably, the decreased glycolysis and impaired embryonic growth induced by the blockade of Notch signaling were restored by activation of PI3K/Akt signaling.

Conclusions: Notch signaling regulates a key glycolytic switch in a PI3K/Akt-dependent manner to supply energy for avian embryonic growth. Our study is the first to demonstrate the role of Notch signaling-induced glycolytic switching in embryonic development, and presents new insight into the energy supply patterns in embryogenesis under hypoxic conditions. In addition, it may also provide a natural hypoxia model for developmental biology studies such as immunology, genetics, virology, cancer, etc.

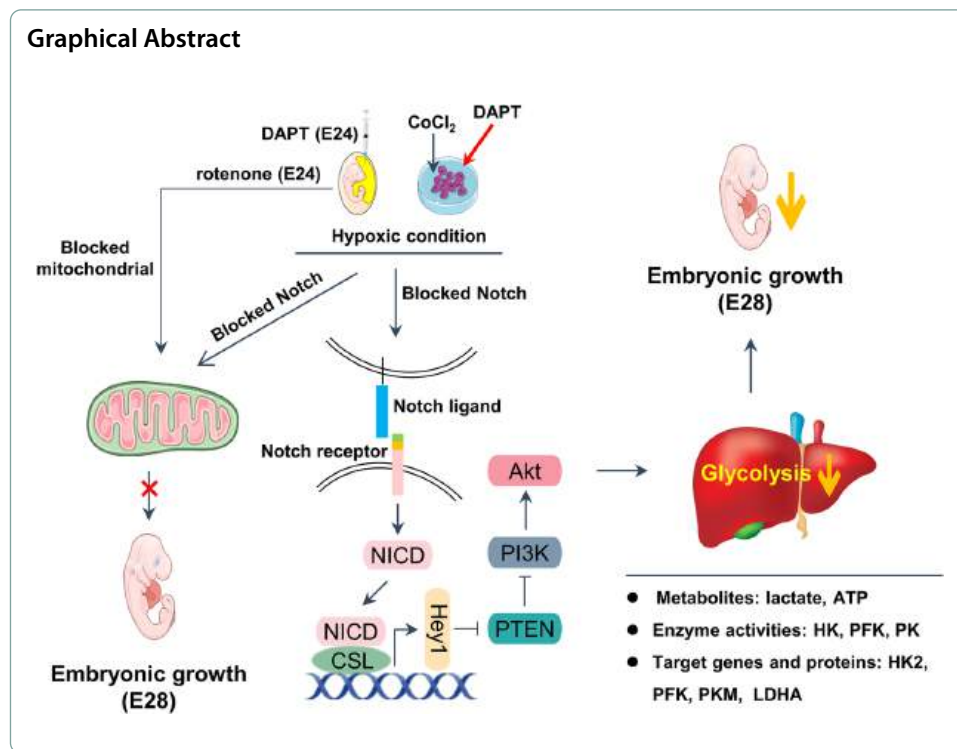
Highlights

- Glycolysis plays a dominant role in the energy supply to support mid-late avian embryonic growth.
- Notch signaling regulates a switch to glycolysis in the liver.
- Notch switched glycolysis in a PI3K/Akt-dependent manner in vivo and in vitro.

Keywords: Notch signaling, Glycolysis, PI3K/Akt, Avian embryonic development



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Introduction

The low hatchability of breeding eggs is an important factor limiting the development of the meat goose industry in China [1]. Unlike the mammalian embryo, the avian embryo develops in an egg that forms a naturally closed chamber [2]. Energy metabolism disorder or insufficient energy supply will affect the development and survival of geese embryos [3]. Dramatic changes in metabolic patterns occur during avian embryogenesis to satisfy different physiological demands of growth [4]. Energy metabolism is vital for maintaining embryonic development, and as the mid-late period coincides with rapid organic development, enhanced oxygen consumption, and increased glycogen storage for pipping and emergence, the energy is heavily utilized [3, 5, 6]. For example, as the embryo develops and O₂ diffusion across the air-cell membrane increases during the early-mid incubation period [7], the substrates that the embryo metabolizes for energy shift from hypoxic glycolysis of carbohydrates (respiratory quotient, RQ ~1.0) to β -oxidation of fatty acids (RQ ~0.7) [8]. In the transition from mid to late incubation, β -oxidation cannot provide all the necessary energy due to the increasing energy demand under hypoxic conditions [7, 9]. We speculate that hypoxic glycolysis might replace β -oxidation and act as the dominant mode of energy supply for avian embryos during the mid-late developmental stages. Thus, studies of hypoxic glycolysis in avian embryogenesis are important for economic developments in poultry breeding, which uses agriculturally significant avian species to provide humans with high-quality animal proteins (e.g., meat and eggs). Compared with other domestic fowl embryos, geese embryos grow fast, have sensitive responses to changes in energy supply, and have longer incubation periods (21 days for chickens versus

28 days for ducks and 30 days for geese). Thus, geese embryos serve as an ideal model to study the role of hypoxic glycolysis in avian embryonic development.

The Notch signaling pathway is a conserved signaling pathway that mediates juxtacrine cell–cell communication [10] and is involved in many key cellular decisions and other core processes during embryogenesis [11]. For example, Notch signaling is required for the generation of hematopoietic stem cells in mice embryos [12] and the induction of central nervous system development in embryos of *Drosophila melanogaster* [13]. Although two Notch proteins of Notch1 and Notch2 of geese are annotated in the National Center for Biotechnology Information (NCBI), the role and underlying mechanisms of Notch signaling in chicken embryogenesis, particularly in the switch to hypoxic glycolysis to provide energy for avian embryonic development are poorly understood. In the present study, the role of glycolysis in embryonic development and the potential mechanisms of Notch signaling in regulating hypoxic glycolysis were evaluated in avian embryos. The results are the first to demonstrate that Notch signaling, in a PI3K/Akt-dependent manner, induces a switch to hypoxic glycolysis, which serves as the main pathway of energy supply during the mid and late stages of avian embryonic development.

Materials and methods

The animal care and use protocol was approved by the Institutional Animal Care and Use Committee of South China Agricultural University (SCAU-10564), and the study was performed following the Regulations for the Administration of Affairs Concerning Experimental Animals.

Incubation procedure

Fertilized geese eggs with similar egg weights were obtained from the same age of breeding female geese fed the same diet to ensure similar nutrient deposition in the yolk for embryonic development. Eggs were incubated in a microcomputer automatic incubator (Keyu, Shandong, China) at a relative humidity of $65 \pm 5\%$ and a temperature of 37.5 ± 0.5 °C. During incubation, eggs were turned at an angle of 60° every 1.5 h until embryonic day (E)28, and then all eggs were transferred to the hatcher with no further egg turning until hatching on E30. Notably, cracked eggs and dead embryos were removed following candling on E8 and E18, and all hatched eggs were wetted and cooled with 38 °C water twice per day from E9 to E28 to eliminate the adverse effects of increased heat production on embryonic development, as described previously [14]. Other incubation management protocols were carried out according to normal incubation procedures. Ten egg embryos, representing the weight distribution of the eggs at the set, were selected on E16, E19, E22, E25, and E28. Embryos were killed by cervical dislocation, and then the egg embryos, yolk sac, and liver were weighed to calculate the average values per replicate from each incubation time. Liver samples were collected for the measurement of indices related to energy metabolism to study the developmental changes in glycolysis and Notch signaling during the mid–late incubation stages.

In ovo injection experiment

The in ovo injection procedure was carried out as described previously [15]. Before injection, the border of the air sac or the yolk sac was identified under candlelight. The fertilized eggs were disinfected with 75% ethanol in the insertion region before injection. A sterile disposable 25.0 × 0.6 mm needle was attached to a 1.0 mL syringe, which was replaced after each egg injection. The holes were sealed with medical adhesive tape (1.0 × 1.0 cm²) immediately after injection. Eggs from the noninjection group were placed outside the incubator environment for 5 min for standardization. Finally, the eggs from each replicate of each treatment were placed on the same egg tray and incubated at 37.5 ± 0.5 °C with a relative humidity of 65 ± 5%. The in ovo injection experiment was carried out as mentioned above at the developmental window of glycolysis on E24. The effects of 2-deoxy-D-glucose (2-DG, glycolysis inhibitor), rotenone (mitochondrial electron transport chain complex I inhibitor), *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-S-phenyl glycine t-butyl ester (DAPT, γ-secretase inhibitor), LY294002 (an inhibitor available for the PI3K family), and 740 Y-P (PI3K family agonist) were tested by in ovo injection to induce avian embryonic models.

Isolation, culture, identification, and hypoxia induction of primary hepatocytes

Hepatocytes were isolated from geese embryos on E24 according to the procedure reported by Osman et al. [16]. In brief, liver tissue was digested with 3 to 5 volumes of 0.1% type IV collagenase (C4-28, Merck, USA) at 37 °C for 20 min. Next, the digestion process was terminated by adding an equal volume (v) of complete medium containing high-glucose Dulbecco's modified Eagle medium (DMEM, Sigma, D0822), 100 IU/mL penicillin, 100 µg/mL streptomycin (Gibco 15140122), 2 mM glutamine (Sigma, G7513), 10% fetal bovine serum (Gibco, 10099141), and 0.02 mL/L EGF (Sigma, E9644) to the mixture. After subsequent centrifugation at 700×*g* for 8 min, the cells were suspended in a complete medium and seeded in cell culture flasks. Hepatocytes were identified by immunofluorescence as described previously [17, 18]. Cell morphology was observed under an inverted microscope (DMIL LED, Leica, Germany). A CCK-8 kit (CA1210, Solarbio Co., Ltd., Beijing, China) was used to determine the viability of hepatocytes. Cell proliferation was measured by a 5-ethynyl-2'-deoxyuridine (EdU) assay using a BeyoClick™ EdU-488 Cell Proliferation Kit (C0071S, Beyotime Biotechnology Co., Ltd., Shanghai, China). According to the description in a previous study, CoCl₂ was selected as a hypoxia mimetic agent to establish the hypoxic environment and metabolic adaptation of primary hepatocytes in vitro [19]. Primary hepatocytes were seeded in six-well plates for 24 h and were then exposed to 0, 100, 200, 300, 400, or 500 µM CoCl₂ for 24 h in a cell incubator with 5% CO₂ at 37 °C to determine the optimum concentration for simulating the hypoxic environment. Each experiment was repeated three times.

Targeted metabolomic analysis

Targeted energy metabolomic profiling of the liver was performed by Applied Protein Technology Co., Ltd. (Shanghai, China) using a liquid chromatography tandem mass spectrometry (LC–MS/MS) system. In brief, 60 mg of the sample was homogenized with 500 µL of a precooled methanol/acetonitrile/H₂O (2:2:1, v/v/v) solution and vortexed for 60 s. The mixture was then sonicated on ice for 30 min and left at –20 °C for 1 h to

precipitate proteins. Next, the mixture was centrifuged at $14,000\times g$ for 15 min at 4 °C, and the supernatant was dried using a lyophilizer. The dried protein extracts were then dissolved in 100 μ L of acetonitrile/ H_2O (1:1, v/v) solution and centrifuged at $14,000\times g$ for 15 min at 4 °C. The analysis was performed with Ultra High-Performance Liquid Chromatography Systems (1290 Infinity LC, Agilent Technologies) and a QTRAP Mass Spectrometer (AB SCIEX 5500). In brief, mobile phases A and B were 10 mM ammonium acetate solution and acetonitrile, respectively. The sample was placed in an autosampler at 4 °C with the column temperature at 45 °C, a flow rate of 0.3 mL/min, and an injection volume of 2 μ L. Gradient of mobile phase B: 90–40% B at 0–18 min, 40–90% B at 18–18.1 min, and 90–90% B at 18.1–23 min. In addition, the MS system operated in negative ion mode at the following conditions: ion spray voltage floating, –4500 V; source temperature, 450 °C; ion source gas 1, 45; ion source gas 2, 45; curtain gas, 30. Analyses were determined by electrospray ionization using multiple reaction monitoring. Peak chromatographic area and retention time were analyzed with Multiquant software.

Biochemical indices related to glycolysis

Glucose and lactate levels were determined with a glucose kit (A154-1-1) and a lactate kit (A019-2-1) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), respectively. The ATP levels in the liver and hepatocytes were determined using an ATP detection kit according to the manufacturer's instructions (S0026, Beyotime Biotechnology Co., Ltd., Shanghai, China). The activities of the rate-limiting enzymes hexokinase (HK) (A077-3-1), phosphofructokinase (PFK) (A129-1-1), and pyruvate kinase (PK) (A076-1-1) were determined by kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. The oxygen consumption rate was measured using an Oxygen Consumption Rate Assay Kit (600800, Cayman Chemical, Ann Arbor, MI, USA).

Quantitative RT–PCR

Total RNA was isolated from liquid nitrogen-frozen liver or hepatocytes using the RNA Purification Kit (B0004DP, EZBioscience Co., Ltd., Beijing, China). The quantity and purity of RNA were assessed using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and agarose gel electrophoresis was used to test the quality of RNA. Total RNA was reverse transcribed into cDNA by the Color Reverse Transcription Kit (A0010CGQ, EZBioscience Co., Ltd., Beijing, China), and a 2 \times Color SYBR Green qPCR Master Mix kit (A0012-R2, EZBioscience Co., Ltd., Beijing, China) was used for subsequent RT–PCR amplification on a Bio-Rad C1000 Touch Thermal Cycler (Bio-Rad, Pleasanton CA, USA). The primers were designed and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China), and the sequences were as follows:

HIF1 α : 5'ATGCCGAAGAAGCAAGGAGT3' and 5'TCCATTTTGGCTTCTGTCTCC3';

Notch1: 5'ATCTCATCAACTGCCACGCA3' and 5'AGCGGGGTCTCCTCCTTATT3';

Notch2: 5'CCAGGGTTCACGGGAGA3' and 5'TTGGCACAAGGTTGAGATG3';

Dll1: 5'CTTTTGGCGACAAACCTGGGG3' and 5'CTGGTTGCAGAAAAGGCC AC3';
Dll4: 5'CTGGTGGCTTTGCTCATA3' and 5'GATTCTTGGGTTTGTAGTTTG3';
Jag1: 5'AAACACCCAAACTGGAC3' and 5'GCTCTTGAGTGCCTTTAT3';
Jag2: 5'TGGAAGGTTGGATGGGAGAA3' and 5'ACAGCCTGGGTAGCGGAC AC3';
Hey1: 5'GCGGGAGGGAAAGGTTAT3' and 5'AGGCGTAGTTATTGAGAT GAGA3';
HK2: 5'GGCAGTCGCTTTCTATCTGG3' and 5'GAAGATGATCAGCGGGAT GT3';
PKF: 5'TCTACAACCTCTACTCCTCC3' and 5'CTTCTTCCTCAGTCCGATCA3';
PKM: 5'CTACAGACCTGTGGCTATTG3' and 5'GAGATTCTTGTAGTCCAG CC3';
NDUFA5: 5'CACTGGGCTCGTAGGATTGG3' and 5'TGCACCAAATTAAGC CGCTG3';
LOC106045434: 5'GGACTGGAGTAACTCAATGG3' and 5'GCTATACTTCAG AGGTCCTG3';
LOC106044242: 5'CAACAAGCCAGATATCGACG3' and 5'GGCCTAAGTTCC TGGATAAC3';
Glut1: 5'CCAAGAGTGTCTCAAGAAG3' and 5'GGTGGAGTAGTAGAAAAC CG3';
GAPDH: 5'TCTGTCTGTGGACCTGACCTGC3' and 5'GCCAGCACCCGCATC AAA3';
LDHA: 5'CCTTTCTGTGGCAGATCTAG3' and 5'GTAGTTCCTTCTGGATTCC C3';
PTEN: 5'GAAGACCATAACCCACCAC3' and 5'GGTCCTTACTTCCCCATA GA3';
PI3K: 5'ACCCAAGCGAGGATGAGG3' and 5'TGTTGCCCGTGTGTAATG3';
Akt: 5'TGCTGGATAAAGATGGAC3' and 5'CTGGTTGTAGAAAGGGAG3';
β-actin: 5'CCATTGGCAATGAGAGGTTTC3' and 5'TGGATACCGCAGGACTCC ATA3';

Western blot analysis

Total protein from the liver or hepatocytes was extracted with radioimmunoprecipitation assay (RIPA) lysis buffer containing 1 mM phenylmethanesulfonyl fluoride (ST506, Beyotime Biotechnology, Shanghai, China). The protein concentration was measured with a bicinchoninic acid (BCA) kit (A045-4-2, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The lysates were heated at 100 °C for 10 min and then stored at −20 °C. The proteins in the lysates were separated by electrophoresis on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and were then transferred onto polyvinylidene difluoride (PVDF) membranes. Next, the membranes were blocked in 5% skim milk for 2 h before being washed three times with tris-buffered saline and polysorbate 20 (TBST) for 10 min each. The primary antibodies used in the present study were mouse anti-β-actin (66009-1-Ig, Proteintech, Wuhan, China),

rabbit anti-HIF-1 α (WL01607, Wanleibio, Shenyang, China), rabbit anti-Hey1 (19929-1-AP, Proteintech, Wuhan, China), rabbit anti-NICD (WL03097a, Wanleibio, Shenyang, China), mouse anti-GAPDH (HC301-01, TransGen Biotech, Beijing, China), rabbit anti-Glut1 (WL03141, Wanleibio, Shenyang, China), rabbit anti-HK2 (WL02454, Wanleibio, Shenyang, China), rabbit anti-PFK (P17858, Cusabio, Wuhan, China), rabbit anti-PKM (D120008, Sangon Biotech, Shanghai, China), rabbit anti-LDHA (D164055, Sangon Biotech, Shanghai, China), rabbit anti-PFKFB2 (D161387, Sangon Biotech, Shanghai, China), rabbit anti-PI3K (WL03380, Wanleibio, Shenyang, China), rabbit anti-Akt (WL0003b, Wanleibio, Shenyang, China), and p-Akt (4060S, Cell Signaling Technology, Massachusetts, USA). The secondary antibodies used in the present study were Horseradish Peroxidase (HRP) goat anti-mouse IgG (SA00001-1, Proteintech, Wuhan, China) and HRP goat anti-rabbit IgG (SA00001-2, Proteintech, Wuhan, China). The PVDF membranes were incubated in enhanced chemiluminescence (ECL) substrate for 2 min and were then visualized by chemiluminescence (5200, Bio-Tanon, Shanghai, China).

Statistical analysis

All data were analyzed using GraphPad Prism version 8.3.0 (GraphPad Software, USA), and a one-way ANOVA followed by Tukey's multiple comparison test was used to compare differences between groups. All data are presented as the mean \pm SEM. The level of significance was set at $P < 0.05$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$.

Results

Glycolysis is the main mode of energy supply during the mid-late embryonic stages

The developmental pattern and correlation between embryonic growth and hepatic glycolysis were explored during the mid-late stages of goose embryogenesis (Fig. 1A). The relative body weight (RBW) and relative liver weight (RLW) increased linearly from embryonic day 16 (E16) to the day of hatching (DOH) (Fig. 1B). Hepatic glycolysis increased during the mid-late embryonic stages, based on the linear increase in the hepatic lactate content from E16 to DOH, the quadratic increase in the ATP content and the activity of HK, which peaked at E25 (Fig. 1C). Correlation analysis revealed a strong positive correlation between embryonic development and glycolysis (Fig. 1D), suggesting that E25 might be a critical window for goose embryonic development. Next, we performed targeted energy metabolomic profiling of the liver at E22, E25, and E28 (Fig. 1E and Additional file 1: Fig. S1A), which revealed that glycolysis was enhanced, as characterized by the increases in glucose 6-phosphate (G-6-P), fructose 6-phosphate (F-6-P), 3-phospho-D-glycerate (3-PG), dihydroxyacetone phosphate (DHAP), pyruvate, and lactate; however, the TCA cycle was downregulated, as characterized by the decreases in cis-aconitate, isocitrate, α -ketoglutarate, fumarate, and malic acid (Fig. 1E).

In ovo injection of 2-DG or rotenone on E24 was used to verify the dominant role of glycolysis in the energy supply during the mid-late goose embryonic stages. The dose effect of in ovo 2-DG injection showed that glycolysis was inhibited on E28 in the groups treated with 20, 40, and 80 mg/kg 2-DG, based on the decreased hepatic lactate and ATP contents (Fig. 1F), along with the decreased mRNA expression of *HK2*, *PFK*, and *PKM* (Fig. 1G). Interestingly, inhibition of glycolysis by in ovo injection of 40 or 80 mg/kg 2-DG impaired embryonic development on E28, as characterized by the

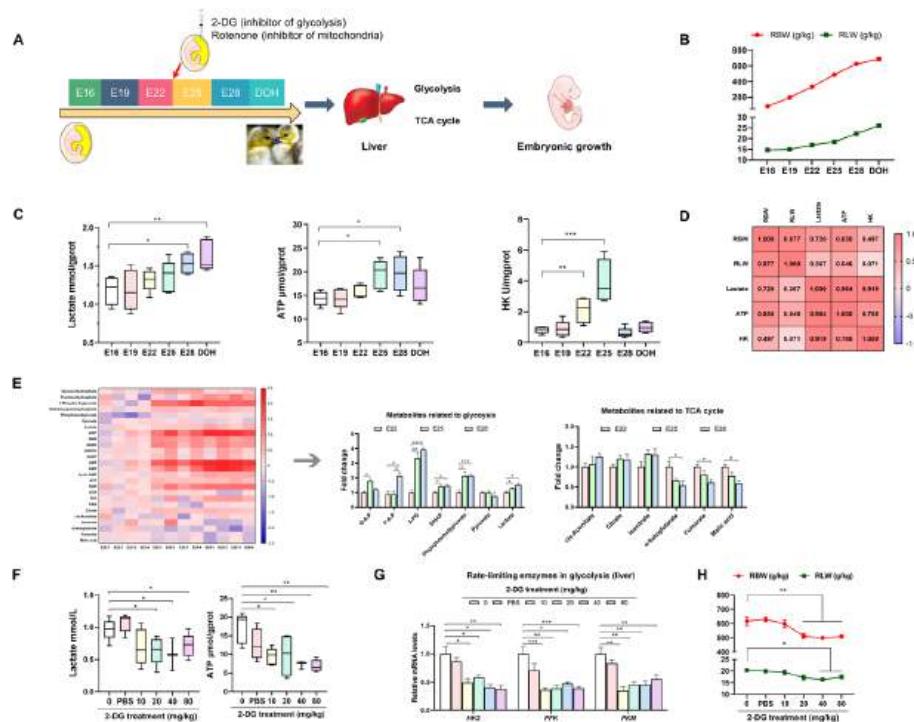


Fig. 1 Glycolysis acted as the main energy supply mode during the mid-late embryonic stages. **A** Schematic depiction of cross-talk between glycolysis and avian embryonic growth. **B** Developmental changes of the geese embryos from E16 to DOH ($n = 10$). **C** Developmental changes in hepatic lactate, ATP content, and activity of HK from E16 to DOH ($n = 6$). **D** Correlation between hepatic glycolysis and embryonic development. **E** Targeted metabolomic profiling of the livers in geese embryos from E22, E25, and E28 ($n = 4$). **F, G** Dose effects of in ovo 2-DG injection in the yolk sac on lactate and ATP levels (**F**) and relative mRNA expressions of HK2, PFK, and PKM (**G**) in the liver of goose embryo ($n = 6$). **H** Developmental changes of the goose embryos after in ovo 2-DG injection in the yolk sac ($n = 10$)

lower RBW and RLW (Fig. 1H). Although there were significant decreases in the activity of mitochondrial electron transport chain complex I (Additional file 1: Fig. S1B), ATP levels (Additional file 1: Fig. S1C), and the mRNA expression of complexes I (*NDUFA5*), III (*LOC106045434*), and IV (*LOC106044242*) (Additional file 1: Fig. S1D), no adverse effects were observed in the growth (Additional file 1: Fig. S1E) and hepatic lactate levels (Additional file 1: Fig. S1C) of embryos subjected to the inhibition of mitochondrial electron transport chain function by in ovo injection of 8, 16, 32, or 64 mg/kg rotenone. In summary, glycolysis acted as the dominant mode of energy supply for the goose embryo during the mid-late developmental stages.

Notch signaling regulates a switch to glycolysis during the mid-late embryonic stages

During embryogenesis, Notch signaling pathway activity was enhanced in a quadratic manner in the embryonic liver from E22 to E28. We observed the greatest mRNA expression (*Notch1*, *Dll1*, *Jag2*, *Hey1*, *Hes2*, and *Hey2*) and protein abundance [hypoxia-inducible factor-1 α (HIF-1 α), Notch intracellular domain (NICD), and Hey1] on E25 (Fig. 2A). Notch signaling and glycolysis were strongly positively correlated (Fig. 2B), suggesting that Notch signaling could play an important role in the regulation of

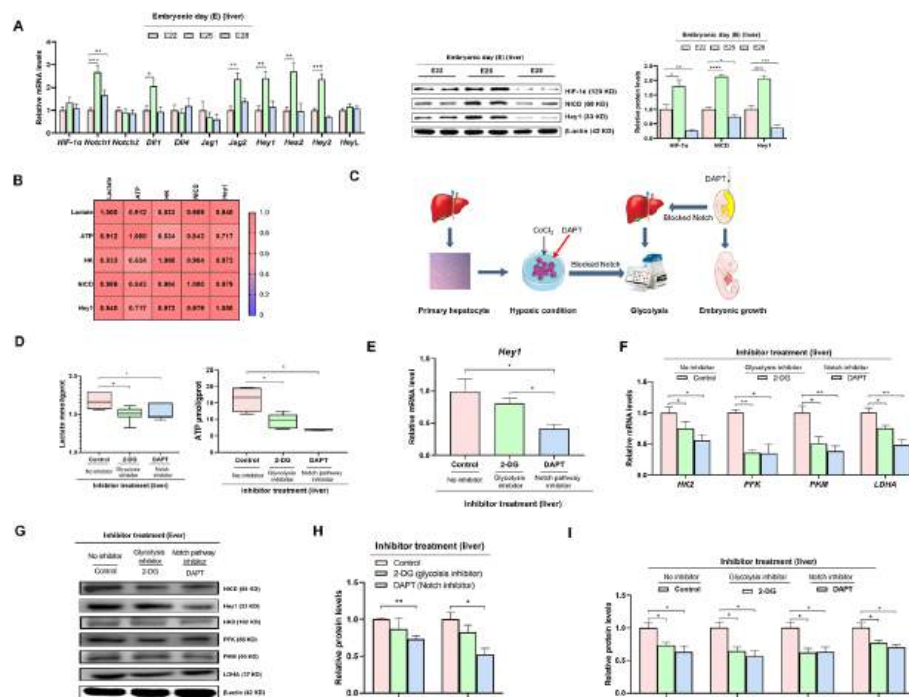


Fig. 2 Notch signaling induced the glycolytic switch as the dominant energy supply mode during the mid-late embryonic stages. **A** Developmental changes of Notch signaling in the liver of geese embryos from E22, E25, and E28 ($n = 6$). **B** Correlation between hepatic glycolysis and Notch signaling. **C** Schematic depiction of Notch signaling regulating glycolysis in vivo and in vitro. **D** Effect of in ovo injection with 20 mg/kg 2-DG or 10 nM DAPT on lactate and ATP content in the liver ($n = 6$). **E, F** Effect of in ovo injection with 20 mg/kg 2-DG or 10 nM DAPT on mRNA expression of *Hey1* (**E**), *HK2*, *PFK*, *PKM*, and *LDHA* (**F**) in the liver ($n = 6$). **G–I** Protein abundances of *Hey1* and *NICD* (**G, H**) as well as *HK2*, *PFK*, *PKM*, and *LDHA* (**G, I**) in the liver of the embryos in ovo injected with 20 mg/kg 2-DG or 10 nM DAPT in the yolk sac ($n = 6$)

glycolysis during the mid–late embryonic stages. Based on the occurrence of a critical switch to Notch signaling during E22–E28, we performed in ovo injection of 10 nM DAPT (an inhibitor of γ -secretase) [20] on E24 (Fig. 2C) and showed inhibition of Notch signaling, as characterized by the decreased hepatic mRNA expression of *Hey1* and protein abundances of *NICD* and *Hey1* (Fig. 2E, G, and H). Notably, and consistent with the results of in ovo 2-DG injection, in ovo DAPT injection on E24 decreased glycolysis levels on E28, as characterized by decreased hepatic lactate and ATP contents (Fig. 2D) and the decreased mRNA expression and protein abundances of *HK2*, *PFK*, *PKM*, and *LDHA* (Fig. 2F, G, and I). Impaired embryonic development but unaffected organ and intestine development was observed based on a decrease in RBW and RLW following 2-DG or DAPT injection (Additional file 1: Fig. S2A, B). No effect was found on mitochondrial function, based on the activity of mitochondrial electron transport chain complex I and the mRNA expression of complexes I (*NDUFA5*), III (*LOC106045434*), and IV (*LOC106044242*) (Additional file 1: Fig. S2C, D). However, inhibition of glycolysis by treatment with 2-DG injection did not affect Notch signaling in the embryonic liver (Fig. 2E, G, and H), suggesting that hepatic glycolytic ability could be switched by Notch signaling.

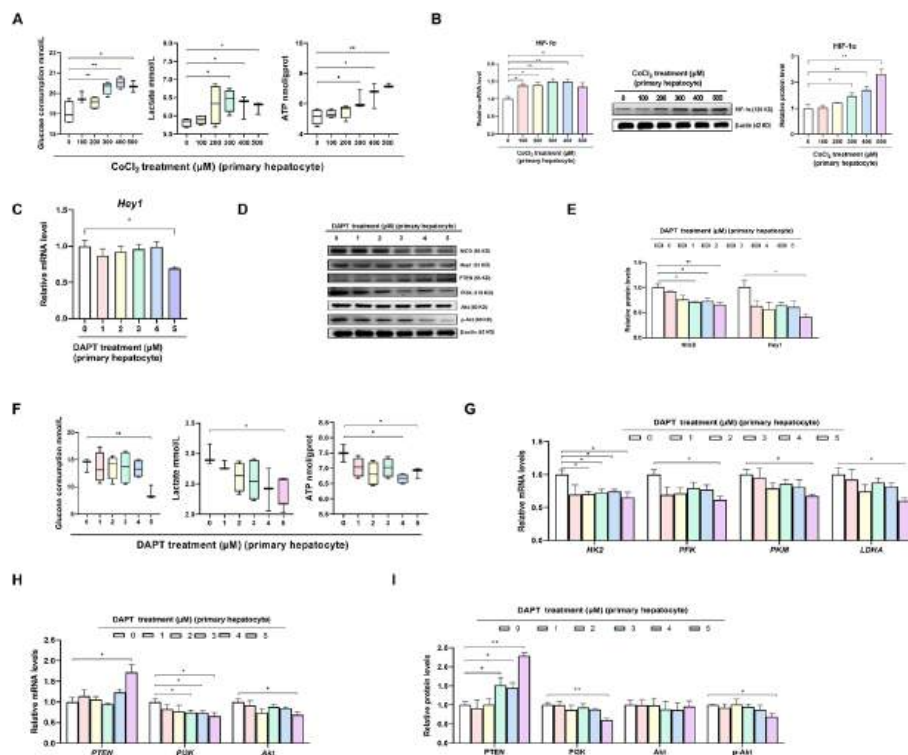


Fig. 3 Blocking of Notch signaling reduces the hypoxic glycolysis level and the PI3K/Akt signaling in primary hepatocytes. **A, B** Dose effect of CoCl_2 on glucose consumption, lactate production, and ATP levels (**A**), and the relative mRNA expression and protein abundance of HIF-1 α (**B**) in goose embryonic primary hepatocytes ($n=6$). **C–I** Dose effect of DAPT on Notch signaling (**C–F**), glucose consumption, lactate production, ATP levels (**F**), relative mRNA expressions of HK2, PFK, PKM, and LDHA (**G**), and PI3K/Akt signaling (**D, H, and I**) in goose embryonic primary hepatocytes ($n=6$)

Blocking Notch signaling suppresses glycolysis and PI3K/Akt signaling in primary hepatocytes

Primary hepatocytes were isolated from the liver tissue of geese embryos on E24 according to the procedure reported by Osman et al. [16]. Hepatocytes were identified (Additional file 1: Fig. S3A), and cell morphology (Additional file 1: Fig. S3B) and viability (Additional file 1: Fig. S3C) were evaluated. CoCl_2 was used to simulate a physiological hypoxic incubation environment for the avian embryo. The dose effect of CoCl_2 (300, 400, or 500 μM) in primary hepatocytes demonstrated that the HIF-1 α mRNA and protein abundance (Fig. 3B), glucose consumption, lactate production, and ATP content (Fig. 3A) were increased. However, cell conjunction (Additional file 1: Fig. S4A), cell proliferation efficiency (Additional file 1: Fig. S4B), and cell viability (Additional file 1: Fig. S4C) were decreased in cells incubated with 400 or 500 μM CoCl_2 . Therefore, 300 μM CoCl_2 (Additional file 1: Fig. S4D) was selected to induce a hypoxic culture condition for primary hepatocytes in our future study.

Under hypoxic conditions, cell conjunction (Additional file 1: Fig. S4E), cell proliferation efficiency (Additional file 1: Fig. S4F), and cell viability (Additional file 1: Fig. S4G) were decreased in cells incubated with 5 μM DAPT. The dose effect of DAPT in primary hepatocytes showed that Notch signaling was inhibited by incubation with

5 μ M DAPT, as characterized by the decreased *Hey1* mRNA expression (Fig. 3C) and NICD and Hey1 protein abundances (Fig. 3D, E), along with reduced glucose consumption, lactate production, ATP content (Fig. 3F), and mRNA expression of *HK2*, *PFK*, *PKM*, and *LDHA* (Fig. 3G). No DAPT effect was found on the mRNA expression of *NUF5A5*, *LOC106045434*, or *LOC1060044242* in hepatocytes (Additional file 1: Fig. S4H). These findings confirmed that glycolysis could be mediated by Notch signaling in vitro. Intriguingly, the blockade of Notch signaling also inhibited PI3K/Akt signaling, as evidenced by the decreases in the mRNA expression of *PTEN*, *PI3K*, and *Akt* and the protein abundances of PTEN, PI3K, and p-Akt (Fig. 3D, H, and I), implying that Notch signaling could mediate glycolysis in a PI3K/Akt-dependent manner.

Notch signaling regulates glycolysis in a PI3K/Akt-dependent manner in primary hepatocytes

To verify the definitive mechanism by which Notch signaling regulates glycolysis under hypoxic conditions in vitro, hepatocytes were treated with 5 μ M DAPT, 20 μ M LY294002 (an inhibitor available for the PI3K family) [21], or 20 μ g/mL 740 Y-P (an agonist of PI3K) [22], according to previous studies (Fig. 4A). Notch signaling was inhibited

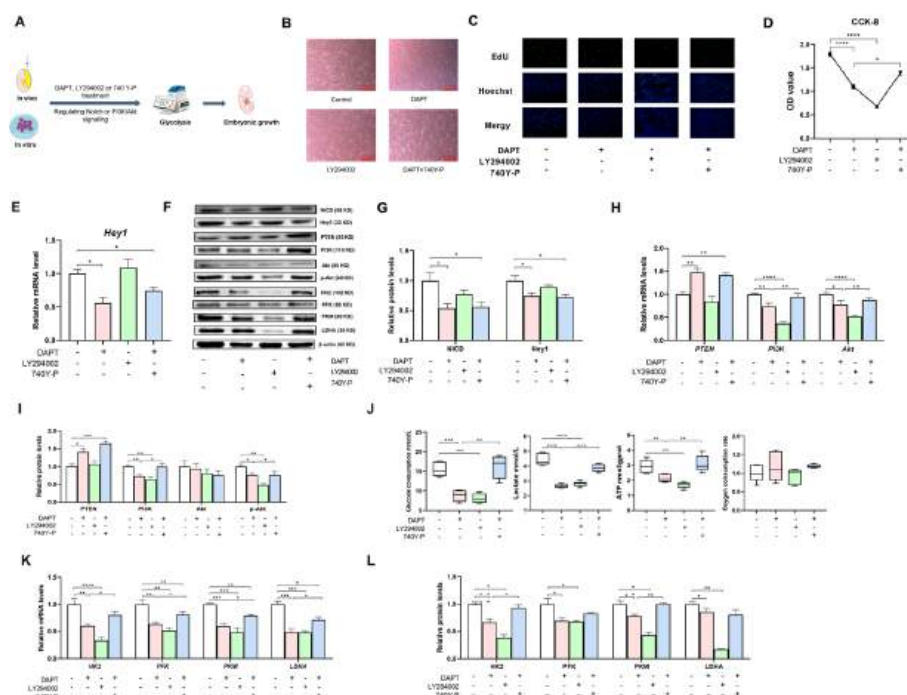


Fig. 4 Notch signaling mediated the glycolytic switch via PI3K/Akt signaling in goose embryonic primary hepatocytes. **A** Schematic representation of the mechanism by which Notch signaling mediates the glycolytic switch to support avian embryonic growth. **B–D** Changes in cell morphology (**B**), cell viability (**C**), and cell proliferation (**D**) after being treated with 5 μ M DAPT, 20 μ M LY294002, or 20 μ g/mL 740Y-P ($n=6$). **E–G** Effect of 5 μ M DAPT, 20 μ M LY294002, or 20 μ g/mL 740Y-P on Notch signaling in the goose embryonic primary hepatocytes ($n=6$). **F, H, and I** Relative mRNA expressions of *PTEN*, *PI3K*, and *Akt* (**H**) and relative protein abundances of *PTEN*, *PI3K*, *Akt*, and p-Akt (**F, I**) in goose embryonic primary hepatocytes treated with 5 μ M DAPT, 20 μ M LY294002, or 20 μ g/mL 740Y-P ($n=6$). **F and J–L** Effect of 5 μ M DAPT, 20 μ M LY294002, or 20 μ g/mL 740Y-P on glucose consumption, lactate production, ATP levels, oxygen consumption rate (**J**), and the relative mRNA expressions and protein abundances of *HK2*, *PFK*, *PKM*, and *LDHA* (**F, K and L**) in goose primary hepatocytes ($n=6$)

by treatment of primary hepatocytes with 5 μ M DAPT (Fig. 4E, F, G), whereas PI3K/Akt signaling was inhibited by treatment with 20 μ M LY294002 and activated by treatment with 20 μ g/mL 740 Y-P, as characterized by the increases and decreases in the mRNA and protein levels of PI3K, Akt, and p-Akt (Fig. 4F, H, I). Blocking PI3K/Akt signaling with LY294002 increased the shrinkage and exacerbated the irregular shape of hepatocytes and decreased the cell proliferation efficiency and cell viability (Fig. 4B–D). Notably, inhibition of PI3K/Akt signaling was also accompanied by a decrease in glycolysis, as evidenced by the decreases in glucose consumption, lactate production, and ATP content (Fig. 4J), along with the mRNA and protein levels of HK2, PFK, PKM, and LDHA (Fig. 4F, K, and L). In contrast, the oxygen consumption rate was unaffected, verifying that primary hepatocytes have a high dependence on glycolysis but not on oxidative phosphorylation, suggesting an important role of PI3K/Akt signaling in regulating glycolysis in primary hepatocytes. Intriguingly, activation of PI3K/AKT by 740 Y-P abrogated the DAPT-induced decrease in glycolysis, confirming that glycolysis was mediated by Notch signaling in a PI3K/Akt-dependent manner *in vitro* under hypoxic conditions. Moreover, the inhibitory or activating effects of DAPT, LY294002, and 740 Y-P on Notch and PI3K/Akt signaling were observed under both normal and hypoxic conditions (Additional file 1: Figs. S4I–K and S5A–C), while no effect was found on glycolysis under normal conditions (Additional file 1: Fig. S5A, D). It was suggested that Notch signaling regulates glycolysis in a PI3K/Akt-dependent manner in primary hepatocytes depending on the hypoxic condition.

Notch signaling mediates glycolysis via PI3K/Akt to improve goose embryonic development

To verify the effects of Notch signaling-mediated hepatic glycolysis on embryonic development *in vivo*, geese embryos were injected *in ovo* with 200 μ L of 10 nM DAPT [20], 60 μ M LY294002 [23], or 50 μ g/mL 740 Y-P [24] on E24 (Fig. 4A) to inhibit Notch signaling (Fig. 5A–C) and PI3K/Akt signaling, and activate PI3K/Akt signaling (Fig. 5B, D, and E), respectively. Blockade of PI3K/Akt signaling by *in ovo* LY294002 injection in the yolk sac decreased hepatic glycolysis, as characterized by the decreases in the lactate and ATP contents; the activities of HK, PFK, and PKM (Fig. 5F); and the mRNA and protein levels of HK2, PFK, PKM, and LDHA (Fig. 5B, G, and H). Additionally, LY294002 treatment impaired embryonic growth on E28, as characterized by the lower RBW and RLW (Additional file 1: Fig. S6A), confirming that PI3K/Akt signaling plays an important role in glycolysis and improves goose embryonic development. Furthermore, the adverse effects on glycolysis and embryo development mediated by blockade of Notch signaling were reversed by activation of PI3K/Akt signaling (Fig. 5B, G, and H, Additional file 1: Fig. S6A, B), confirming that Notch signaling mediates glycolysis via PI3K/Akt signaling to improve goose embryonic development *in vivo*.

Discussion

Cellular metabolism is switched from oxidative phosphorylation to anaerobic glycolysis under hypoxic conditions [25]. Due to the limited oxygen availability during avian embryogenesis, as observed in a naturally closed chamber [26], it was speculated that glycolysis could be the main mode of energy supply supporting the formation and

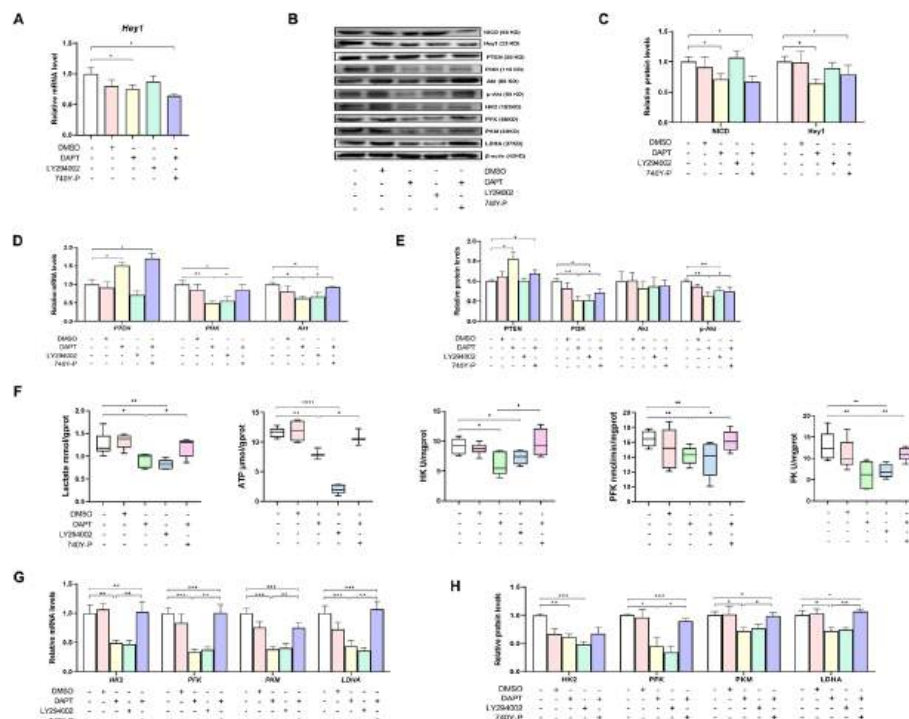


Fig. 5 Notch signaling mediated glycolysis switch via PI3K/Akt signaling to support goose embryonic development. **A–E** Effect of in ovo 10 nM DAPT, 60 μ M LY294002, or 50 μ g/mL 740Y-P injection in the yolk sac on Notch signaling (**A–C**) and PI3K/Akt signaling (**B, D, and E**) in the liver of geese embryos ($n=6$). **B and F–H** Lactate content, ATP levels, activities of HK, PFK, and PK (**F**), and the relative mRNA expressions (**G**) and protein abundances (**B, H**) of HK2, PFK, PKM, and LDHA in the liver of embryos in ovo injected with 10 nM DAPT, 60 μ M LY294002, or 50 μ g/mL 740Y-P in the yolk sac ($n=6$)

growth of the avian embryo. However, the role and underlying mechanism of hepatic glycolysis in avian embryonic development remains unclear. We found that hepatic glycolysis was enhanced during E22–E28 and that inhibition of glycolysis on E24 was followed by a blockade of embryonic growth at later incubation periods. This might be because the induction of HIF-1 α , subjected to the aggravated natural hypoxic environment during the incubation process, could be recruited to the Notch-responsive promoter to activate Notch signaling by the interaction between HIF-1 and NICD [27], ultimately leading to the elevated hepatic glycolysis level. However, no differences in hepatic pyruvate levels between E22 and E28 were observed, which might be due to the dynamic balance between glycolytic pyruvate production and TCA cycle pyruvate consumption [28]. The previous study showed that glycolysis supported embryonic muscle growth in *Drosophila* and zebrafish by promoting myogenic cell fusion [29]; however, a transient reduction in glycolysis reduced pathological angiogenesis [30]. In addition, Notch signaling regulates the expression of glycolysis-related genes in an environment-dependent manner in zebrafish embryonic development [31]. Therefore, no conclusive or reliable evidence exists for the role of glycolysis in avian embryonic development. Our results of in ovo 2-DG injection confirmed that the inhibition of glycolysis reduced embryonic growth in an avian embryo model, whereas the inhibition of mitochondrial electron transport chain complex I failed to impair embryonic development. Therefore,

a new perspective of glycolysis in energy supply was proposed in our study: hypoxic glycolysis could act as a main energy supply mode to maintain normal development during the mid–late avian embryonic stages. Intriguingly, the inhibition of Notch signaling by in ovo DAPT injection impaired glycolytic ability and embryonic development, whereas the inhibition of glycolysis by in ovo 2-DG injection did not display significant feedback effects on Notch signaling. Our results are the first to demonstrate that there is a Notch signaling-mediated switch to glycolysis, which serves as the main energy supply mode during the mid–late embryonic stages in geese.

Previous studies reported that a switch to glycolysis can be controlled by Notch signaling via several different mechanisms [31–34]. In our study, Notch-mediated inhibition of glycolysis was accompanied by the blockade of PI3K/Akt signaling in primary hepatocytes, consistent with the finding indicating that hyperactivated Notch signaling enhanced glycolysis via activation of the PI3K/Akt signaling pathway in cancer cells [32]. In addition, PTEN is a well-known cell cycle repressor that can be negatively regulated by canonical Notch signaling [35]. As reported previously, Notch signaling has been reported to activate the PI3K/Akt pathway by inhibiting transcription of PTEN [36, 37], which was consistent with the increased PTEN mRNA and protein expression following the inhibition of Notch signaling by DAPT in the present study. Notably, the decrease in glycolysis mediated by inhibition of Notch signaling was reversed by activation of PI3K/Akt signaling. These in vitro data suggested that the switch to glycolysis was controlled by Notch signaling in a PI3K/Akt-dependent manner in embryonic primary hepatocytes. The synergism between Notch and PI3K/Akt signaling also supported the idea that Notch signaling is necessary for the regulation of glycolysis under hypoxic conditions [38] and for the maintenance of the undifferentiated cell state [27].

In our study, geese embryos were selected as an ideal avian model to verify the effect and mechanism of the Notch signaling-induced glycolytic switch in vitro. Notch signaling has been reported to be required for the development of epidermal [39] and ventricular chambers [40] during embryogenesis in mammals [41]; however, the mechanism by which Notch signaling regulates the development of avian embryos remains unclear. In our study, the reduced hepatic glycolysis resulting from the inhibition of either Notch or PI3K/Akt signaling in vivo was accompanied by impaired embryonic development, which was reversed by the activation of PI3K/Akt signaling. This finding suggests that Notch signaling induces a switch to glycolysis in the liver via PI3K/Akt signaling, which is beneficial for the embryonic development of avian species. These in vivo data provided a new perspective on the role of glycolysis in supplying energy for embryonic development; namely, a switch to hypoxic glycolysis is controlled by Notch signaling in a PI3K/Akt-dependent manner to supply energy for the mid–late embryonic stages, as demonstrated using the goose embryo model. Our findings also provide a new way to improve avian embryonic development via the regulation of either maternal dietary nutrient supplementation or in ovo feeding of exogenous nutrients. On the one hand, avian embryogenesis is important for the economic development of poultry breeding and for providing humans with high-quality animal proteins such as meat and eggs. On the other hand, avian embryos in a naturally hypoxic environment might be ideal models in developmental biology to investigate immunology, genetics, virology, and cancer under hypoxic conditions. In conclusion, our study is the first to demonstrate the role of

Notch signaling in inducing a switch to glycolysis during embryonic development. Our findings present new insight into the energy supply patterns in embryogenesis under hypoxic conditions, and open up the possibility of improving avian embryonic development through exogenously mediated Notch signaling to benefit the poultry industry.

Abbreviations

DHAP	Dihydroxyacetone phosphate
DOH	Day of hatching
DAPT	<i>N</i> -[<i>N</i> -(3,5-Difluorophenacetyl)- <i>L</i> -alanyl]- <i>S</i> -phenyl glycine <i>t</i> -butyl ester
EdU	5-Ethynyl-2'-deoxyuridine
E	Embryonic day
F-6-P	Fructose 6-phosphate
G-6-P	Glucose 6-phosphate
HIF-1 α	Hypoxia-inducible factor-1 α
HK	Hexokinase
NICD	Notch intracellular domain
PFK	Phosphofructokinase
PK	Pyruvate kinase
RQ	Respiratory quotient
RBW	Relative body weight
RLW	Relative liver weight
2-DG	2-Deoxy-D-glucose
3-PG	3-Phospho-D-glycerate

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s11658-023-00459-4>.

Additional file 1: Figure S1. Inhibition of the mitochondrial electron transport chain does not affect goose embryonic development. Metabolites besides those associated with glycolysis and the TCA cycle in the liver of goose embryos from E22, E25 and E28. Dose effect of rotenone on the activity of electron transport chain complex I, hepatic lactate and ATP levels, and the relative mRNA expressions of electron transport chain complexes I, III, and IV in the liver of the goose embryo. Dose effect of rotenone on developmental changes of goose embryos. **Figure S2.** In ovo injected with 20 mg/kg 2-DG or 10 nM DAPT impaired goose embryonic development. Effect of in ovo injected with 20 mg/kg 2-DG or 10 nM DAPT on developmental changes of goose embryo and embryonic organs and intestines, and the activity of ETC complex I, and the relative mRNA expressions of NUDFA5, LOC106045434, and LOC1060044242. **Figure S3.** Identification, cell morphology, and cell viability of goose embryonic primary hepatocytes. Identification of goose embryonic primary hepatocytes by immunofluorescence of cytokeratin-8 and cytokeratin-18. CK18 and CK18, DAPI, scale bar: 50 μ m. Morphology of goose embryonic primary hepatocytes from 0 to 72 h after isolation. Changes in cell viability within one week of goose embryo primary hepatocytes. **Figure S4.** Dose effect of CoCl₂ or DAPT on goose embryonic primary hepatocytes. Dose effect of CoCl₂ on cell morphology, cell proliferation, and cell viability. Hepatocyte viability within 1 week after being treated with 300 μ M CoCl₂. Dose effect of DAPT on cell morphology, cell proliferation, cell viability, and the relative mRNA expressions of NUDFA5, LOC106045434, and LOC1060044242. Effect of CoCl₂ and DAPT on relative protein abundances of NICD, Hey1, PTEN, PI3K, Akt, and p-Akt in hepatocytes under normal condition. **Figure S5.** Effects of DAPT, LY294002 and 740Y-P treatment on Notch signaling, PI3K/Akt signaling, and glycolysis in hepatocytes under normal condition. **Figure S6.** In ovo injected with 10 nM DAPT or 60 μ M LY294002 impaired goose embryonic development. Effect of in ovo 10 nM DAPT, 60 μ M LY294002, or 50 μ g/mL 740Y-P injection on developmental changes of goose embryos and embryonic organs and intestines.

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Author contributions

All authors read and approved the final manuscript. HW and WL designed research and analyzed data; HW, WL, XW, and YZ performed the research; WW, LY, and YZ contributed unpublished reagents/analytical tools; HW, WL, and YZ wrote the manuscript.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The animal care and use protocol was approved by the Animal Care and Use Committee of South China Agricultural University (SCAU-10564), and this study was conducted in accordance with the Regulations on the Administration of Affairs Concerning Experimental Animals.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Original Research Article

Developmental changes in lipid and fatty acid metabolism and the inhibition by in ovo feeding oleic acid in Muscovy duck embryogenesis



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ABSTRACT

Hepatic lipid and fatty acid (FA) metabolism are critical for regulating energetic homeostasis during embryogenesis. At present, it remains unclear how an exogenous FA intervention affects embryonic development in an avian embryo model. In Exp. 1, 30 fertilized eggs were sampled on embryonic days (E) 16, 19, 22, 25, 28, 31 and the day of hatch (DOH) to determine the critical period of lipid metabolism. In Exp. 2, a total of 120 fertilized eggs were divided into two groups (60 eggs/group) for in ovo feeding (IOF) procedures on E25. Eggs were injected into the yolk sac with PBS as the control group and with oleic acid (OA) as the IOF-OA treatment group. Samples were collected on E28 and E31. In Exp. 1, hepatic triacylglycerol (TG) and cholesterol (CHO) contents increased while serum TG content decreased from E16 to DOH ($P < 0.05$). Both serum and liver displayed an increase in unsaturated FA and a decrease in saturated FA ($P < 0.05$). There was a quadratic increase in the target gene and protein expression related to hepatic FA de novo synthesis and oxidation ($P < 0.05$), whose inflection period was between E22 and E28. In Exp. 2, compared with the control embryos, IOF-OA embryos had an increased yolk sac TG content on E28 and E31, and a decreased serum TG and CHO content on E28 ($P < 0.05$). The IOF-OA embryos had less OA in the yolk sac and liver on E28, and less unsaturated FA in the serum and liver on E31 than did the control embryos ($P < 0.05$). Hepatic gene mRNA expression related to FA uptake, synthesis, and oxidation on E28 was lower in IOF-OA than in control embryos ($P < 0.05$), not on E31 ($P > 0.05$). Maximal metabolic changes in lipid and FA metabolism occurred on E22–E28 in Muscovy duck embryogenesis, along with the altered target gene and protein expression related to lipogenesis and lipolysis. IOF-OA intervention on E25 could inhibit the target gene expression related to FA uptake, synthesis, and oxidation, which may influence the normal FA metabolism on E28 during embryogenesis. © 2023 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

The avian embryo is a multifaceted model system for studying developmental mechanisms, such as organ morphogenesis,

nervous system development and maternal effects on embryogenesis (Clayton and Emery, 2015; Groothuis et al., 2019; Hirst and Marcelle, 2015; Kulesa et al., 2013). During incubation, the development of poultry embryos depends on the nutrient composition of the eggs, and these functional molecules exert their effects systematically (Meng et al., 2021a; Moran, 2007). Lipid distribution in the yolk sac varies at different embryonic stages and is transferred to embryos through the highly vascularized yolk sac membrane or directly into the intestine via the yolk stalk (Meng et al., 2021b; van der Wagt et al., 2020). The liver is responsible for lipogenesis and lipolysis during the embryonic and neonatal periods (Alves-Bezerra and Cohen, 2017; Cai et al., 2017; Wang et al., 2015). Plasma lipoprotein particles are produced by very-low-density lipoprotein

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(VLDL) in the yolk sac's endodermal epithelial cells and then transported to the liver via vitelline circulation (Hermann et al., 2000; Yadgary et al., 2010). As lipids are transferred gradually from the yolk sac into the embryo, liver lipid deposition progressively accumulates in the form of lipid droplets, typically with a core of triacylglycerols (TG) and other neutral lipids (e.g., cholesterol and retinyl esters) surrounded by a phospholipid monolayer (Noble et al., 1984; Speake et al., 1998; Wang et al., 2017a).

The rapid growth of genetically improved birds can make the quantity of nutrients contained in the egg insufficient for the complete development of embryonic tissues (Grodzick et al., 2013). Moreover, the practice of post-hatch fasting until the chicks are housed can further limit the nutritional reserves contained in the yolk. In ovo feeding (IOF) as a tool is being developed to deliver growth-promoting compounds and nutrients during the embryonic stage to improve the performance and gut health of poultry (Das et al., 2021; Peebles, 2018; Wong and Uni, 2021). For example, IOF of carbohydrates and amino acids could improve the energy status of the embryos by increasing glycogen storage and enhancing jejunal nutrient uptake and digestion (Foye et al., 2006). Besides, IOF vitamin C and vitamin D₃ resulted in improved bone characteristics (tibia breaking strength and bone mineralization, etc.) of birds at post-hatch (Bello et al., 2014; Santos et al., 2019). However, there was little available information on the effect of IOF of fatty acids (FA). FA is a major source via enhancing mitochondrial β -oxidation for ATP generation when energy demand increases during embryonic development (Bradley and Swann, 2019; Noble and Cocchi, 1990). Moreover, FA and their metabolites are involved in cell growth and development, cell signaling, and modulating the structural and functional processes at each stage of embryonic development (Duttaroy and Basak, 2020). Furthermore, the egg yolk is the sole source of n-6 and n-3 polyunsaturated FA (PUFA) in the chick embryo due to the absence of desaturases that insert double bonds beyond the δ -9 carbon (Cherian, 2015). Oleic acid (OA) is the most abundant FA in the yolk of developing chick embryos, which is key for energy supply during incubation (Şahan et al., 2014; Su et al., 2020). We hypothesized that IOF-OA could achieve a beneficial effect on embryonic development by altering FA profile and lipid metabolism and modifying the regulation of transcription of different genes.

In this study, the dynamic patterns of lipid metabolism, FA profile, and target gene and protein expression in tissues were investigated to determine the critical period of lipid metabolism during the embryonic development of Muscovy duck. Then, based on the critical period of lipid metabolism, the effect of IOF-OA on the alteration of lipid metabolism and FA profile during embryonic development was investigated using a Muscovy duck embryo model.

2. Materials and methods

2.1. Animal ethics statement

The animal care and use protocol was approved by the Animal Care and Use Committee of South China Agricultural University (SCAU-10564), and the study was conducted following the Regulations for the Administration of Affairs Concerning Experimental Animals.

2.2. Animals and incubation

In Exp. 1, a total of 350 Muscovy duck eggs were obtained from a commercial hatchery (Wen's Food Group Co., Ltd, Yunfu, Guangdong, China). These eggs were incubated in an automatically controlled incubator (Dezhou Keyu Hatching Equipment Co., Ltd,

Dezhou, China) at an incubation temperature of 37.5 ± 0.5 °C and relative humidity of $55\% \pm 5\%$ until the 31st day of incubation (E31). Eggs were turned at a 90-degree angle every 90 min from the beginning of incubation through to E31. After that, all eggs were transferred to hatching crates and moved to hatchers set at a temperature of 37.0 ± 0.5 °C, which declined to 36.0 ± 0.5 °C by the end of incubation. All eggs were candled on E15, and any unfertilized and unviable eggs were discarded. A total of 180 viable eggs (75.2 ± 1.8 g) and 30 newly-hatched ducklings (43.2 ± 0.7 g) were used for testing. Thirty eggs were selected for sampling on E16, E19, E22, E25, E28 and E31, and 30 ducklings on the day of hatch (DOH, within 24 h after external piping), consisting of 6 replicates each with 5 embryos.

In Exp. 2, a total of 200 Muscovy duck eggs were incubated and 120 viable eggs (78.9 ± 1.6 g) were selected and divided into two groups (60 eggs each) on E25, each group consisting of six replicates of 10 embryos per replicate. The control and treatment groups were injected with a 100 μ L volume of phosphate-buffered saline (PBS, #G4202, Servicebio, Wuhan, China) and OA ($\geq 99\%$, #75090, Sigma-Aldrich, Wyoming, USA) into the yolk sac, respectively. To do this, a sterile disposable 25.0-mm \times 0.6-mm needle was attached to a 1.0-mL syringe, which was replaced after each egg injection. Eggs were sanitized with 75% ethanol in the needle insertion region before their injection. Immediately after injection, the hole was sealed with medical adhesive tape (1.0 cm \times 1.0 cm), and the eggs move into an incubator. Two embryos of each treatment per replicate were selected for sampling on E28 and E31, respectively. The number of unviable embryos was recorded during E26 to DOH, and the embryonic mortality in the control group and IOF-OA group was 16.67% and 21.67%, correspondingly.

2.3. Sample collection

To obtain sufficient samples for analysis, it was necessary to pool the serum and liver samples together, and likewise for the yolk sac samples, so that the results would be comparable (Noble and Moore, 1964). Samples of the yolk sac, serum and liver from 5 or 2 embryos were pooled together for each replicate in Exp. 1 or Exp. 2, respectively. Yolk sac (without membrane) samples were collected and stored at -20 °C for their biochemical index and FA analyses. Blood samples from E16 to E31 were collected from the umbilical vein by using glass Pasteur pipettes (7 mm \times 150 mm). The tip of a glass Pasteur pipette was melted on the outer flame of an alcohol lamp and then drawn out with tweezers to make a needle with a diameter of < 0.3 mm. Blood samples on DOH were collected from the jugular vein using disposable syringes (16.0-mm \times 0.45-mm needle attached to a 1.0-mL syringe). The serum was separated via centrifuging at $664 \times g$ for 10 min at room temperature and stored at -20 °C until the biochemical index and FA analyses. From Exp.1, approximately 0.5 to 1 cm³ of each liver sample was fixed in 4% formaldehyde for its hematoxylin-eosin (H&E) and oil-red O staining histological analysis. The rest of the liver samples were rinsed with ice-cold PBS, frozen in liquid nitrogen and stored at -80 °C for later analyses of biochemical index, FA composition and relative gene mRNA and protein expression levels.

2.4. Morphological examination

After fixing, paraffin sections and cryosections were taken from the liver and these were used for H&E staining and oil-red O staining, respectively. The procedure consisted of dehydration, paraffin embedding, sectioning and staining. The sections were observed and photographed under a microscope (Eclipse E100 and DS-U3, Nikon, Tokyo, Japan). The ratio of red-stained area to picture

area in oil-red O-stained sections was calculated in Image J (National Institutes of Health, Maryland, USA).

2.5. Biochemical index detection

Biochemical indices were measured according to the manufacturer's instructions for each assay kit used (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), including TG, cholesterol (CHO), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), lipoprotein lipase (LPL), hepatic lipase (HL) and total lipase (TL). VLDL was measured with an ELISA assay kit according to the manufacturer's instructions (Shanghai Enzyme-linked Biotechnology Co., Ltd. Shanghai, China).

2.6. Fatty acid analysis

Lipid extraction and FA analysis of yolk sac, serum and liver samples were conducted as previously reported (Li et al., 2020). In brief, lipids from the yolk sac and liver tissues were extracted with chloroform and methanol (2:1, vol/vol) and FA methyl esters (FAME) were prepared by transesterification with boron trifluoride etherate. The lipids present in serum samples were directly converted to FAME by transesterification. Next, the FAME were determined by a gas chromatography system (GC-7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with a hydrogen flame ionization detector. The GC system was fitted with a capillary column (60 m × 0.25 mm CP7487, 0.20-μm film thickness, Agilent). Next, the FAME were identified by comparing the FAME profiles of samples with those of FAME standards (#CRM47885, Sigma–Aldrich). FA profiles of the yolk sac, serum, and liver tissues are reported here as percentages of total FA.

2.7. Relative gene mRNA expression analysis

Total RNA was extracted from liver tissue with Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to protocols for using this reagent. Reverse transcription was performed as described in the Primer Script RT Reagent Kit (TaKaRa, Dalian, China). Primer sequences used in the current study were obtained from GenBank (Table 1) and synthesized by the Sangon Biotechnology Co., Ltd. (Shanghai, China). The samples were analyzed in duplicate by real-time quantitative reverse transcription polymerase chain reaction

(qRT-PCR) performed on a detection system (Applied Biosystems QuantiStudio 7 Flex, Life Technologies, Carlsbad, CA, USA). Each qRT-PCR reaction consisted of 5 μL of SYBR Green Realtime PCR Master Mix kit (#QPK-201, TOYOBO, Osaka, Japan), 0.4 μL of each forward primer and reverse primer, 3.2 μL of ddH₂O, and 1.0 μL of template cDNA into a total volume of 10 μL. The PCR program went as follows: 95 °C for 1 min, followed by 40 cycles each at 95 °C for 15 s, then 60 °C for 15 s, and finally 72 °C for 45 s. The amplification specificity was verified by a melting curve analysis done at the end of every PCR run. Beta-actin was highly expressed in the liver with a stable Cq value and was selected as the reference gene. Gene expression was normalized to the reference gene and the mRNA transcripts were quantified using the comparative CT method ($2^{-\Delta\Delta C_T}$). The data were analyzed using Cq, where $\Delta\Delta C_T = (C_{T,Target} - C_{T,Actin})_{Time\ x} - (C_{T,Target} - C_{T,Actin})_{Time\ 0}$. Time x is the time point ranging from E19 to DOH and Time 0 represents the 1 × expression of the target gene on E16 normalized to β-actin (Livak and Schmittgen, 2001).

2.8. Western blotting analysis

Liver samples collected on E16, E22, E28 and DOH in Exp. 1 were used for western blotting analysis. Each tissue sample was homogenized with ice-cold RIPA lysis buffer (#AWB0136, Abiowell, Changsha, China) containing 1 mmol/L phenylmethylsulfonyl fluoride (#AWH0650, Abiowell). The mixture was placed on ice for 10 min and the homogenate was centrifuged at 12,000 × g for 5 min at 4 °C. Protein concentration in the collected supernatant was measured as described in the BCA assay kit (Beyotime Biotechnology, Shanghai, China). The protein supernatant was mixed with a loading buffer, boiled for 5 min and then stored at 4 °C for Western blotting analysis. Antigenic proteins were separated via electrophoretic SDS-PAGE on 10% gel for 130 min at 75 V (DY-6C, Liuyi, Beijing, China). After transferring to a nitrocellulose filter membrane at 300 mA with Trans-Blot (DYCZ-40D, Liuyi) in a transfer buffer (#AWC0114, Abiowell), the membrane was blocked for 1.5 h at room temperature in a 5% skim milk powder solution (#AWB0004, Abiowell), then incubated overnight at 4 °C with rabbit monoclonal antibodies for SREBP1 (#ab28481, Abcam, Cambridge, England), FASN (#10624-2-AP, Proteintech, Chicago, USA), PPARα (#ab24509, Abcam), CPT1 (#15184-1-AP, Proteintech) and β-actin (#66009-1-Ig, Proteintech). The membrane was then incubated with HRP goat anti-rabbit IgG (#SA00001-2, Proteintech)

Table 1
Forward and reverse primer sequences for PCR analysis.

Target genes	Forward primer (5' → 3')	Reverse primer (5' → 3')	GenBank accession No.	Product size (bp)
CD36	TATCGTTTCGAGTTCCTCGTGAAG	AGTTCCTGGGATATGACCTCTCTGTAC	XM_038183702.1	94
SLC27A4	GCCTGATGACGTGATGACGACTG	AGAACTTCTGCGGATGACGATGG	XM_027470622.2	113
FABP1	TGGGAATAAGTTCAAGGTTACCGTCAC	GGTCTTGGCTTCTCTCTCTGTCAG	XM_005023289.5	112
Lpin 1	ACAAAGCAAGAACAATGACACAGG	GAGAAATGGCAATGGAGGGCAAATC	XM_038176802.1	137
SREBP1	TGGTGGTGGACGCCGAGAAG	TCGTTGATGGAGGAGCGGTAGC	XM_038187023.1	133
ACC1	CACAGATCCAGAGCAGCACTTC	GGCAGGCAGTATCCGTTTCATCAC	XM_038165892.1	100
FASN	TCTCTGCCATCTCCGAACTTC	TTAGCCACTGTGCCAACTCAAGC	XM_027471234.2	96
SCD1	AGTTCCTCTCCGCTTCCAGC	TTCTCCATGACGGCATCCCC	XM_027460089.2	82
ELOVL2	ACCGGAAAGCACCTTCAAGAACAG	TTCCAGGAATCCATTGGCAGCAGTG	XM_038175034.1	106
ELOVL3	TCCTGGAACTGGGCGACACC	GCGTAGATGAGAGTGGCGATGTG	XM_038181654.1	94
PPARα	ACCATCTCTGATGATACCTTCTCTTC	AAGTTGAGCATGTCTGTGACAAGTTG	NM_001310383.1	86
RXRα	TGGGAGCCATTGCTCTCTTCAAC	GATGCGTACACCTTCTCCGTAAC	XM_027471073.2	88
CPT1A	CCGCCATCTGTCTGCTCTATG	TGTGTTGCTGTGGTGTCTGACTTG	XM_027457809.2	119
ACADL	TGGTGCCATTGCCATGACAGAAC	TCCTCCGTTAAGAATCCAGTCACTTC	XM_027461394.2	99
ACOX3	GAAGGAGAAGCAGTCAGGGCAAAG	GCAATGGCTAGTGACCGACAGTAG	XM_038178270.1	81
β-actin	TACGCCAACACGGTGCTG	GATTCATCATCTCTGCTTG	NM_00131042.1	215

CD36 = CD36 molecule; SLC27A4 = solute carrier family 27 member 4; FABP1 = fatty acid-binding protein 1; Lpin 1 = phosphatidate phosphatase; SREBP1 = sterol regulatory element-binding transcription factor 1; ACC1 = acetyl-CoA carboxylase alpha; FASN = fatty acid synthase; SCD1 = stearoyl-CoA desaturase; ELOVL2 = elongation of very-long-chain fatty acids protein 2; ELOVL3 = elongation of very-long-chain fatty acids protein 3; PPARα = peroxisome proliferator-activated receptor alpha; RXRα = retinoid X receptor alpha; CPT1A = carnitine palmitoyltransferase 1 A; ACADL = long-chain-acyl-CoA dehydrogenase; ACOX3 = acyl-CoA oxidase 3.

diluted in PBST buffer (#AWI0130, Abiowell) and also with ECL chemiluminescence solution (#AWB0005, Abiowell) for 1 min. Ensuing bands were detected by a chemiluminescence imaging system (ChemiScope6100, Qinxiang, Shanghai China) and their density was determined using Image J software.

2.9. Statistical analysis

Data were expressed as mean \pm standard error of the mean (SEM). Data from Exp. 1 were analyzed with a one-way analysis of variance (ANOVA) using the PROC GLM procedure in Statistical Analysis System (SAS) v9.2 (SAS Inst. Inc., Cary, NC, USA). The differences in means were considered significant at $P < 0.05$, and notable differences between groups were identified using Duncan's multiple comparisons test. Orthogonal polynomial contrasts were used to identify the form of the effect (linear or quadratic) over time (embryonic development days). The data for each sampling time point from Exp. 2 were analyzed with a t -test, using the PROC TTEST procedure in SAS v9.2, for which differences were considered significant at $P < 0.05$.

3. Results

3.1. Hepatic morphological change

Representative hepatic H&E stained and oil-red stained sections in the course of Muscovy duck embryogenesis are presented in Fig. 1A and B, respectively. With morphological post-processing of liver tissue, boundaries of indicative fat droplets were identified as white cavities and red dots for quantitative analysis by H&E and oil-red O staining, respectively. The H&E staining results showed that fat shapes increasingly erased small regions by filling holes and gaps and eventually separated adjacent fat droplets as the incubation period progressed. The red-stained area percentage of the hepatic oil-red O-stained sections increased ($P < 0.05$) linearly from E16 through to DOH (Fig. 1C).

3.2. Developmental changes in biochemical indices related to lipid metabolism

During embryogenesis, the TG content of the yolk and liver increased ($P < 0.05$) linearly or quadratically, while the serum TG content decreased ($P < 0.05$) linearly or quadratically during the embryonic period (Fig. 2A and B). The HDLC level of yolk increased ($P < 0.05$) linearly, while the LDLC level of yolk decreased ($P < 0.05$) linearly (Fig. 2A). There were significant increases in hepatic content of CHO and LDLC and a decrease in hepatic TL activity ($P < 0.05$) in a linear or quadratic manner with prolonged incubation (Fig. 2B). The level of HDLC and HL both increased ($P < 0.05$) in serum yet decreased ($P < 0.05$) in liver linearly or quadratically in response to the longer incubation period (Fig. 2B). The VLDL content and the TL activity in serum increased ($P < 0.05$) in a quadratic manner and hepatic VLDL content increased ($P < 0.05$) in a linear manner (Fig. 2B). Quadratic changes ($P < 0.05$) were evident for the LPL activity of liver and serum, which plateaued in periods corresponding to E19 to E25 and E28 to E31, respectively (Fig. 2B).

3.3. Developmental changes in fatty acid profiles in serum and tissues

The OA varied in a linear ($P < 0.05$) or quadratic ($P < 0.05$) manner in the yolk sac (Table 2), serum (Table 3), and liver (Table 4), with its level peaking on DOH, E25, and E31, respectively. Similarly,

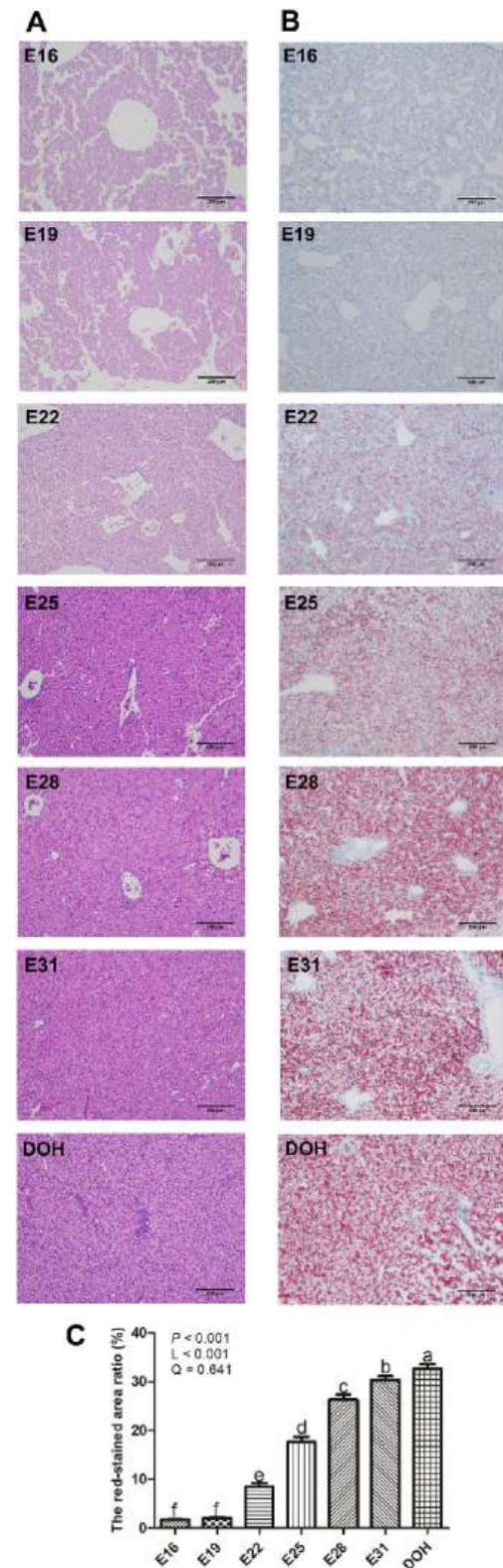


Fig. 1. Representative hepatic H&E stained sections and oil-red O stained sections in Muscovy duck embryogenesis. (A) H&E stained sections. (B) Oil-red O stained sections. The pictures are at $200\times$ magnification and the ruler in the lower right corner of the picture (A, B) is $200\mu\text{m}$. (C) The percentage of red-stained area to picture area in the oil red-stained O section was calculated using software Image J. a–f Data are expressed as mean \pm SEM ($n = 6$), and bars with different lowercase letters indicate statistically significant differences (one-way ANOVA, $P < 0.05$). L, linear; Q, quadratic.

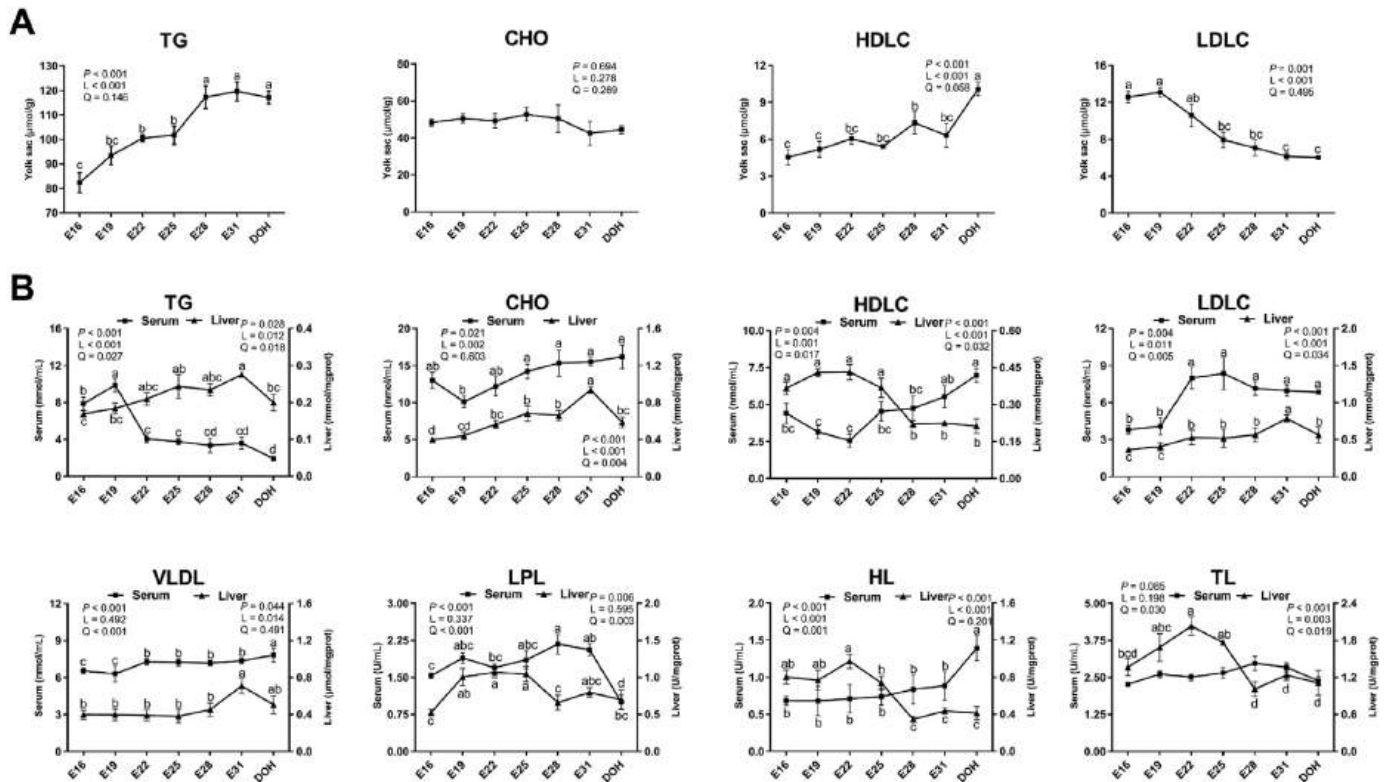


Fig. 2. Dynamic changes of the yolk, serum and hepatic biochemical index in Muscovy duck embryogenesis. (A) yolk sac, as a fresh basis. (B) serum and liver. TG = triacylglycerol contents; CHO = total cholesterol contents; HDLC = high-density lipoprotein cholesterol contents; LDLC = low-density lipoprotein cholesterol contents; VLDL = very-low-density lipoprotein contents; LPL = lipoprotein lipase activity; HL = hepatic lipase activity; TL = total lipase activity. ^{a-d} Data are expressed as mean \pm SEM ($n = 6$), and values on the same line with different lowercase letters indicate statistically significant differences (one-way ANOVA, $P < 0.05$). L, linear; Q, quadratic.

monounsaturated FA (MUFA) increased ($P < 0.05$) linearly or quadratically, attaining their highest level on E31 to DOH, E25 and E31 in the yolk sac, serum and liver, respectively. The PUFA increased ($P < 0.05$) linearly from E16 to DOH in serum, but no differences ($P > 0.05$) in PUFA were observed in either the yolk sac or liver. Unsaturated FA (UFA) increased ($P < 0.05$) linearly or quadratically, whereas saturated FA (SFA) decreased ($P < 0.05$) linearly or quadratically in the yolk sac, serum and liver from E16 to DOH.

3.4. Developmental changes in hepatic gene and protein expression levels related to fatty acid metabolism

Hepatic mRNA expression of the genes *CD36*, *SLC27A4*, *Lipin1*, *FASN*, *SCD1*, *ELOVL2*, *PPAR α* , *RXR α* , *CPT1A*, *ACADL* and *ACOX3* increased ($P < 0.05$) all linearly or quadratically from E16 to DOH, for which the maximal values were observed between E25 and E28 (Fig. 3A). Hepatic mRNA expression levels of *FABP1*, *SREBP1*, *ACC1* and *ELOVL3* genes decreased ($P < 0.05$) from E16 to E31 but significantly increased ($P < 0.05$) on DOH (Fig. 3A). Hepatic protein expression of *SREBP1*, *FASN*, *PPAR α* and *CPT1* increased ($P < 0.05$) linearly or quadratically, with maximal values arising between E22 and E28 (Fig. 3B and C).

3.5. Effects of IOF-OA on biochemical indices of lipid and fatty acid profiles

Compared with the control group, the IOF-OA group had a greater ($P < 0.05$) content of TG content in its yolk sac on E28 and E31 (Fig. 4A). On E28, the IOF-OA group also showed augmented ($P < 0.05$) serum activities of LPL and TL, in addition to a greater

hepatic HDLC content as well as HL and TL activities (Fig. 4B and C). The IOF-OA group had a reduced ($P < 0.05$) serum content of TG and CHO on E28 (Fig. 4B), and a lower ($P < 0.05$) hepatic VLDL content on E31 vis-à-vis the control group (Fig. 4C). Regarding their FA profiles, relative to the control group, the IOF-OA group was characterized by lower ($P < 0.05$) OA percentages in its yolk sac and liver on E28 (Tables 5 and 7), less ($P < 0.05$) n-6 PUFA in its serum on E28 and E31 (Table 6), and reductions ($P < 0.05$) in UFA in both its serum and liver on E31 (Tables 6 and 7).

3.6. Effects of IOF-OA on hepatic gene expression related to fatty acid metabolism

The hepatic mRNA expression of genes related to FA uptake, synthesis and oxidation, namely *CD36*, *SLC27A4*, *Lipin 1*, *SREBP1*, *ACC1*, *FASN*, *SCD1*, *ELOVL2*, *ELOVL3*, *PPAR α* , *RXR α* , *CPT1* and *ACOX3* were all lower ($P < 0.05$) in the IOF-OA group than the control group on E28 (Fig. 5). No significant differences ($P > 0.05$) were found in the above gene mRNA expression between the control group and the IOF-OA group on E31.

4. Discussion

Hepatic lipids accumulate progressively during embryogenesis in our study, as evinced by the greater number and area of fat droplets present in the histological sections and the higher hepatic contents of TG and CHO, which were consistent with previous studies of chicken embryos (Guedes et al., 2014; Kim et al., 2017; Liu et al., 2020; Zhao et al., 2007). There were similar developmental patterns between serum and liver in the changes of their CHO, LDLC, VLDL, LPL and TL. Greater hepatic CHO biosynthesis leads to

Table 2
Dynamic changes of the yolk sac fatty acid composition in Muscovy duck embryogenesis (%).

Item		E16	E19	E22	E25	E28	E31	DOH	SEM	P	Linear	Quadratic
Myristic acid	C14:0	0.69 ^a	0.60 ^{ab}	0.56 ^b	0.66 ^{ab}	0.57 ^b	0.55 ^{bc}	0.45 ^c	0.032	0.001	<0.001	0.304
Palmitic acid	C16:0	30.27 ^{ab}	28.51 ^{abc}	31.44 ^a	28.38 ^{abc}	30.79 ^{ab}	26.72 ^{bc}	25.94 ^c	0.017	0.024	0.011	0.088
Palmitoleic acid	C16:1	2.36	2.39	2.48	2.55	2.19	2.46	2.11	0.533	0.547	0.325	0.245
Stearic acid	C18:0	7.43	6.91	6.93	7.60	6.86	7.54	7.40	0.063	0.171	0.408	0.306
Oleic acid	C18:1n-9	40.59 ^d	43.82 ^{bc}	40.77 ^d	42.43 ^{cd}	40.58 ^d	44.97 ^{ab}	46.48 ^a	0.099	<0.001	<0.001	0.003
Linoleic acid	C18:2n-6	11.67	11.36	11.73	11.54	10.46	11.15	11.29	0.433	0.872	0.379	0.708
Linolenic acid	C18:3n-3	0.58	0.39	0.59	0.55	0.51	0.58	0.61	0.220	0.335	0.310	0.510
Eicosatrienoic acid	C20:3n-6	0.42 ^{ab}	0.31 ^{bc}	0.30 ^c	0.46 ^a	0.36 ^{abc}	0.37 ^{abc}	0.42 ^{ab}	0.027	0.023	0.343	0.235
Eicosatrienoic acid	C20:3n-3	3.19 ^a	3.11 ^{ab}	2.82 ^{bc}	2.90 ^{abc}	2.63 ^c	2.80 ^{bc}	2.62 ^c	0.015	0.009	<0.001	0.253
Nervonic acid	C24:1n-9	0.37 ^a	0.32 ^{ab}	0.30 ^{ab}	0.28 ^b	0.29 ^b	0.38 ^a	0.37 ^a	0.050	0.034	0.303	0.002
Docosahexaenoic acid	C22:6n-3	0.63 ^a	0.55 ^a	0.42 ^b	0.41 ^b	0.39 ^b	0.40 ^b	0.45 ^b	0.011	<0.001	<0.001	<0.001
MUFA		43.31 ^b	46.53 ^{ab}	43.55 ^b	44.84 ^b	43.06 ^b	47.81 ^a	48.97 ^a	0.455	<0.001	<0.001	0.007
PUFA		16.49	15.72	15.85	15.86	14.26	15.21	15.22	0.268	0.557	0.094	0.476
n-6 PUFA		12.09	11.67	12.03	12.00	10.73	11.52	11.71	0.017	0.834	0.402	0.628
n-3 PUFA		4.40 ^a	4.05 ^{ab}	3.82 ^b	3.87 ^b	3.53 ^b	3.69 ^b	3.50 ^b	0.225	0.010	<0.001	0.211
n-6/n-3		2.75	2.89	3.14	3.11	3.04	3.14	3.41	0.075	0.125	0.007	0.918
UFA		59.80 ^{bc}	62.25 ^{ab}	59.39 ^{bc}	60.70 ^{abc}	57.31 ^c	63.02 ^{ab}	64.18 ^a	0.063	0.007	0.048	0.017
SFA		38.38 ^a	36.02 ^{ab}	38.93 ^a	36.64 ^{ab}	38.21 ^a	34.93 ^{ab}	33.60 ^b	0.527	0.039	0.012	0.122
UFA/SFA		1.57 ^c	1.73 ^{abc}	1.54 ^c	1.66 ^{bc}	1.55 ^c	1.81 ^{ab}	1.92 ^a	0.522	0.004	0.003	0.030

E16 to E31 = embryonic day 16 to 31; DOH = day of hatch.

MUFA is the sum of monounsaturated fatty acids that include C16:1, C18:1n-9 and C24:1n-9. PUFA is the sum of polyunsaturated fatty acids that include C18:2n-6, C20:3n-6, C18:3n-3, C20:3n-3 and C22:6n-3. N-6 PUFA is the sum of n-6 polyunsaturated fatty acids that include C18:2n-6 and C20:3n-6. N-3 PUFA is the sum of n-3 polyunsaturated fatty acids that include C18:3n-3, C20:3n-3 and C22:6n-3. UFA is the sum of unsaturated fatty acids that include MUFA and PUFA. SFA is the sum of saturated fatty acids that include C14:0, C16:0 and C18:0.

^{a-d} Data are expressed as mean \pm SEM ($n = 6$), and data on the same line with different lowercase letters indicate statistically significant differences (one-way ANOVA, $P < 0.05$).

more VLDL being secreted into the blood, thereby increasing the total plasma CHO and LDLC concentrations (Wang et al., 2017b). The liver secretes TG-rich VLDL that is delivered to peripheral tissues for oxidation or storage (Xiao et al., 2011). The HDLC content, regulated by HL activity, underwent a pronounced increase in serum from E22 to DOH, but a sharp decline in the liver from E28 to DOH. It has been suggested that HL serves as a ligand to facilitate lipoprotein uptake by hepatic cell surface receptors and proteoglycans in the middle period of embryogenesis (E16–E25) (Thuren, 2000). In addition, HL functions as a lipolytic enzyme to hydrolyze TG and phospholipids in circulating plasma lipoproteins in the late period of embryogenesis (E28–DOH) (Santamarina-Fojo et al., 2004). Plasma TG is emulsified and packaged into chylomicrons and then delivered to the liver, where they are taken up via receptor-mediated endocytosis, releasing FA when the lysosomes process these particles (Kawano and Cohen, 2013). Interestingly, the hepatic TG content increased during embryogenesis yet the serum TG content declined markedly from E19 to DOH. The LPL activity varies with the changes to its main substrates, TG and VLDL (Li et al., 2020; Olivecrona, 2016). Additionally, relative expression levels of hepatic LPL mRNA increased from E9 through E19 in chick embryos (Zhao et al., 2010). It is presumed that TG is hydrolyzed by LPL to release FA for β -oxidation to satisfy the energy demands arising in mid-to-late embryogenesis.

Hepatic FA metabolism is a key step in the regulation of energy homeostasis in the course of embryogenesis. Parallel changes in FA composition were found between the liver and serum in the current study. As the incubation proceeded, hepatic and serum UFA increased and SFA decreased, which was consistent with the FA changes reported in chicken and turkey embryos (Ding and Lilburn, 1996; Su et al., 2020). In contrast to the FA composition of the liver or serum, that of the yolk sac stayed constant throughout embryogenesis, especially distinguished by a steady level of PUFA. In liver and serum, the most abundant FA (OA) was remarkably increased going from E16 to E31 but exhibited a notable decrease on DOH. Except for de novo synthesis, the rising hepatic OA percentage was affected by the accumulation within hepatocytes of large lipid droplets consisting mainly of cholesteryl oleate transferred from the yolk sac (Decrock et al., 2001; Noble and Moore,

1964; Speake et al., 1998). It has been suggested that OA acts as fuel in FA oxidation to generate ATP during mid-to-late embryonic development (Wajner and Amaral, 2015). As the parent compound for the family of n-6 PUFA, C18:2n-6 was the predominant n-6 PUFA present in the yolk sac, serum and liver during embryogenesis, whose content rose from E16 to E28 and fell from E31 to DOH in our study. This parallel change of n-6 PUFA and C18:2n-6 also occurs in chick embryos (Ding and Lilburn, 1996; Kuksis, 1992; Su et al., 2020; Xiao et al., 2020). Generally, C18:2n-6 can be esterified to form neutral and polar lipids such as phospholipids, a structural component of membranes, or they can be elongated and desaturated to produce other bioactive n-6 PUFA (Das, 2006; Whelan and Fritsche, 2013). The main n-3 PUFA are transferred from the yolk sac to the liver, and the developmental maturation of hepatic n-3 PUFA metabolism may supply DHA to the retina and brain during embryogenesis (Cherian et al., 1997; Cherian and Sim, 1992; Martin et al., 1994). This explains why the main n-3 PUFA, composed of C20:3n-3 and C22:6n-3 (DHA), decreased in the serum and liver of duck embryos. A similar changing pattern of n-3 PUFA was also observed in king penguin embryos between E33 and E55 (Decrock et al., 2001). Hepatic UFA increased with the absorption of the yolk sac as well as their de novo synthesis, and serum UFA changed dynamically in parallel with hepatic UFA in response to vital requirements for embryonic development, such as energy demands, organogenesis and cellular homeostasis regulation.

The energy derived from FA β -oxidation is essential for maintaining the normal development and growth of poultry embryos. This process involves the expression of key genes related to FA uptake, de novo biosynthesis and oxidation within the cell. Hepatic FA de novo synthesis increased during E25–DOH, and this might be regulated by the gene and protein expression of SREBP1 and FASN. In addition, ACC1, FASN and SCD1 were subjected to SREBP1 which coordinates the synthesis of FA (Khesht and Hassanabadi, 2012). ACC1 catalyzes the rate-limiting step of the FA biosynthesis pathway by converting acetyl-CoA to malonyl-CoA (Salie and Thelen, 2016). Both acetyl-CoA and malonyl-CoA serve as substrates for FASN to produce C16:0 (Jensen-Ustad and Semenkovich, 2012). SCD1 is the enzyme responsible for the synthesis of MUFA, especially OA (Lounis and Bergeron, 2017; Piccinin et al., 2019). The

Table 3
Dynamic changes of the serum fatty acid composition in Muscovy duck embryogenesis (%).

Item		E16	E19	E22	E25	E28	E31	DOH	SEM	P	Linear	Quadratic
Myristic acid	C14:0	0.77	0.53	0.53	0.58	0.62	0.91	0.72	0.041	0.115	0.231	0.088
Palmitic acid	C16:0	27.51 ^a	27.37 ^a	24.92 ^b	23.86 ^{bc}	23.91 ^{bc}	23.65 ^{bc}	23.33 ^c	0.270	<0.001	<0.001	0.003
Palmitoleic acid	C16:1	1.90 ^a	1.08 ^b	1.15 ^b	1.34 ^b	1.00 ^b	1.08 ^b	1.14 ^b	0.058	<0.001	0.001	0.003
Stearic acid	C18:0	12.73 ^a	11.79 ^{ab}	11.64 ^{ab}	10.12 ^{bc}	10.54 ^{bc}	9.83 ^{bc}	9.52 ^c	0.280	0.011	<0.001	0.482
Oleic acid	C18:1n-9	30.78 ^e	33.08 ^d	34.14 ^{cd}	38.60 ^a	36.57 ^{ab}	37.01 ^{ab}	35.39 ^{bc}	0.413	<0.001	<0.001	<0.001
Linoleic acid	C18:2n-6	8.62 ^e	9.6 ^{de}	11.67 ^{ab}	11.89 ^a	11.01 ^{abc}	10.13 ^{cd}	10.78 ^{bc}	0.189	<0.001	0.001	<0.001
Eicosatrienoic acid	C20:3n-6	0.64	0.82	0.68	0.73	0.67	0.71	0.61	0.032	0.807	0.535	0.402
Eicosatrienoic acid	C20:3n-3	10.73 ^c	9.25 ^{de}	9.55 ^d	8.43 ^e	11.00 ^{bc}	12.37 ^a	11.81 ^{ab}	0.217	<0.001	<0.001	<0.001
Nervonic acid	C24:1n-9	1.07 ^{ab}	0.88 ^{bc}	0.70 ^{cd}	0.55 ^d	0.74 ^{cd}	0.69 ^{cd}	1.16 ^a	0.042	<0.001	0.838	<0.001
Docosahexaenoic acid	C22:6n-3	3.53 ^a	3.30 ^{bc}	3.01 ^{cd}	2.63 ^d	2.01 ^d	2.00 ^d	3.10 ^{ab}	0.110	<0.001	<0.001	<0.001
MUFA		33.74 ^e	35.05 ^{de}	35.87 ^{cd}	40.50 ^a	38.11 ^b	38.77 ^{ab}	37.56 ^{bc}	0.382	<0.001	<0.001	<0.001
PUFA		23.41 ^{bcd}	22.97 ^{cd}	24.91 ^{abc}	22.26 ^d	24.69 ^{abc}	25.20 ^{ab}	26.02 ^a	0.300	0.002	0.002	0.138
n-6 PUFA		9.15 ^d	10.42 ^c	12.35 ^a	12.62 ^a	11.68 ^{ab}	10.84 ^{bc}	11.12 ^{bc}	0.197	<0.001	0.004	<0.001
n-3 PUFA		14.26 ^a	12.55 ^b	12.56 ^b	11.21 ^c	13.01 ^b	14.37 ^a	14.90 ^a	0.222	<0.001	0.007	<0.001
n-6/n-3		0.64 ^e	0.83 ^{cd}	0.99 ^{ab}	1.12 ^a	0.91 ^{bc}	0.76 ^{de}	0.75 ^{de}	0.025	<0.001	0.730	<0.001
UFA		57.15 ^c	58.02 ^c	60.78 ^b	62.07 ^{ab}	62.80 ^{ab}	63.98 ^a	63.59 ^a	0.414	<0.001	<0.001	0.017
SFA		40.76 ^a	39.60 ^{ab}	37.09 ^b	33.30 ^c	33.77 ^c	32.86 ^c	33.40 ^c	0.576	<0.001	<0.001	0.019
UFA/SFA		1.40 ^c	1.47 ^c	1.64 ^b	1.82 ^a	1.80 ^a	1.87 ^a	1.88 ^a	0.030	<0.001	<0.001	0.002

E16 to E31 = embryonic day 16 to 31; DOH = day of hatch.

MUFA is the sum of monounsaturated fatty acids that include C16:1, C18:1n-9 and C24:1n-9. PUFA is the sum of polyunsaturated fatty acids that include C18:2n-6, C20:3n-6, C20:3n-3 and C22:6n-3. N-6 PUFA is the sum of n-6 polyunsaturated fatty acids that include C18:2n-6 and C20:3n-6. N-3 PUFA is the sum of n-3 polyunsaturated fatty acids that include C20:3n-3 and C22:6n-3. UFA is the sum of unsaturated fatty acids that include MUFA and PUFA. SFA is the sum of saturated fatty acids that include C14:0, C16:0 and C18:0.

^{a-e} Data are expressed as mean \pm SEM ($n = 6$), and data on the same line with different lowercase letters indicate statistically significant differences (one-way ANOVA, $P < 0.05$).

mRNA expression levels of *FASN* and *SCD1* increased during E25–DOH, which was consistent with the increased OA in the liver. FA elongation occurs predominantly in microsomes, with the rate-limiting step of condensation catalyzed by the ELOVL family (Zhang et al., 2016). ELOVL2 prefers PUFA, and ELOVL3 prefers SFA or MUFA as substrates, respectively (Guillou et al., 2010). The mRNA expression levels of *ELOVL2* and *ELOVL3* were higher on E28–DOH and DOH, which respectively coincided with an increase in PUFA and a decrease in SFA and MUFA. The hepatic uptake rate of FA is regulated by FA transport proteins, namely FA translocase (CD36), FA transport protein (FATP/SLC27A4) and FA-binding protein (FABP) (Canbay et al., 2007; Gimeno, 2007; Kazantzis and Stahl, 2012; Pepino et al., 2014). There were increases in hepatic mRNA

expression levels of *CD36* and *SLC27A4* on E25, and *FABP1* on DOH, indicating the uptake of hepatic FA from serum was enhanced, resulting in the reduced serum TG content. There was rapid uptake of yolk in avian embryos during the late term of incubation, especially on DOH (Liu et al., 2020). FABP1 is associated with fatty acid transportation and its dramatic increase on DOH implied the accelerated utilization of yolk fat by embryos. Lipin 1 was found to have important functions in glycerolipid biosynthesis and its enhanced expression led to stimulation of TG synthesis and secretion (Reue and Dwyer, 2009; Zhang and Reue, 2017). The greater mRNA expression of *Lipin1* on E25 and DOH could upregulate the activities of glycerolipid biosynthetic enzymes, thereby increasing the hepatic TG content. The expression levels of *PPAR α* ,

Table 4
Dynamic changes of the hepatic fatty acid composition in Muscovy duck embryogenesis (%).

Item		E16	E19	E22	E25	E28	E31	DOH	SEM	P	Linear	Quadratic
Myristic acid	C14:0	8.63 ^a	8.59 ^a	5.25 ^b	4.06 ^{bc}	3.29 ^{bc}	2.76 ^{bc}	2.31 ^c	0.489	<0.001	<0.001	0.075
Myristoleic acid	C14:1	0.91 ^{ab}	0.94 ^a	0.70 ^{abc}	0.47 ^c	0.56 ^{bc}	1.01 ^a	0.53 ^c	0.052	0.011	0.072	0.136
Palmitic acid	C16:0	19.67 ^a	18.44 ^a	15.48 ^b	14.93 ^b	14.70 ^b	13.65 ^b	14.32 ^b	0.433	<0.001	<0.001	0.008
Palmitoleic acid	C16:1	0.44 ^c	0.42 ^c	0.72 ^b	0.84 ^b	1.10 ^a	0.67 ^{bc}	0.64 ^{bc}	0.048	<0.001	0.004	<0.001
Stearic acid	C18:0	14.46 ^a	12.67 ^{abc}	12.95 ^{abc}	10.86 ^{bcd}	10.26 ^d	12.09 ^{cd}	13.50 ^{ab}	0.335	0.002	0.086	<0.001
Oleic acid	C18:1n-9	20.07 ^e	22.98 ^e	32.61 ^d	36.79 ^{bc}	39.84 ^{ab}	40.26 ^a	36.30 ^c	1.263	<0.001	<0.001	<0.001
Linoleic acid	C18:2n-6	5.18 ^c	5.87 ^c	8.18 ^b	9.92 ^a	10.00 ^a	9.10 ^{ab}	8.70 ^{ab}	0.330	<0.001	<0.001	<0.001
Eicosatrienoic acid	C20:3n-6	0.38 ^c	0.33 ^c	0.34 ^c	0.53 ^{bc}	0.88 ^a	0.61 ^b	0.46 ^{bc}	0.038	<0.001	0.001	0.010
Eicosatrienoic acid	C20:3n-3	17.96 ^a	15.23 ^{abc}	16.10 ^{ab}	13.05 ^{cd}	11.41 ^d	14.00 ^{bcd}	14.42 ^{bc}	0.461	0.001	0.001	0.002
Nervonic acid	C24:1n-9	0.88	0.75	0.83	0.76	0.72	0.78	0.87	0.026	0.502	0.836	0.081
Docosahexaenoic acid	C22:6n-3	7.15 ^a	5.09 ^b	5.64 ^b	5.45 ^b	5.05 ^b	5.79 ^b	4.73 ^b	0.184	0.003	0.005	0.121
MUFA		22.22 ^d	24.79 ^d	34.72 ^c	38.65 ^b	42.12 ^{ab}	42.47 ^a	38.86 ^b	1.278	<0.001	<0.001	<0.001
PUFA		30.67	26.52	30.21	28.93	27.33	29.50	28.30	0.434	0.098	0.488	0.496
n-6 PUFA		5.56 ^d	6.19 ^d	8.47 ^c	10.44 ^{ab}	10.88 ^a	9.71 ^{abc}	9.15 ^{bc}	0.350	<0.001	<0.001	<0.001
n-3 PUFA		25.11 ^a	20.32 ^b	21.74 ^b	18.49 ^{bc}	16.45 ^c	19.79 ^{bc}	19.15 ^{bc}	0.575	<0.001	<0.001	0.002
n-6/n-3		0.22 ^e	0.31 ^{de}	0.41 ^{cd}	0.57 ^{ab}	0.69 ^a	0.49 ^{bc}	0.48 ^{bc}	0.029	<0.001	<0.001	<0.001
UFA		52.96 ^d	51.31 ^d	64.93 ^c	67.58 ^{bc}	69.45 ^{ab}	71.97 ^a	67.16 ^{bc}	1.280	<0.001	<0.001	<0.001
SFA		42.77 ^a	39.70 ^a	32.80 ^b	29.84 ^{bc}	28.25 ^c	28.51 ^c	30.13 ^{bc}	0.988	<0.001	<0.001	<0.001
UFA/SFA		1.24 ^d	1.31 ^d	1.99 ^c	2.27 ^b	2.48 ^{ab}	2.53 ^a	2.25 ^b	0.084	<0.001	<0.001	<0.001

E16 to E31 = embryonic day 16 to 31; DOH = day of hatch.

MUFA is the sum of monounsaturated fatty acids that include C14:1, C16:1, C18:1n-9 and C24:1n-9. PUFA is the sum of polyunsaturated fatty acids that include C18:2n-6, C20:3n-6, C20:3n-3 and C22:6n-3. N-6 PUFA is the sum of n-6 polyunsaturated fatty acids that include C18:2n-6 and C20:3n-6. N-3 PUFA is the sum of n-3 polyunsaturated fatty acids that include C20:3n-3 and C22:6n-3. UFA is the sum of unsaturated fatty acids that include MUFA and PUFA. SFA is the sum of saturated fatty acids that include C14:0, C16:0 and C18:0.

^{a-e} Data are expressed as mean \pm SEM ($n = 6$), and data on the same line with different lowercase letters indicate statistically significant differences (one-way ANOVA, $P < 0.05$).

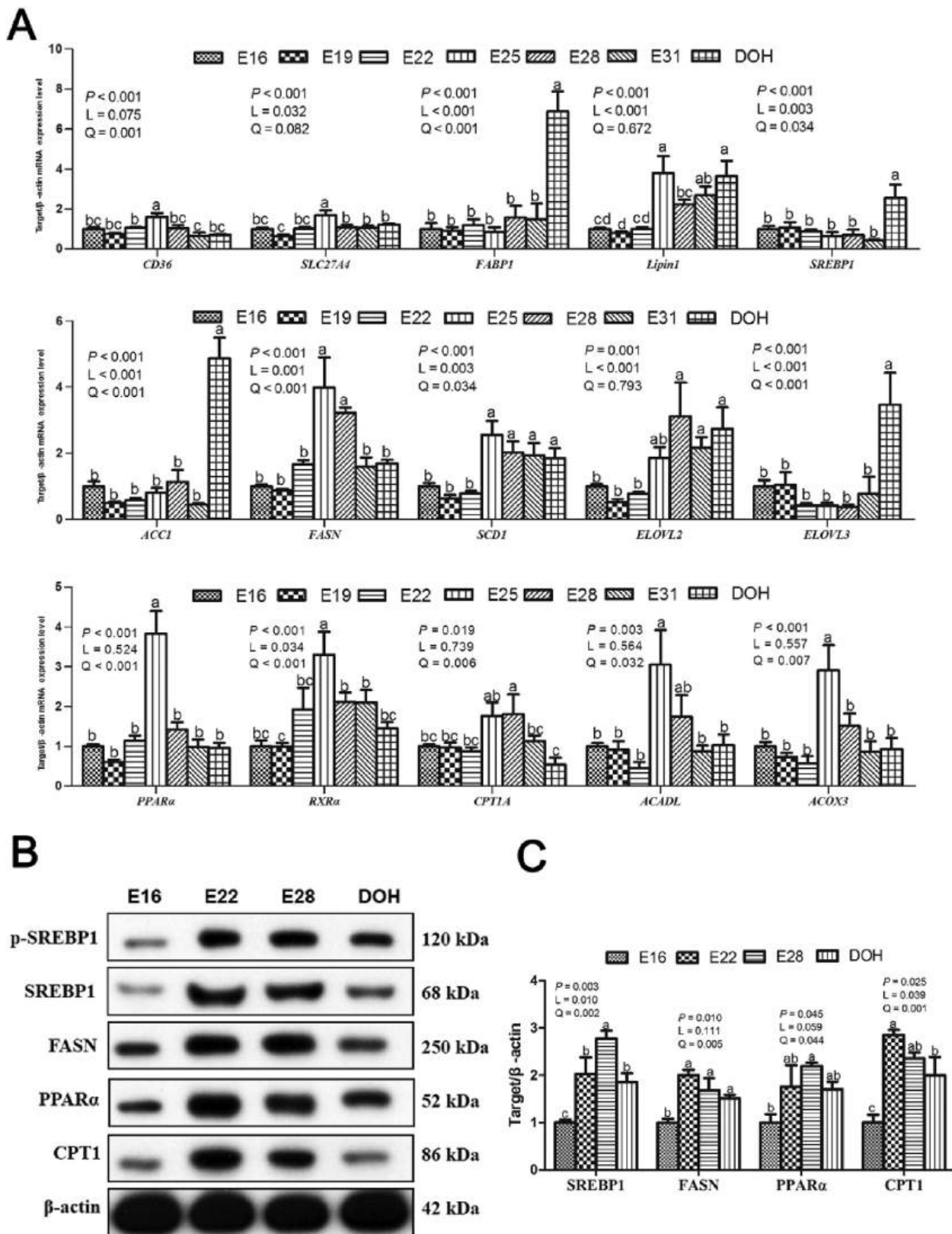


Fig. 3. Dynamic changes in hepatic gene and protein expression related to fatty acid metabolism in Muscovy duck embryogenesis. (A) Targeted gene relative expression ($n = 6$). (B, C) The band density in western blotting analysis was determined using Image J software ($n = 3$). *CD36* = CD36 molecule; *SLC27A4* = solute carrier family 27 member 4; *FABP1* = fatty acid-binding protein 1; *Lipin 1* = phosphatidate phosphatase 1; *SREBP1* = sterol regulatory element-binding transcription factor 1; *ACC1* = acetyl-CoA carboxylase alpha; *FASN* = fatty acid synthase; *SCD1* = stearoyl-CoA desaturase; *ELOVL2* = elongation of very-long-chain fatty acids protein 2; *ELOVL3* = elongation of very-long-chain fatty acids protein 3; *PPARα* = peroxisome proliferator-activated receptor alpha; *RXRα* = retinoid X receptor alpha; *CPT1A* = carnitine palmitoyltransferase 1A; *ACADL* = long-chain-acyl-CoA dehydrogenase; *ACOX3* = acyl-CoA oxidase 3. ^{a-d} Data are expressed as mean \pm SEM, and bars with different lowercase letters indicate statistically significant differences (one-way ANOVA, $P < 0.05$). L, linear; Q, quadratic.

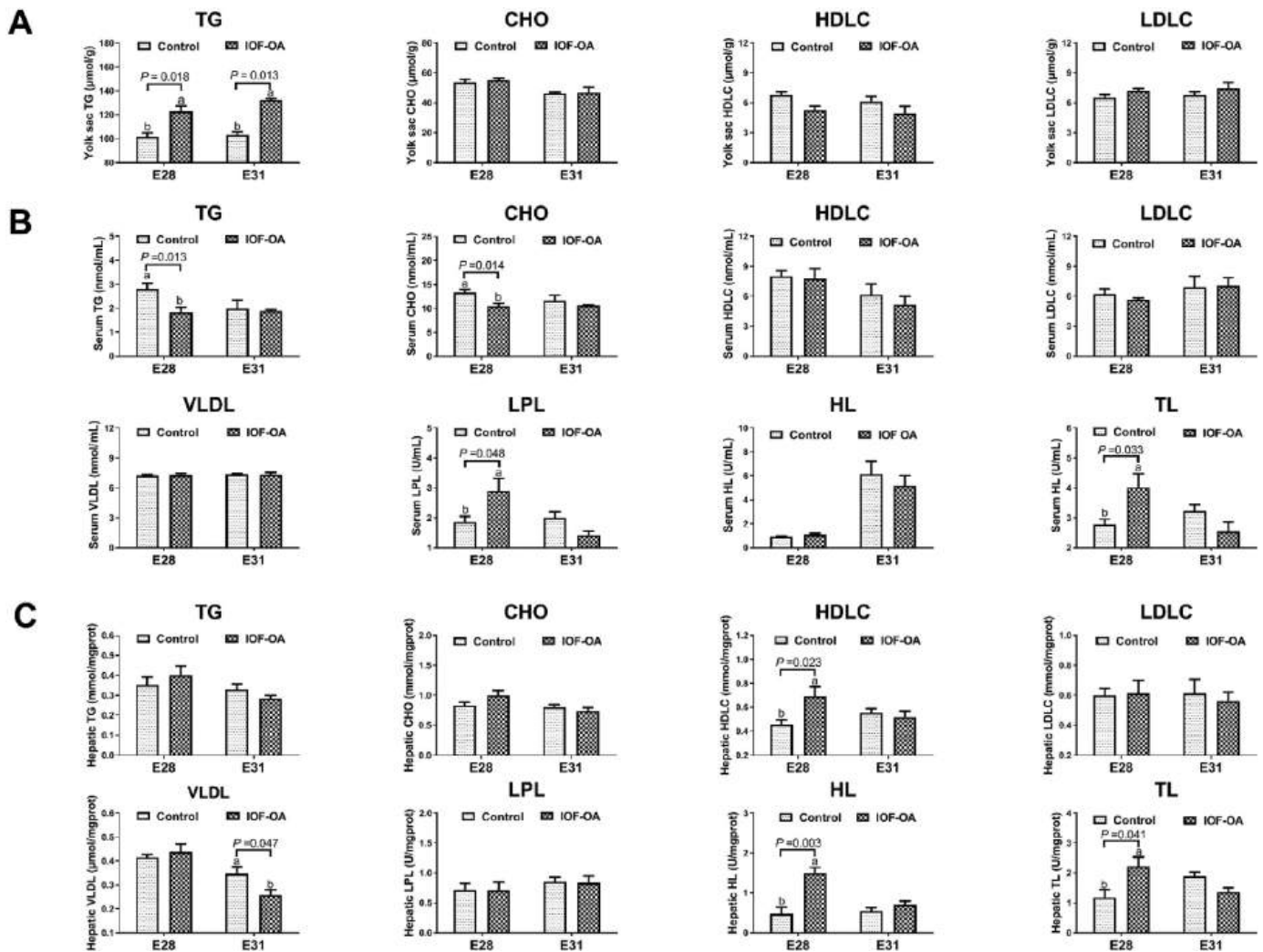


Fig. 4. Effect of in ovo feeding oleic acid on the changes in biochemical index in Muscovy duck embryogenesis. (A) yolk sac, as a fresh basis; (B) serum; (C) liver. Control group was in ovo injected with PBS, and IOF-OA was the treatment group which had in ovo feeding with oleic acid on E25. TG = triacylglycerol contents; CHO = total cholesterol contents; HDLC = high-density lipoprotein cholesterol contents; LDLC = low-density lipoprotein cholesterol contents; VLDL = very-low-density lipoprotein cholesterol contents; LPL = lipoprotein lipase activity; HL = hepatic lipase activity; TL = total lipase activity. ^{a-b} Data are expressed as mean \pm SEM ($n = 6$), and bars on the same sampled day with different lowercase letters indicate statistically significant differences (t -test, $P < 0.05$).

RXR α , *CPT1A*, *ACADL* and *ACOX3* genes related to FA oxidation all increased during E25–E28, which was partly regulated by the expressions of PPAR and CPT1. PPAR is of critical importance to catabolism and clearance of FA, and retinoid X receptors are obligate heterodimeric partners for PPAR action (Cai et al., 2021; Wang et al., 2020), while CPT1A is required for the transport of long-chain FA into mitochondria (Broadway et al., 2001). Accordingly, protein expression levels of SREBP1, FASN, PPAR α and CPT1 increased quadratically and peaked in the period of E22 to E28. Altogether, the results of mRNA and protein expression analyses suggest that FA synthesis and oxidation increased during E22 to E28 to meet the energy demands of embryo development.

During incubation, embryonic growth and development are dependent on nutrients deposited in the egg. However, the rapid growth of genetically improved birds can make the amount of nutrients contained in the egg insufficient for the complete development of embryonic tissues (Grodzick et al., 2013). In order to overcome these problems, IOF was developed to increase the availability of nutrients for the embryo. Previous studies have demonstrated the beneficial effects of IOF carbohydrates, amino acids, minerals, and vitamins on embryonic development but the

consequences of an intervention by FA are rarely reported (Foye et al., 2006; Tangara et al., 2010; Yair et al., 2015). In our study, the IOF-OA treatment increased the TG content of the yolk sac on E28 and E31, as well as the hepatic HDLC content and HL activity on E28, but decreased the TG and CHO contents of serum on E28 and hepatic VLDL content on E31. As a key component of the cell membrane, CHO is pivotal to the growth process of embryos, including the regulation of cellular signaling and production of bile acids and steroid hormones, and deficiencies in CHO during embryogenesis and organogenesis cause severe abnormalities (Connor et al., 1969; Roux et al., 2000; Woollett, 2008; Yoshida and Wada, 2005). In cell models, OA inhibits the synthesis of CHO and reduces its accumulation by decreasing the CHO biosynthesis rate and the influx of low-density lipoprotein while increasing the high-density lipoprotein-mediated efflux of CHO (Natali et al., 2007; Priore and Gnoni, 2017; Rosenblat et al., 2016). Hepatic FA metabolism is typically associated with energy homeostasis in growth and development, such that metabolic disorders of FA often lead to dysfunction and adverse physiological effects (Hodson, 2019; Migrenne et al., 2007). In avian embryo models, the FA composition in embryonic tissues could be affected by the maternal FA supply

Table 5

Effect of in ovo feeding oleic acid on changes in yolk sac fatty acid composition in Muscovy duck embryogenesis (%).

Item		E28				E31			
		Control	IOF-OA	SEM	P	Control	IOF-OA	SEM	P
Myristic acid	C14:0	0.97	0.99	0.082	0.782	0.96 ^b	1.30 ^a	0.151	0.049
Palmitic acid	C16:0	27.45	29.30	1.357	0.202	27.12	26.37	0.755	0.346
Palmitoleic acid	C16:1	2.79 ^b	4.35 ^a	0.860	0.010	3.51 ^a	2.29 ^b	0.512	0.038
Stearic acid	C18:0	4.78	4.09	0.326	0.060	4.90	4.27	0.751	0.421
Oleic acid	C18:1n-9	46.27 ^a	43.61 ^b	1.051	0.022	45.42	46.78	1.606	0.417
Linoleic acid	C18:2n-6	12.37	12.39	0.571	0.977	12.79	13.61	1.211	0.515
Linolenic acid	C18:3n-3	0.35	0.33	0.018	0.527	0.35	0.37	0.039	0.736
Eicosatrienoic acid	C20:3n-6	0.25	0.23	0.028	0.425	0.23	0.22	0.028	0.561
Eicosatrienoic acid	C20:3n-3	3.07	3.13	0.180	0.756	3.22 ^b	3.52 ^a	0.125	0.038
Nervonic acid	C24:1n-9	0.40	0.40	0.046	0.972	0.38	0.35	0.039	0.428
Docosahexaenoic acid	C22:6n-3	0.56 ^a	0.46 ^b	0.034	0.016	0.49 ^b	0.58 ^a	0.014	<0.001
MUFA		49.40	48.36	1.649	0.544	49.31	49.35	1.673	0.981
PUFA		16.60	16.51	0.641	0.885	17.08	18.19	1.269	0.401
n-6 PUFA		12.62	12.58	0.575	0.939	13.02	13.82	1.216	0.526
n-3 PUFA		3.98	3.93	0.204	0.811	4.05	4.37	0.192	0.134
n-6/n-3		3.19	3.22	0.197	0.876	3.20	3.20	0.313	0.998
UFA		66.00	64.87	1.641	0.506	66.39	67.54	1.285	0.393
SFA		33.20	34.39	1.567	0.466	32.98	31.73	1.286	0.353
UFA/SFA		2.01	1.90	0.137	0.447	2.02	2.14	0.126	0.363

Control group was in ovo injected with PBS, and IOF-OA was the treatment group which had in ovo feeding with oleic acid on embryonic day 25 (E25). MUFA is the sum of monounsaturated fatty acids that include C16:1, C18:1n-9 and C24:1n-9. PUFA is the sum of polyunsaturated fatty acids that include C18:2n-6, C20:3n-6, C18:3n-3, C20:3n-3 and C22:6n-3. N-6 PUFA is the sum of n-6 polyunsaturated fatty acids that include C18:2n-6 and C20:3n-6. N-3 PUFA is the sum of n-3 polyunsaturated fatty acids that include C18:3n-3, C20:3n-3 and C22:6n-3. UFA is the sum of unsaturated fatty acids that include MUFA and PUFA. SFA is the sum of saturated fatty acids that include C14:0, C16:0 and C18:0.

^{a,b} Data are expressed as mean \pm SEM ($n = 6$), and data on the same sampled day on the same line with different lowercase letters indicate statistically significant differences (t -test, $P < 0.05$).

and lipid utilization (Akbari et al., 2020; Cherian et al., 1997; Speake et al., 1999). Maternal dietary conjugated linoleic acid increased the SFA in the yolk sac and decreased hepatic UFA in chick or quail embryos, resulting in their lower hatchability rate and diminished chick weight upon hatching (Aydin and Cook, 2004; Fu et al., 2019; Leone et al., 2010). Here, the IOF-OA in duck embryos on E25 decreased the UFA percentage in serum and liver and down-regulated the hepatic mRNA expression of *CD36*, *SLC27A4* and *FABP1* genes related to FA uptake, and *Lipin1*, *SREBP1*, *ACCI*, *FASN*, *SCD1*, *ELOVL2* and *ELOVL3* genes related to FA synthesis on E28,

while there was no difference on these genes mRNA expression on E31. These inconsistent results between E28 and E31 implied that exogenous OA intervention may lose effectiveness on FA metabolism and target gene expression as development progresses. Additionally, the decreased hepatic mRNA expression of *PPAR α* , *RXR α* , *CPT1A* and *ACOX3* genes related to FA oxidation induced by the IOF-OA treatment might generate less energy to support embryonic growth and development on E28, contributing to higher embryonic mortality (control group vs IOF-OA group: 16.67% vs 21.67%).

Table 6

Effect of in ovo feeding oleic acid on changes in serum fatty acid composition in Muscovy duck embryogenesis (%).

Item		E28				E31			
		Control	IOF-OA	SEM	P	Control	IOF-OA	SEM	P
Myristic acid	C14:0	3.71	5.02	0.860	0.159	6.32	7.58	1.244	0.344
Palmitic acid	C16:0	20.94	20.77	1.081	0.876	21.24 ^a	19.47 ^b	0.904	0.039
Palmitoleic acid	C16:1	0.91	1.04	0.204	0.555	0.81	0.63	0.173	0.314
Stearic acid	C18:0	8.36	8.40	0.419	0.932	8.86 ^b	9.96 ^a	0.511	0.046
Oleic acid	C18:1n-9	35.08	37.08	1.740	0.280	35.29	33.32	1.549	0.231
Linoleic acid	C18:2n-6	12.48	11.18	0.696	0.093	10.42 ^a	8.77 ^b	0.549	0.013
Eicosatrienoic acid	C20:3n-6	1.16	1.02	0.308	0.671	0.51	0.75	0.251	0.361
Eicosatrienoic acid	C20:3n-3	11.37	10.12	2.062	0.557	10.67	11.94	1.187	0.312
Nervonic acid	C24:1n-9	1.06	0.54	0.368	0.233	0.64	0.69	0.094	0.604
Docosahexaenoic acid	C22:6n-3	2.03	2.10	0.128	0.580	1.89	2.09	0.309	0.529
MUFA		36.45	38.13	1.761	0.366	36.53	34.29	1.677	0.212
PUFA		27.03	24.08	2.143	0.199	23.32	23.42	1.513	0.949
n-6 PUFA		13.64 ^a	11.87 ^b	0.641	0.020	10.76 ^a	9.39 ^b	0.633	0.047
n-3 PUFA		13.40	14.09	1.673	0.511	12.56	14.03	1.210	0.254
n-6/n-3		1.05	0.85	0.120	0.128	0.88 ^a	0.68 ^b	0.073	0.021
UFA		63.68	62.21	1.753	0.424	61.95 ^a	59.99 ^b	0.471	0.049
SFA		34.33	33.35	1.839	0.608	35.36 ^b	38.00 ^a	0.728	0.047
UFA/SFA		1.86	1.89	0.116	0.797	1.71 ^a	1.58 ^b	0.061	0.048

Control group was in ovo injected with PBS, and IOF-OA was the treatment group which had in ovo feeding with oleic acid on embryonic day 25 (E25). MUFA is the sum of monounsaturated fatty acids that include C16:1, C18:1n-9 and C24:1n-9. PUFA is the sum of polyunsaturated fatty acids that include C18:2n-6, C20:3n-6, C20:3n-3 and C22:6n-3. N-6 PUFA is the sum of n-6 polyunsaturated fatty acids that include C18:2n-6 and C20:3n-6. N-3 PUFA is the sum of n-3 polyunsaturated fatty acids that include C20:3n-3 and C22:6n-3. UFA is the sum of unsaturated fatty acids that include MUFA and PUFA. SFA is the sum of saturated fatty acids that include C14:0, C16:0 and C18:0.

^{a,b} Data are expressed as mean \pm SEM ($n = 6$), and data on the same sampled day on the same line with different lowercase letters indicate statistically significant differences (t -test, $P < 0.05$).

Table 7

Effect of in ovo feeding oleic acid on the changes of hepatic fatty acid composition in Muscovy duck embryogenesis (%).

Item		E28				E31			
		Control	IOF-OA	SEM	P	Control	IOF-OA	SEM	P
Myristic acid	C14:0	5.77	5.90	0.644	0.854	5.08 ^b	8.23 ^a	0.528	<0.001
Myristoleic acid	C14:1	0.57 ^b	0.73 ^a	0.078	0.045	0.67	0.86	0.127	0.162
Palmitic acid	C16:0	16.96 ^a	14.95 ^b	0.799	0.033	16.91	17.10	1.020	0.850
Palmitoleic acid	C16:1	0.59	0.46	0.098	0.244	0.47	0.40	0.092	0.454
Stearic acid	C18:0	12.65 ^b	16.26 ^a	0.820	0.001	16.28	14.85	1.028	0.194
Oleic acid	C18:1n-9	30.18 ^a	27.47 ^b	1.536	0.048	26.86	27.49	1.665	0.714
Linoleic acid	C18:2n-6	8.68	8.41	0.607	0.668	8.09	8.24	0.887	0.871
Eicosatrienoic acid	C20:3n-6	0.34	0.45	0.073	0.182	0.41	0.43	0.061	0.708
Eicosatrienoic acid	C20:3n-3	14.14	16.19	1.053	0.084	15.40	16.32	1.323	0.504
Nervonic acid	C24:1n-9	0.89	0.69	0.210	0.375	0.95	1.01	0.112	0.595
Docosahexaenoic acid	C22:6n-3	6.21	6.14	1.007	0.947	6.49 ^a	3.00 ^b	0.737	<0.001
MUFA		31.73	29.36	1.606	0.173	28.87	29.69	1.790	0.656
PUFA		30.01	31.19	1.001	0.267	30.39	27.98	1.567	0.156
n-6 PUFA		9.03	8.86	0.615	0.797	8.50	8.67	0.867	0.847
n-3 PUFA		20.98	22.33	1.122	0.260	21.89	19.32	1.768	0.176
n-6/n-3		0.43	0.40	0.043	0.523	0.41	0.46	0.077	0.556
UFA		61.74	60.55	0.783	0.162	59.25 ^a	57.67 ^b	0.552	0.017
SFA		36.16	37.10	0.624	0.163	38.26	38.81	0.871	0.543
UFA/SFA		1.71	1.63	0.048	0.156	1.55	1.49	0.045	0.196

Control group was in ovo injected with PBS, and IOF-OA was the treatment group which had in ovo feeding with oleic acid on embryonic day 25 (E25). MUFA is the sum of monounsaturated fatty acids that include C14:1, C16:1, C18:1n-9 and C24:1n-9. PUFA is the sum of polyunsaturated fatty acids that include C18:2n-6, C20:3n-6, C20:3n-3 and C22:6n-3. N-6 PUFA is the sum of n-6 polyunsaturated fatty acids that include C18:2n-6 and C20:3n-6. N-3 PUFA is the sum of n-3 polyunsaturated fatty acids that include C20:3n-3 and C22:6n-3. UFA is the sum of unsaturated fatty acids that include MUFA and PUFA. SFA is the sum of saturated fatty acids that include C14:0, C16:0 and C18:0.

^{a,b} Data are expressed as mean \pm SEM ($n = 6$), and data on the same sampled day on the same line with different lowercase letters indicate statistically significant differences (t -test, $P < 0.05$).

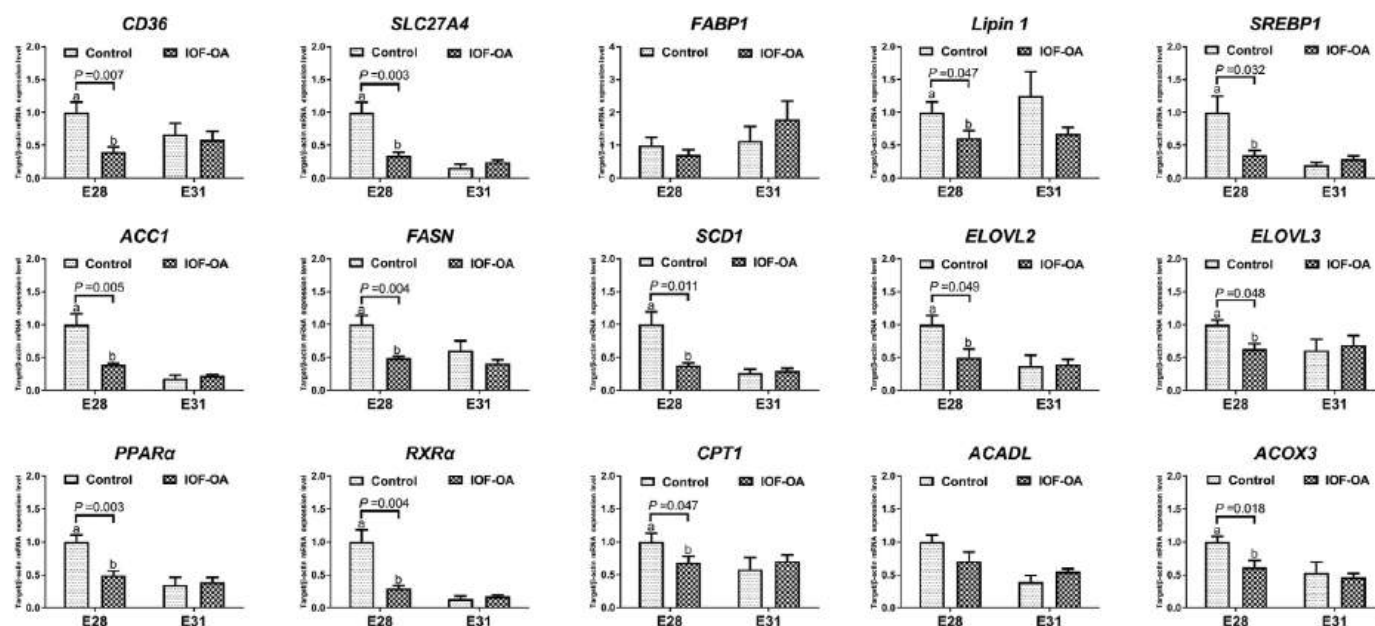


Fig. 5. Effect of in ovo feeding oleic acid on the gene expression related to hepatic fatty acid metabolism in Muscovy duck embryogenesis. Control group was in ovo injected with PBS, and IOF-OA was the treatment group which had in ovo feeding with oleic acid on E25. *CD36* = CD36 molecule; *SLC27A4* = solute carrier family 27 member 4; *FABP1* = fatty acid-binding protein 1; *Lipin 1* = phosphatidate phosphatase 1; *SREBP1* = sterol regulatory element-binding transcription factor 1; *ACC1* = acetyl-CoA carboxylase alpha; *FASN* = fatty acid synthase; *SCD1* = stearoyl-CoA desaturase; *ELOVL2* = elongation of very-long-chain fatty acids protein 2; *ELOVL3* = elongation of very-long-chain fatty acids protein 3; *PPARα* = peroxisome proliferator-activated receptor alpha; *RXRα* = retinoid X receptor alpha; *CPT1A* = carnitine palmitoyltransferase 1A; *ACADL* = long-chain-acyl-CoA dehydrogenase; *ACOX3* = acyl-CoA oxidase 3. ^{a,b} Data are expressed as mean \pm SEM ($n = 6$), and bars on the same sampled day with different lowercase letters indicate statistically significant differences (t -test, $P < 0.05$).

5. Conclusion

In summary, the maximal metabolic changes in lipid metabolism in both the liver and serum of Muscovy duck embryos occurred from E22 to E28, along with altered levels of target gene and protein expression related to lipogenesis and lipolysis. In ovo feeding with oleic acid on E25 could inhibit the target gene

expression related to FA uptake, synthesis and oxidation, which may influence normal FA metabolism of duck embryos on E28.

Author contributions

Xiufen Zhang: conceptualization, formal analysis, data curation, writing original draft. **Qilin Wu:** formal analysis, project

administration. **Wenxuan Zheng**: formal analysis, methodology. **Chuang Liu**: resources, software. **Liang Huang**: methodology, data curation. **Xin Zuo**: formal analysis. **Wenquan Xiao**: formal analysis. **Xiaofeng Han**: formal analysis. **Hui Ye**: data curation. **Wence Wang**: data curation. **Lin Yang**: supervision, funding acquisition, and project administration. **Yongwen Zhu**: supervision, funding acquisition, project administration, writing-reviewing, and editing.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Nutrition and Disease

Evidence from an Avian Embryo Model that Zinc-Inducible MT4 Expression Protects Mitochondrial Function Against Oxidative Stress



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ABSTRACT

Background: Metallothioneins (MTs) have a strong affinity for zinc (Zn) and remain at a sufficiently high level in mitochondria. As the avian embryo is highly susceptible to oxidative damage and relatively easy to manipulate in a naturally closed chamber, it is an ideal model of the effects of oxidative stress on mitochondrial function. However, the protective roles and molecular mechanisms of Zn-inducible protein expression on mitochondrial function in response to various stressors are poorly understood.

Objectives: The study aimed to investigate the mechanisms by which Zn-induced MT4 expression protects mitochondrial function and energy metabolism subjected to oxidative stress using the avian embryo and embryonic primary hepatocyte models.

Methods: First, we investigated whether MT4 expression alters mitochondrial function. Then, we examined the effects of Zn-induced MT4 overexpression and MT4 silencing on embryonic primary hepatocytes from breeder hens fed a normal Zn diet subjected to a *tert*-butyl hydroperoxide (BHP) oxidative stress challenge during incubation. In vivo, the avian embryos from hens fed the Zn-deficient and Zn-adequate diets were used to determine the protective roles of Zn-induced MT4 expression on the function of mitochondria exposed to oxidative stress induced by in ovo BHP injection.

Results: An in vitro study revealed that Zn-induced MT4 expression reduced reactive oxygen species accumulation in primary hepatocytes. MT4 silencing exacerbated BHP-mediated mitochondrial dysfunction whereas Zn-inducible MT4 overexpression mitigated it. Another in vivo study disclosed that maternal Zn-induced MT4 expression protected mitochondrial function in chick embryo hepatocytes against oxidative stress by inhibiting the peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α)/peroxisome proliferators-activated receptor- γ (PPAR- γ) pathway.

Conclusion: This study underscores the potential protective roles of Zn-induced MT4 expression via the downregulation of the PGC-1 α /PPAR- γ pathway on mitochondrial function stimulated by the stress challenge in the primary hepatocytes in an avian embryo model. Our findings suggested that Zn-induced MT4 expression could provide a new therapeutic target and preventive strategy for repairing mitochondrial dysfunction in disease.

Keywords: avian embryo model, metallothionein, mitochondrial function, oxidative stress, zinc

Abbreviations: 8-OHdG, 8-hydroxy-2-deoxyguanosine; BHP, *tert*-butyl hydroperoxide; E18, 18-d embryo age; HEPES, N-2-hydroxyethylpiperazine-n-ethane-sulphonic acid; MMP, mitochondrial membrane potential; MT4, metallothionein 4; mtDNA, mitochondrial DNA; OCR, oxygen consumption rate; PBS, phosphate buffer saline; PGC-1 α , peroxisome proliferator-activated receptor γ coactivator-1 α ; PPAR- α , peroxisome proliferators-activated receptor- α ; PPAR- γ , peroxisome proliferators-activated receptor- γ ; PVDF, polyvinylidene difluoride; ROS, reactive oxygen species; Zn, zinc.

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Introduction

Mitochondria are energy-generating organelles that occur in most eukaryotic cells [1]. They are also major reactive oxygen species (ROS) producers and targets [2]. Adaptation to various physiological demands requires an optimal balance between ROS production and scavenging [3]. Various stressors may cause mitochondria to generate excessive ROS which, in turn, impairs their integrity and biogenesis and reduces their energy metabolism by suppressing oxidative phosphorylation and adenosine triphosphate (ATP) production [4]. The avian embryo develops in an egg. Although the latter is a naturally closed chamber, it is also nonetheless highly vulnerable to oxidative damage induced by external and internal stressors during embryogenesis [5]. During the mid-late phase of embryo formation, the internal organs rapidly develop, mitochondrial β -oxidation is enhanced, and energy demand increases to accommodate physiological demands [6]. The exposure of saturated fatty acids to atmospheric oxygen and the accumulation of polyunsaturated fatty acids in the lipids of mitochondrial membranes increase the susceptibility of chick embryos to oxidative stress [7]. Excessive ROS causes mitochondrial dysfunction and retards or arrests growth [8]. Moreover, as the embryos of birds are easier to maintain and manipulate than those of most other vertebrate species, they are ideal as models for investigating the effects of oxidative stress on mitochondrial function. The peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α)-peroxisome proliferators-activated receptor (PPAR)-dependent signaling pathway [9] is implicated in the regulation of mitochondrial genes [10]. Nevertheless, the mechanisms of mitochondria dysfunction remain to be elucidated.

Metallothioneins (MTs) are cysteine-rich (30%–33% of total amino acid), low molecular weight protein (6–7kDa) with a strong affinity for zinc (Zn) and other heavy metals [11]. Mitochondria have the highest MT content of all organelles [12]. Intracellular MT concentrations are very low in normal cells. In response to stress, however, MTs are rapidly induced and remain at high levels long enough to alter mitochondrial function [13]. The MTs family is widespread in mammals [14], plants [15], and microorganisms [16], but is slightly under-reported in avian studies. Metallothioneins 4 (MT4) as the main isoform of MTs in poultry is predicted to maintain cellular Zn ion homeostasis and participate in the cellular response subjected to stress challenge [17]. MT4 upregulation has been detected in the liver, pancreas, kidneys, and intestinal mucosae of chickens under oxidative stress [18]. MT4 may protect the mitochondria against oxidative damage by enhancing their antioxidant capacity [19]. An in vitro study demonstrated that MTs modulate mitochondrial function by transferring electrons to cytochrome and ATP to maintain energy homeostasis by regulating mitochondrial respiration and ATP production [20]. A prior study showed that MT-I and MT-II knockout mice exhibited mild obesity and hyperleptinaemia [21]. In contrast, other investigators demonstrated that MTs reduce oxygen consumption [22] and inner membrane permeability [12] in distressed cells and isolated mitochondria, respectively. Hence, there are conflicting conclusions regarding the mechanisms by which MTs regulate mitochondrial function, especially in response to stressors. Furthermore, it is unknown whether MT4 is implicated in the regulation of mitochondrial function via the PGC-1 α /peroxisome proliferators-activated

receptor- γ (PPAR- γ) signaling pathway. In this work, we used avian embryo and embryonic primary hepatocyte models to explore the putative mechanisms by which Zn-induced MT4 expression protects mitochondrial function and energy metabolism against oxidative stress.

Methods

Ethic statement

The animal care and use protocol was approved by the Animal Care and Use Committee of South China Agricultural University (SCAU-10564), and this study was conducted following the Regulations on the Administration of Affairs Concerning Experimental Animals.

Primary hepatocytes culture and treatments

Primary hepatocytes was isolated from the chick on 18-d embryo age (E18) of breeder hens fed a normal Zn diet and then cultured in a Zn-deficient medium as described previously [23]. In brief, liver tissue was digested with 3–5 volumes of 0.1% type IV collagenase (C4-28, Merck) at 37°C for 20 min. Next, an equal volume (v) of complete medium was added to terminate the digestion process. After subsequent centrifugation at $700 \times g$ for 8 min, cells were suspended in a complete medium and seeded in cell culture flasks, with the medium changed 3 times a week. To investigate the effect of Zn on MT4 expression, cells were treated with different Zn concentrations at 0, 20, and 50 μ M using Zn sulfate (7733-02-0, Sigma-Aldrich) in a Zn-deficient medium. A CCK-8 kit (CA1210, Solarbio Co., Ltd.) was used to determine cell viability. The dose effects of 0, 10, 20, 50, and 100 μ M *tert*-butyl hydroperoxide (BHP) incubation (100 μ M H₂O₂ as positive control) on ROS production as well as 0 μ M Zn incubation on MT4 expression in embryonic primary cells, respectively (Exp. 1).

Cell transfection for MT4 silencing

Primary hepatocytes from hens fed a normal Zn diet were transfected using JetPRIME Transfection Reagent according to the manufacturer's instructions (Polyplus Transfection). Before transfection, 4 μ L of siRNA (20 μ M) and 200 μ L of jetPRIME were mixed and vortexed for 10 s, then supplemented with 4 μ L jetPRIME RNAiMAX transfection reagent, mixed for 1 s and incubated at room temperature for 10 min. Finally, 200 μ L of the mixture was added to the well and mixed gently by rocking the plate back and forth. FAM-siRNA was used to confirm the transfection efficiency of siRNAs in broiler chick primary hepatocytes. The siRNAs used in the present study were synthesized by a commercial company (GenePharma), and primer sequences are listed in Supplemental Table 1. First, primary hepatocytes with and without MT4 silencing were treated with 50 μ M Zn to study the effect of Zn-induced MT4 expression on mitochondrial function (Exp. 2). The BHP as a short-chain analog of lipid hydroperoxide has been explored to induce oxidative damage on mitochondrial homeostasis and energy metabolism in the rat and cell [24–26]. Therefore, BHP has been used to induce mitochondrial oxidative stress in vivo and in vitro in this study [27]. Therefore, to verify the protective effect of Zn-induced MT4 expression against BHP-mediated cellular oxidative stress, cells were pretreated in the presence or absence of 50 μ M Zn for 12 h

and subsequently treated BHP for 2 h afterward according to the published study [23] (Exp. 3).

Animal feeding experiments

The feeding experimental procedures were described in detail in our previous study [28]. In brief, a total of 144 21-wk-old yellow-feathered broiler breeders were obtained from a commercial farm (Jilong Group) and reared in the caged for an adaptation period from 21 to 28 wk old. Subsequently, all female breeders were fed with a semipurified diet containing 26.34 mg Zn/kg from 29 to 30 wk of age to deplete Zn stores; the recommendation is 220 mg/kg of Zn sulfate in hen diets. After that, laying broiler breeders were randomly allotted into 2 dietary treatments with unsupplemented (control group; 0 mg Zn/kg) and supplemental 220 mg Zn/kg diet as Zn sulfate (Zn group) to meet the Zn requirement of laying female broiler breeders according to the Nutrient requirements of Yellow Chickens in China (NY/T 3645-2020), each treatment with 6 replicates of 12 birds per replicate for the experimental period from 31 to 36 wk old. The corn–soybean meal basal diet contained 11.63 MJ metabolizable energy/kg, 185.1 g crude protein/kg, 9.1 g lysine/kg, 8.4 g methionine + cysteine/kg, 37.0 g calcium/kg, and 4.4 g available phosphorus/kg. The measured Zn contents were 29.2 and 245.6 mg Zn/kg diet in the control and Zn-supplemented diets, respectively. To study the dynamic change of Zn-induced MT4 expression during embryogenesis, a total of 24 embryos (4 embryos per replicate) from hens fed low (control group) and high Zn (Zn group) diets on the first 3 d of 35 wk of age were collected from embryos at E13, E15, E17, and E19, respectively (Exp. 4). On the last 3 d of 35 wk of age, 60 fertilized eggs were collected from hens fed the control diet and then were divided into 2 groups by in ovo injections of phosphate buffer saline (PBS) and Zn solutions (Exp. 5). On the last 3 d of 36 wk of age, a total of 120 fertilized eggs from each treatment were subjected to in ovo injections of PBS and BHP (Exp. 6), respectively. Injection concentrations and methods are described in the following section.

In ovo injection procedure

The procedure of in ovo injection procedure was carried out in chick embryos from yellow-feathered breeders on E18 according to the description previously [29]. Before injections, the outline of the air cell and the yolk sac was identified under the candle, and unfertilized eggs were removed. Fertilized eggs were sterilized with 75% ethanol and then injected with sterile disposable 25.0 × 0.6 mm needles attached to a 1.0 mL syringe, the syringe was changed after each egg injection. The Zn sulfate (Superior Purity, 99.5%, Tianjin Fuchen Chemical Reagent Co.) and BHP (458139, Sigma) were dissolved in a PBS solution to prepare a stock solution containing 50 µM Zn (Exp. 5) and 600 µM BHP (Exp. 6), respectively. All solutions were filtered using a 0.22-µm acetate filter (MSI). After injection of 0.1 mL of the stock solutions of either BHP or Zn sulfate, respectively, the holes were sealed with medical adhesive tape (1.0 × 1.0 cm²), and the eggs from each replicate of each treatment were placed on the same egg tray and incubated at 38.0°C and 55% relative humidity. At E19, 24 embryos (4 per replicate) from each treatment were killed by cervical dislocation. The liver from the embryos was immediately dissected and frozen in liquid nitrogen and then stored at −80°C for further analyses.

Isolation of hepatic mitochondria

In Exp. 4–6, the liver tissues from each replicate were sampled and then were pooled with the equal weight for the isolation of hepatic mitochondria. The mix liver samples were homogenized in 10 vol cold buffer A [1 mg/mL fatty acid-free bovine serum albumin, 75 mM sucrose, 225 mM mannitol, 10 mM N-2-hydroxyethylpiperazine-n-ethane-sulphonic acid (HEPES), and 0.1 mM ethyleneglycotetraacetic acid (EGTA), pH 7.4] with a polytetrafluoroethylene Dounce homogenizer and then subjected to differential centrifugation to separate mitochondria (700 × g for 15 min and then 10,000 × g for 15 min at 4°C). The supernatant containing cytoplasm was used for analyses related to oxidative damage, whereas the precipitate containing mitochondria was resuspended in buffer B (10 mM HEPES, 0.1 mM EGTA, and 395 mM sucrose, pH 7.4) to determine the total protein and oxygen consumption (VO₂) using the quantitative assay kit (A045-4-2, Jiancheng Institute of Bioengineering) and Clark-type oxygen electrode, respectively. All experiments were performed at 25°C. Additions were made at intervals of 4 min.

Determination of ROS, mitochondrial membrane potential, and ATP contents

Intracellular ROS of cells in Exp. 1–3 and liver samples in Exp. 4–6 was measured using a nonfluorescent probe that can freely penetrate the intracellular matrix and be oxidized by ROS to dichloride fluorescein (DCF), which was namely 2,7-dichloride-hydro fluorescein diacetate (DCFH-DA; CT0045, Leigen Institute of Biotechnology). The fluorescence intensity of DCF reflecting the cellular level of ROS was measured by excitation and emission at 485 and 525 nm, respectively. The mitochondrial membrane potential (MMP) was measured in Exp. 2, 3, and 5 with JC-1 (5 µg/mL, DF0080, Leigen Institute of Biotechnology) as described in the manufacturer's instructions. JC-1 forms J-aggregates and emits red fluorescence at high MMP, whereas the JC-1 monomeric form emits green fluorescence at low MMP. Both colors were detected using a fluorescence microplate reader (FLx800, Bio-Tek). The ATP level was analyzed (Exp. 2, 3, 5, and 6) using a fluorescence microplate reader, and the ATP was determined by the bioluminescence method using a commercial assay kit (S0026, Beyotime Biotechnology Co., Ltd.) as described by Hung et al. [30].

Determination of Zn, 8-OHdG, MT4, lactate, and pyruvate contents

The embryonic liver samples were collected to analyze Zn contents (Exp. 4 and 5), which were measured using an inductively coupled plasma emission spectroscope (IRIS Intrepid II, Thermal Jarrell Ash) after wet digestions with HNO₃ and HClO₄, as described previously [31]. The contents of 8-hydroxy-2-deoxyguanosine (8-OHdG) (Exp. 5 and 6) and MT4 (Exp. 1–6) were measured using commercial kits according to the manufacturer's instructions (H165 and A006-2-1, Jiancheng Institute of Bioengineering). Lactate and pyruvate levels (Exp. 5 and 6) were determined with a lactate kit (A019-2-1) and a pyruvate kit (A081-1-1) (Nanjing Jiancheng Bioengineering Institute), respectively. The concentrations of total protein in cytoplasm and mitochondria were determined as described before and all indices were expressed as units per milligram of protein.

Determination of OCR

The oxygen consumption rate (OCR) of hepatocytes without BHP treatment (Exp. 2) was measured using Seahorse XF96 equipment (Seahorse Bioscience Inc., Institute of Molecular and Cell Biology). Hepatocytes (25,000 per well) were equilibrated for 1 h in a non-CO₂ incubator at 37°C after 24 h of seeding before measurement. Oligomycin (1 μM; Agilent, 103015–100), CCCP (0.5 μM; Sigma-Aldrich, C2759), rotenone (1 μM; Agilent 103015–100), and antimycin (1 μM; Agilent, 103015–100) were subsequently injected into cells at the indicated time points. The OCR of hepatocytes subjected to the BHP incubation was measured (Exp. 3) using an Oxygen Consumption Rate Assay Kit (600800, Cayman Chemical).

Histological analysis

The histological study was performed as described previously [32]. Briefly, formalin-fixed embryonic liver samples were embedded in paraffin and sectioned at 5 μM for staining with hematoxylin and eosin. For the ultrastructural study, tissue samples were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer for 1 h at 48°C. After washing in 0.1 M phosphate buffer, tissue samples were embedded in agar chips and further postfixed with 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2). They were dehydrated, then embedded in Araldite/Epon (Electron Microscopy Sciences), and sectioned into semithin slices for toluidine blue staining. The ultrathin sections were contrasted with uranyl acetate and lead citrate and then observed by transmission electron microscopy at 200 kV.

Real-time quantitative PCR analysis

Total RNA from samples was isolated with an RNA Purification Kit (B0004DP, EZBioscience Co., Ltd.). cDNA synthesis was performed using the Color Reverse Transcription Kit (A0010CGQ, EZBioscience Co., Ltd.). A 2 × Color SYBR Green qPCR Master Mix kit (A0012-R2, EZBioscience Co., Ltd.) was used for subsequent RT-qPCR amplification on a Bio-Rad C1000 Touch Thermal Cycler (Bio-Rad). The expression of each gene was normalized to that of the reference gene of β-actin according to the 2^{−ΔΔCt} method. The primers used are listed in [Supplemental Table 1](#).

Mitochondrial DNA copy number quantification

Mitochondrial DNA (mtDNA) copy number was determined using the RT-qPCR method as described previously [33]. Genomic DNA was extracted using TIANamp Genomic DNA Kit (D1700, Solarbio) according to the manufacturer's instructions. The concentration of DNA in the extracts was measured by a NanoDrop 2000 Spectrophotometer (Thermo Scientific). DNA primer was designed to detect ATP6 as the maker for mtDNA ([Supplemental Table 1](#)). The quantification of mtDNA copy number was normalized to that of the reference gene of GCG.

Western blot

Total protein was extracted with radioimmunoprecipitation assay buffer containing 1 mM phenylmethanesulfonylfluoride (ST506, Beyotime Biotechnology) and quantified with a bicinchoninic acid assay kit (A045-4-2, Nanjing Jiancheng Bioengineering Institute). The lysates were heated at 100°C for 10 min and then stored at −20°C. A total of 60 μg of protein was

loaded on a 1.5 mm SDS–PAGE gels and blotted onto a polyvinylidene difluoride (PVDF) membrane at 260 mA for 1.5 h. After blocking in 5% skim milk for 2 h, PVDF membranes were incubated with primary antibody at 4°C overnight. The following primary antibodies were used according to [Supplemental Table 1](#). After incubation with a proper secondary HRP-labeled antibody (Vector Laboratories), western blot signals were detected via chemiluminescence with ChemiDoc Imaging Systems (Bio-Rad). The information on the antibodies used for western blot is listed in [Supplemental Table 2](#).

Statistical analysis

Data were presented as the mean ± SEM. GraphPad Prism was applied for graphing and statistical analysis (version 7, GraphPad Software). Data were compared using an unpaired Student's *t*-test or a 1-way or 2-way ANOVA followed by a Tukey's multiple-comparison post-hoc test. *P* < 0.05 was considered to indicate a statistically significant difference.

Results

Zn-induced *MT4* expression augments the ability of the mitochondria in embryonic primary hepatocytes to reduce ROS accumulation

Here, we investigated whether Zn-induced *MT4* overexpression and *MT4* silencing alter mitochondrial function in embryonic primary hepatocytes. Primary hepatocytes were isolated from chick embryos and cultivated on an E18 medium ([Supplemental Figure 1](#)). *MT4* overexpression and silencing models were established in embryonic primary hepatocytes to determine the role of *MT4* in mitochondrial function in vitro. Relative *MT4* mRNA and protein expression increased 2 or 3-fold in embryonic primary hepatocytes incubated with Zn in a Zn-deficient medium ([Figure 1C, D](#)). In contrast, relative *MT4* mRNA and protein expression had decreased by ~45% at 48 h after *MT4* siRNA transfection in embryonic primary hepatocytes ([Figure 1A, B](#)).

Zn-induced *MT4* expression reduces ROS accumulation in primary hepatocytes ([Figure 1E, F](#)) and the ROS and 8-OHdG levels in embryonic livers ([Supplemental Figure 2D, F](#)). In ovo Zn injection confirmed these findings ([Supplemental Figure 2A–D, F](#)). Conversely, *MT4* silencing promotes mitochondrial ROS accumulation in embryonic primary hepatocytes. Hence, *MT4* loss may intensify the sensitivity of embryonic primary hepatocytes to oxidative stress. Zn treatment restores ATP biosynthesis in particular and mitochondrial function in general in *MT4*-silenced embryonic primary hepatocytes ([Figure 1H](#)). Moreover, increased hepatic ATP and decreased PA and LD levels were detected in chick embryos injected with Zn in ovo ([Supplemental Figures 2G–I](#)). The induced changes in *MT4* expression had no apparent impact on the matrix metalloprotein (MMP) levels or OCR in embryonic primary hepatocytes ([Figure 1G, I](#)) or the MMP levels in embryonic livers ([Supplemental Figure 2E](#)).

MT4 silencing exacerbates BHP-mediated mitochondrial dysfunction and the latter is restored by Zn-induced *MT4* expression

As Zn-induced *MT4* expression alters mitochondrial properties, we investigated its role in protecting mitochondrial function

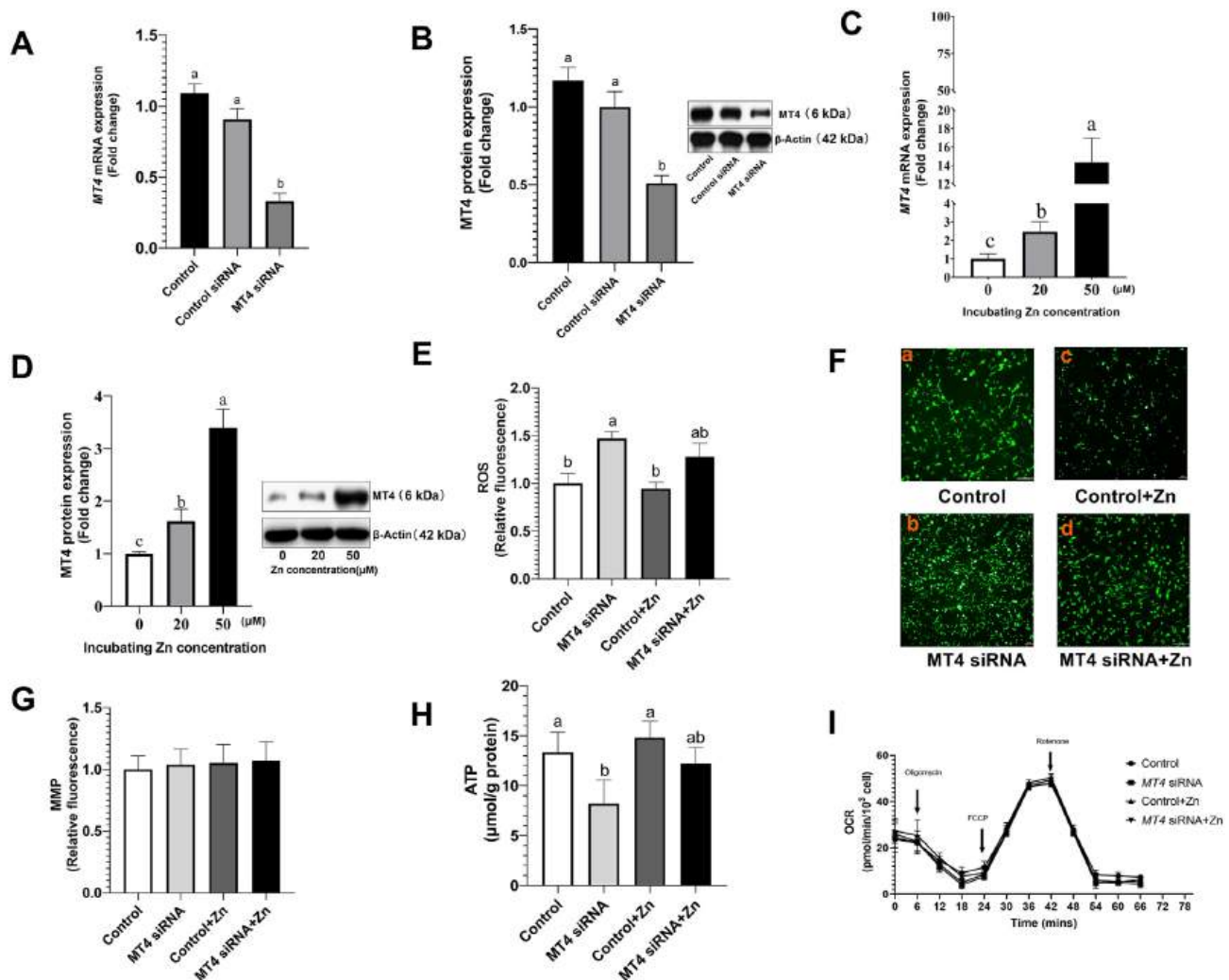


FIGURE 1. Zn-induced *MT4* expression enhances mitochondrial function in embryonic primary hepatocytes. (A, B) Effect of *MT4* siRNA transfection on mRNA (A) and protein (B) expressions of *MT4* in embryonic primary hepatocytes. (C, D) Effect of incubation Zn concentration from 0 to 50 μM on mRNA (C) and protein (D) expressions of *MT4* in embryonic primary hepatocytes and 50 μM Zn concentration was selected for Zn-induced *MT4* overexpression. (E–I) Effect of *MT4* siRNA transfection or Zn on intracellular ROS accumulation (E and F), MMP (F), ATP (H), and OCR (I) in embryonic primary hepatocytes. Graph bars marked with different letters on top represent statistically significant results ($P < 0.05$, $n = 4–6$) based on 'Tukey's post-hoc analysis, whereas bars labeled with the same letter correspond to results that show no statistically significant differences. Data were mean \pm SEM.

against the negative effects of various stressors. The oxidant BHP is used to induce oxidative stress in mammalian cell mitochondria [34]. Here, BHP-mediated oxidative damage markedly increased the ROS (Figure 2A) and decreased the MMP (Figure 2D, E) levels (Exp. 1 and 3). The loss of mitochondrial integrity and the inhibition of mitochondrial respiration were associated with decreased ATP levels and OCR (Figure 2F, G). Furthermore, *MT4* silencing promoted intracellular ROS accumulation (Figure 2B, C) and the reduction of MMP (Figure 2D, E), ATP, and OCR (Figure 2F, G) in embryonic primary hepatocytes incubated with BHP. However, Zn pretreatment alleviated BHP-mediated mitochondrial dysfunction in the embryonic primary hepatocytes (Figure 2B–G).

Maternal Zn-induced *MT4* expression in avian embryos protects hepatic mitochondrial function and energy metabolism against oxidative stress

As reported previously [29], the maximal rate of Zn mobilization occurred in chick embryos at the later embryonic stage between E18 and E21, associated with the significant increases of gene expression related to some key Zn transporters (such as *MTs*) [35]. Our results confirmed that maternal Zn supply was deposited in the egg yolk and transferred to the liver (target organ) (Figure 3A) of the developing embryo. Maternal Zn supplementation increased hepatic *MT4* mRNA expression seven-fold in the embryonic liver on E19 (Figure 3B). The Zn-induced *MT4* expression was confirmed following the in ovo Zn injection

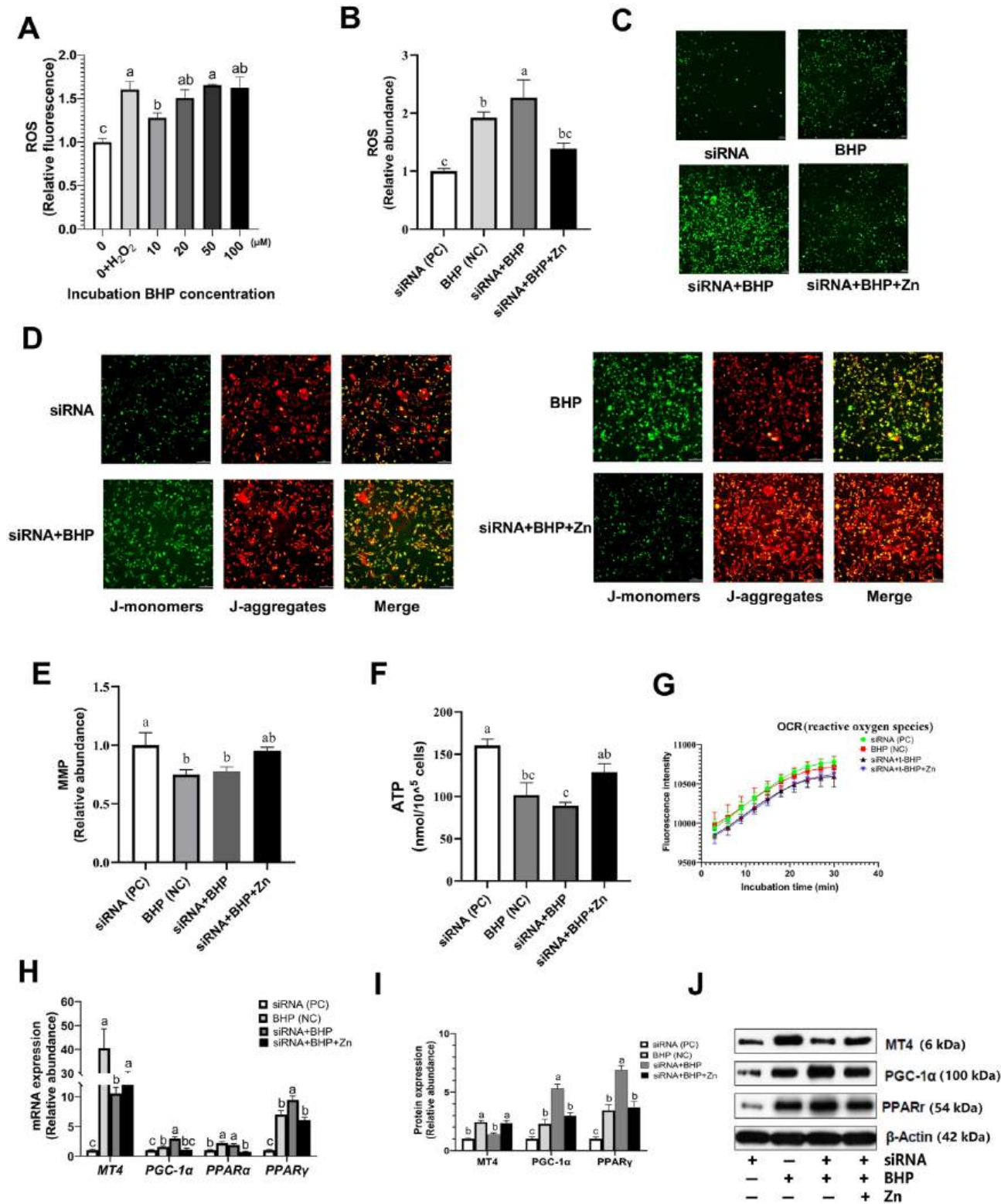
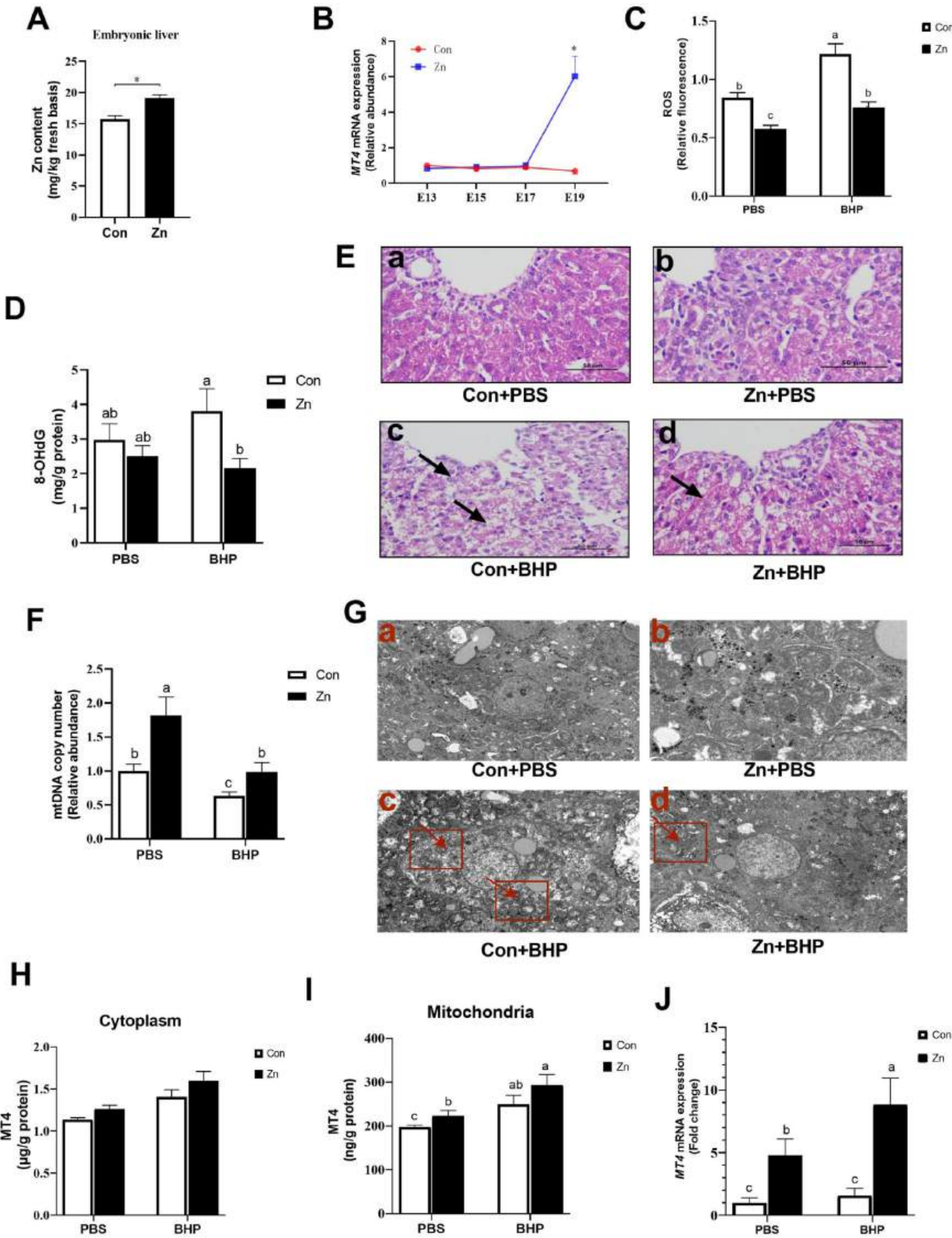


FIGURE 2. *MT4* silence aggravated BHP-mediated mitochondrial dysfunction and was restored by Zn-induced *MT4* expression. (A) Effect of incubation BHP concentration from 0 to 100 μ M on ROS generation in embryonic primary hepatocytes. 100 μ M H_2O_2 as positive control and 50 μ M BHP was selected for BHP-mediated oxidative stress. (B–G) Effect of *MT4* siRNA transfection, BHP, or Zn on intracellular ROS accumulation (B, C), MMP (D, E), ATP (F), and OCR (G) in embryonic primary hepatocytes. (H–J) Effect of *MT4* siRNA transfection, BHP, or Zn on mRNA and protein expression of *MT4* and genes related to the PGC-1 α /PPAR- γ pathway in embryonic primary hepatocytes. Intracellular ROS generation and MMP were measured by DCFH-DA and JC-1 staining, respectively. In the presence of a high MMP, JC-1 forms J-aggregates that emit red fluorescence, whereas JC-1 monomeric form emits green fluorescence at low MMP. Graph bars marked with different letters on top represent statistically significant results ($P < 0.05$, $n = 4–6$) based on Tukey’s post-hoc analysis, whereas bars labeled with the same letter correspond to results that show no statistically significant differences. Data were mean \pm SEM.



(caption on next page)

(Exp. 5, [Supplemental Figure 2A](#)). Therefore, in ovo BHP injection on E18 was then selected to clarify the protective role of maternal Zn supplementation on mitochondrial function. In ovo BHP injection (Exp. 6) decreased mtDNA copy number ([Figure 3F](#)) and increased ROS ([Figure 3C](#)) and *MT4* mRNA expression ([Figure 3J](#)) in liver, and increased the *MT4* contents in the hepatic cytoplasm and mitochondria ([Figure 3H, I](#)) regardless of in ovo treatments. Histological staining showed that in ovo BHP injection caused necrosis and inflammatory cell infiltration while reducing mitochondrial density and condensing mitochondrial membranes in the hepatocytes ([Figure 3E](#)). This abnormal mitochondrial morphology resulted in decreased ATP production ([Figure 4A](#)), upregulation of the target genes related to energy metabolism, namely, *L-CPT1* and *L-CPT2* ([Figure 4D, E](#)), and increased hepatic LD but not PA content ([Figure 4B, C](#)).

Maternal Zn supplementation increased hepatic *MT4* mRNA ([Figure 3J](#)) and protein expression ([Figure 4J](#)) as well as cytoplasmic and mitochondrial *MT4* ([Figure 3H, I](#)) regardless of stressor interventions. However, it had no apparent influence on laying performance ([Supplemental Figure 3](#)). In the absence of stress, maternal Zn supplementation had no positive impact on mitochondrial structure or function ([Figure 3G](#)). However, maternal Zn-induced *MT4* expression offset any in ovo BHP injection-induced increase in hepatic mitochondrial ROS or 8-OHdG ([Figure 3C, D](#)) or morphological damage to the embryos ([Figure 3E](#)). Moreover, it restored hepatic ATP to normal levels in the embryos subjected to in ovo BHP injection ([Figure 4A](#)). In chick embryos, then, maternal Zn-induced *MT4* expression protected hepatic mitochondrial function and energy metabolism against oxidative stress.

Mechanism by which zinc-inducible *MT4* expression protects mitochondrial function against oxidative stress involves inhibition of the PGC-1 α /PPAR- γ pathway

We then examined the molecular mechanism by which maternal Zn supplementation promotes *MT4* synthesis and protects chick embryos against stress-induced mitochondrial dysfunction. The in vivo (Exp. 3) and in vitro (Exp. 6) studies revealed that the PGC-1 α -PPAR- γ signaling pathway was activated in response to oxidative stress and intensified in *MT4*-silenced cells ([Figure 2H–J](#)) and the livers of the embryos in Zn-deficient eggs ([Figure 4G, I–L](#)). The addition of Zn to *MT4*-silenced cells and maternal Zn supplementation prevented ROS increase ([Figures 2B and 3C](#)) and recovered normal mitochondrial function following oxidative stress by upregulating the PGC-1 α /PPAR- γ signaling pathway ([Figures 2H–J and 4G, I–J, L](#)). However, BHP-induced oxidative stress had no apparent

effect on *SDHA* or peroxisome proliferators-activated receptor- α (PPAR- α) mRNA expression in chick embryos ([Figure 4F, H](#)).

Discussion

It has been proposed that MTs protect mitochondria against the destructive effects of oxidative stress by eliminating hydroxyl radicals [36]. Nevertheless, there are conflicting conclusions regarding the mechanism by which MTs regulate mitochondrial function, especially in response to stressors. Intracellular MT concentrations are very low in normal cells. In this study, BHP as a short-chain analog of lipid hydroperoxide has been used to induce oxidative damage on mitochondrial homeostasis and energy metabolism [24,26,37,38]. Similar levels of ROS were produced in embryonic primary hepatocytes between the incubations of 100 μ M H₂O₂ and 50–100 μ M BHP. In addition, Slamenova et al. [39,40] also found that the incubation of BHP enhanced the activities of SOD (<70 μ M), catalase (<140 μ M), and glutathione peroxidase (<210 μ M), which displayed greater stimulative effect on peroxide metabolizing enzymes than H₂O₂ at the same incubation concentrations in human hepatoma cell line (HepG2) cells. Then, MTs were rapidly induced in response to ROS stress in the mitochondria of the embryonic primary hepatocytes and protected the cells against oxidative damage. Subsequently, targeted *MT* gene disruption promoted ROS accumulation and potentiated the cytotoxic effects of oxidative stress in embryonic primary hepatocytes. Similar discoveries were reported for *MT*-null cells [41,42] and mice [21]. Our results indicated that *MT4* silencing aggravated BHP-mediated mitochondrial dysfunction in the embryonic primary hepatocytes as these cells displayed dramatic decreases in MMP, OCR, and ATP biosynthesis. An earlier study [43] disclosed that MTs inhibit oxidative stress-induced ROS formation by altering 1 of the nuclear-encoded subunits of mitochondrial complex IV and increasing the capacity of the latter to utilize molecular oxygen. However, another investigator demonstrated that MTs reduced oxygen consumption and inner membrane permeability in both isolated mitochondria and those in intact cells subjected to oxidative stress [22]. This discrepancy may be explained by the relative differences in the responses of various cell types, treatment dosages, and timing of events. Overall, the results suggest that Zn-induced *MT4* expression enhances the ability of the mitochondria in embryonic primary hepatocytes to reduce ROS accumulation.

MTs are small (6–7kDa) cysteine-rich proteins that are induced by Zn and freely penetrate outer mitochondrial membranes [44]. In embryos, MTs also protect against free radicals and other oxidants and regulate energy metabolism [31,45]. In vitro and in ovo studies demonstrated that Zn-inducible *MT*

FIGURE 3. Maternal Zn-inducible *MT4* expression attenuated in ovo injected BHP-induced mitochondrial oxidative damage. (A) Effect of maternal Zn on Zn content of the embryonic liver at E19. (B) Effect of maternal Zn on *MT4* expression in the embryonic liver from E13 to E19. (C, D, and F) Effect of maternal Zn or in ovo BHP injection on intracellular ROS accumulation (C), 8-OHdG (D), and mtDNA copy number (F) in embryonic liver. (E) Representative hepatic morphological changes in embryo by HE staining. Red arrows represent the swollen and damaged hepatic tubule and glomerulus (red arrows). All micrographs 200 \times magnification, hematoxylin/eosin staining. (G) Representative electron microscopy micrographs of mitochondrial ultrastructure. The mitochondria from the BHP treated embryo was smaller with a condensed mitochondrial membrane density and the adverse effects were eliminated by maternal Zn treatment (red arrows). (H–J) Effect of maternal Zn with or without in ovo BHP injection on *MT4* contents in cytoplasm (H), mitochondria (I), and *MT4* expression (J) in the embryonic liver. Data were presented as the mean \pm SEM and were analyzed using a 2-way ANOVA with a post-hoc Tukey's multiple-comparison test. Graph bars marked with different letters on top represent statistically significant results ($P < 0.05$, $n = 6$).

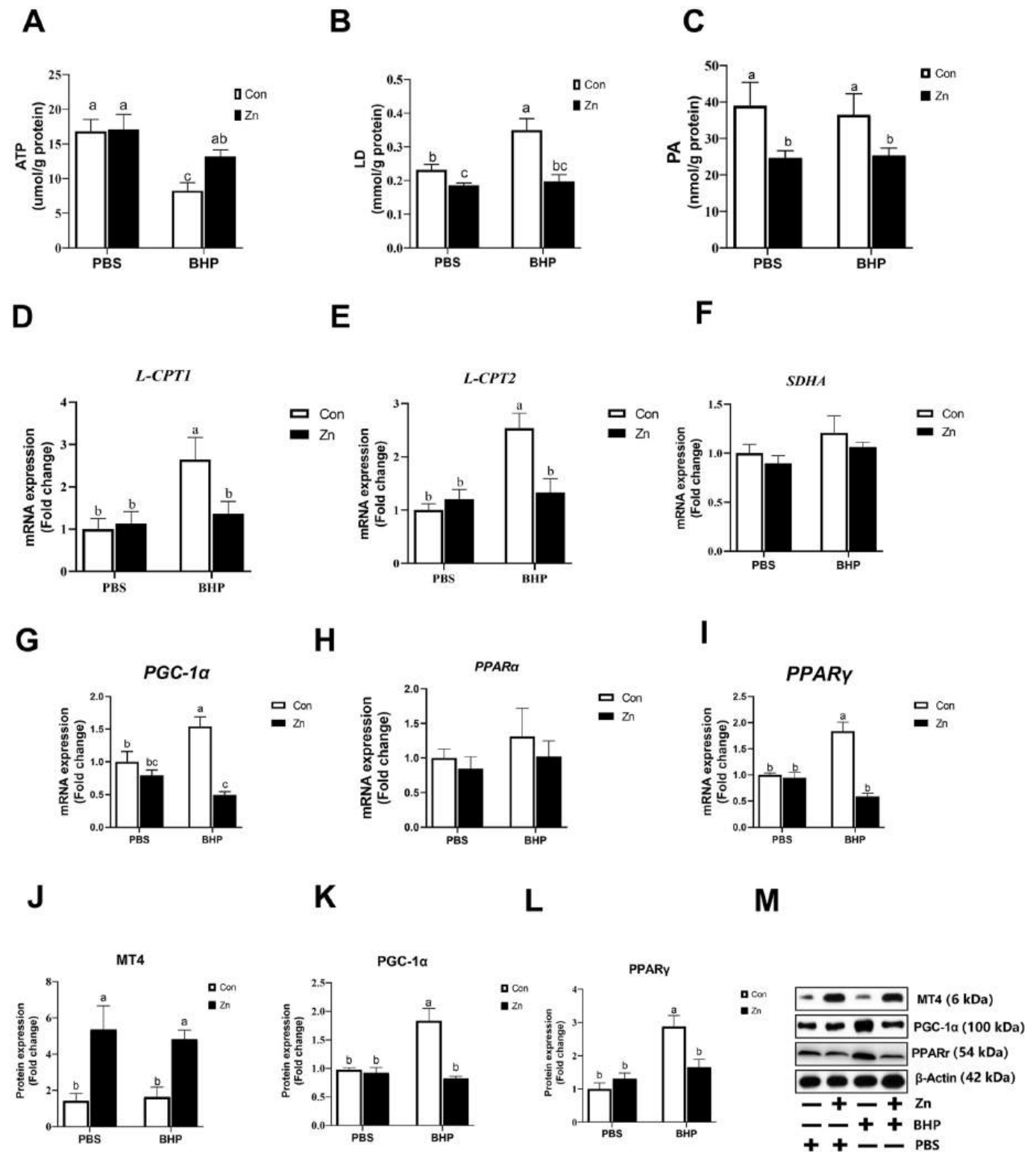


FIGURE 4. Maternal Zn-inducible MT4 regulates mitochondrial function in a stress-response manner in avian embryos. (A–C) Effect of maternal Zn with or without in ovo BHP injection on ATP (A), LD (B), and PA (C) contents in the embryonic liver. (D–I) Effect of maternal Zn with or without in ovo BHP injection on mRNA expression of *L-CPT1* (D), *L-CPT2* (E), *SDHA* (F), *PGC-1α* (G), *PPAR-α* (H), and *PPAR-γ* (I) in the embryonic liver (E19). (J–M) Effect of maternal Zn with or without in ovo BHP injection on protein expression of MT4 (J), PGC-1α (K), and PPAR-γ (L) in the embryonic liver. Data were presented as the mean ± SEM and were analyzed using a 2-way ANOVA with a post-hoc Tukey’s multiple-comparison test. Graph bars marked with different letters on top represent statistically significant results ($P < 0.05$, $n = 6$).

overexpression potentiates mitochondria. This finding was also confirmed in mitochondrial disease models [46–48]. We found that Zn supplementation upregulated embryonic *MT4* expression and abrogated ROS-induced oxidative damage and mitochondrial dysfunction both in vitro. The developing avian embryo is highly vulnerable to oxidative damage caused by external and internal stressors [49,50]. In vivo results showed that maternal Zn-induced *MT4* expression protects mitochondrial structure and function in Zn-deficient avian embryo models subjected to oxidative stress. The results of this work indicated that whereas *MT4* silencing promoted BHP-mediated mitochondrial dysfunction, Zn-induced *MT4* expression inhibited it.

It was proposed that the PGC-1 α /PPAR- γ signaling pathway is a key regulator of mitochondrial function [51]. PGC-1 α induction might upregulate DNA-binding transcription factors [52] such as PPARs (PPAR- α , PPAR β , and PPAR- γ) [53], promote the transcription of mitochondrial regulatory proteins, and stabilize energy metabolism and mitochondrial homeostasis [54]. Here, oxidative stress-induced ROS accumulation activated PGC-1 α signaling and restored BHP-mediated mitochondrial dysfunction. This discovery was consistent with the finding that PGC-1 α is upregulated in neurons under oxidative stress [55]. Furthermore, PGC-1 α -PPAR- γ signaling is intensified in *MT4*-silenced cells subjected to oxidative stress possibly because PGC-1 α trans-activates antioxidant genes [56]. Here, however, Zn-induced *MT4* expression in *MT4*-silenced cells and chick embryos prevented excessive PGC-1 α /PPAR- γ signaling pathway activation in response to oxidative damage. This research underscores the potential protective roles of Zn-inducible *MT4* expression and PGC-1 α /PPAR- γ signaling pathway down-regulation against mitochondrial dysfunction in embryonic primary hepatocytes exposed to oxidative stress.

Mitochondria are critical regulators of many aspects of cellular metabolism [57], including energy harvesting, health, and life span [58]. The increasing evidence suggests that mitochondrial dysfunction induced by oxidative stress contributes to aging and a variety of pathologies, including metabolic, cardiovascular, neurodegenerative, and neuromuscular diseases [59–62]. The studies on the role of *MTs* in the past 3 decades were limited to primary functions like antioxidants and anti-apoptosis. This study revealed a new exploration of *MT4* function on the regulation of mitochondrial processes using an avian model, such as energy metabolism, ROS generation, or metabolic PGC-1 α /PPAR- γ pathways related to mitochondrial function. As such, the findings linking the capability to specifically manipulate Zn-induced *MT4* levels in cells and animals suggest that a new preventive strategy in mitochondrial dysfunction could be explored by therapeutic targeting *MTs*.

In conclusion, this study underscores the potential protective roles of Zn-induced *MT4* expression via the downregulation of PGC-1 α /PPAR- γ signaling pathway on mitochondrial function stimulated by the stress challenge in the primary hepatocytes in an avian embryo model (Graphical Abstract). The findings suggest that the *MTs* could be applied as a new therapeutic target for repairing mitochondrial dysfunction in disease. Nevertheless, it remains to be determined through further research whether *MT4* regulates the PGC-1 α signaling pathway directly by coupling with regulatory element-binding proteins or indirectly by regulating ROS signaling.

Author contributions

The authors' responsibilities were as follows – YWZ, HL: designed the experiments; HL, LH, XFZ, HW, HQZ: conducted the experiments and sample analyses; LH, TY, QLW, WXZ: analyzed the data; JL, WHX: provided some critical suggestions for feeding experiment; JT, WCW, LY: revised the manuscript; all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Ethics approval

All animal experiments were performed in accordance with the guidelines of the local animal ethics committees.

Consent for publication

All authors have read and approved the submission of the manuscript.

Data availability

All data, analytical methods, and study materials are available from the corresponding author on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tjn.2024.01.011>.

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Research Article

Effect of Maternal Marginal Zinc Deficiency on Development, Redox Status, and Gene Expression Related to Oxidation and Apoptosis in an Avian Embryo Model

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Maternal severe zinc (Zn) deficiency resulted in growth retardation and high mortality during embryonic development in human. Therefore, this study is aimed at evaluating the effect of maternal marginal Zn deficiency on the development and redox status to avoid severe Zn deficiency using an avian model. A total of 324 laying duck breeders at 214 days old were randomly allotted into 3 dietary Zn levels with 6 replicates of 18 ducks per replicate. The birds were fed experimental diets including 3 dietary supplemental Zn levels of 0 mg/kg (maternal Zn-deficient group, 29.2 mg Zn/kg diet), 60 mg/kg (maternal Zn-adequate group), and 120 mg/kg (maternal Zn-high group) for 6 weeks. Dietary Zn levels had no effect on egg production and fertility ($P > 0.05$), whereas dietary Zn deficiency decreased breeder plasma Zn concentration and erythrocytic alkaline phosphatase activity at week 6 and inhibited erythrocytic 5'-nucleotidase (5'-NT) activity at weeks 2, 4, and 6 ($P < 0.05$), indicating that marginal Zn-deficient status occurred after Zn depletion. Maternal marginal Zn deficiency increased embryonic mortality and contents of superoxide anion radical, MDA, and PPC and reduced MT content and CuZnSOD activity in duck embryonic livers on E29. The MDA content was positively correlated with embryonic mortality. Maternal marginal Zn deficiency increased *BCL2-associated X protein* and *Caspase-9* mRNA expressions as well as decreased *B-cell lymphoma-2* and *MT1* mRNA and signal AKT1 and ERK1 protein expressions ($P < 0.05$). Breeder plasma Zn concentration and erythrocytic 5'-NT activities at week 6 were positively correlated with GSH-Px activity and GPx, *MT1*, and *BCL2* mRNA expressions in embryonic livers on E29. In conclusion, erythrocytic 5'-NT activity could be more rapid and reliable to monitor marginal Zn-deficient status. Marginal Zn deficiency impaired hatchability and antioxidant defense system and then induced oxidative damage and apoptosis in the embryonic liver, contributing to the greater loss of duck embryonic death.

1. Introduction

Zinc (Zn) is an essential trace mineral required for maintaining the normal growth and development of embryos [1]. Maternal marginal Zn deficiency could lead to the susceptibility of embryonic death predominantly [2]. Furthermore,

severe Zn deficiency in maternal diets resulted in growth retardation, abnormal development, and increased mortality of embryos [3]. Therefore, it is crucial to assess or predict maternal marginal Zn nutritional status to prevent embryos subjected to severe Zn deficiency. Some traditional and reliable biochemical or functional indicators (e.g., tissue Zn

contents and bone mineralization) have been proposed to estimate maternal Zn status [4]. Only when Zn deficiency is relatively severe is it possible to detect changes in tissue Zn concentrations. It is necessary to select some specific sensitive biomarkers to predict maternal marginal Zn status. Some studies in rats and humans revealed that plasma Zn concentration could be used as a sensitive biomarker in response to Zn status [5, 6]. Alkaline phosphatase (ALP) is a Zn metalloenzyme, and its activity in blood was decreased by 80% when the Zn content was reduced from 96 mg/kg to 1.2 mg/kg in the rat [7]. The 5'-nucleotidase (5'-NT) activity, like a cell membrane enzyme in erythrocyte and thymulin [8], was more sensitive to mild Zn deficiency than plasma Zn concentration [9, 10]. It is speculated that the activities of ALP and 5'-NT could be developed as specific sensitive biomarkers for predicting the marginal Zn-deficient status.

Maternal inadequate Zn supply decreased Zn deposition in egg yolk and then reduced the Zn mobilization from storage sites to the tissues of the developing embryos [11]. Zn as a cofactor of some distinct metalloenzymes, such as metallothioneins (MTs) and copper-zinc superoxide dismutase (CuZnSOD), has a diverse range of biological reactions for maintaining embryonic development [12, 13]. Marginal Zn deficiency in maternal diet could induce some adverse effects on antioxidant ability and antiapoptosis during embryonic development [14]. In vivo studies revealed that marginal Zn deficiency throughout gestation caused induction of oxidative stress and impaired the normal development of the fetal brain in the rat [15]. Maternal dietary Zn supplementation could effectively eliminate chick embryonic mortality induced by maternal hyperthermia via enhancing antioxidant ability [12]. In vitro studies also have demonstrated that Zn deficiency in cell culture was conducive to the production of reactive oxygen (ROS) and caspase activation [16]. In addition, Zn deficiency induced apoptosis involving the inhibition of growth factor signaling pathways during embryonic and fetal development [2]. It is hypothesized that a deficient in Zn availability could cause alterations in redox status and then lead to oxidative damage and cell apoptosis in tissues, contributing to abnormal embryo development. In the current study, effect of maternal marginal Zn deficiency on embryonic development, redox status, and gene expressions related to antioxidant and antiapoptosis abilities were studied in an avian embryo model.

2. Methods and Materials

2.1. Animals and Diets. All animal protocols used in the present study were approved by the South China Agricultural University Institutional Animal Care and Use Committee. A total of 370 186-day-old Muscovy duck breeders were obtained from a commercial duck breeder farm (WENS Group, Yunfu, Guangdong, China) and housed in the caged system for 4-week adaptation period. During the adaptation period, welfare-related assessments and interventions were carried out to meet the requirements of the South China Agricultural University Institutional Animal Care and Use Committee. All breeder ducks were fed restrictively (160 g/d/bird) with a commercial feed at the nutritional level

(11.32 MJ metabolizable energy/kg, 180 g crude protein/kg, 7.0 g lysine/kg, 7.2 g methionine+cysteine/kg, 24.0 g calcium/kg, 3.8 g available phosphorus/kg, and 40 mg Zn/kg) during adaptation period. Then, 324 laying duck breeders aged 214 days were selected, balanced for laying rate, and then randomly allotted into 3 dietary Zn levels with 6 replicates of 18 ducks per replicate. The experimental period lasted for 6 weeks. The experimental diets included 3 dietary supplemental Zn levels of 0 mg/kg (maternal Zn-deficient group, MZD), 60 mg/kg (maternal Zn-adequate group, MZA), and 120 mg/kg (maternal Zn-high group, MZH) as inorganic Zn sulfate. The diets were formulated to meet or exceed the nutritional requirements of laying duck breeders according to the national agricultural industry standard of China (NY/T 2122-2012). According to the experimental treatments, single batch of basal diet was mixed and then divided into 3 aliquots with or without supplementation of Zn sulfate (10024018, Sinopharm Chemical Reagent Co., Ltd., Beijing, China). The composition of the basal diet is shown in Table 1. The analyzed values of Zn contents in MZD, MZA, and MZH diets were 29.2, 87.4, and 163.4 mg/kg, respectively. All birds had diet restrictions (160 g/d/bird) and access to water *ad libitum*. The breeders received 16 h of daily lighting from 04:30 am to 08:30 pm. Room temperature and humidity were controlled by the air-conditioner and recorded daily. Manure was removed through an automatic belt system daily. All male duck breeders were fed the same diet formulated to meet the nutritional requirements throughout the experimental period. The practice of semen collection started at 175 days of age, and the quality of semen was determined by the volume and numbers of semen and sperm motility. During the experimental feeding period, semen was collected and mixed from male duck breeders from 182 to 224 days of age. Artificial insemination was performed every four days. At the end of the experiment, all breeders were removed and were fed restrictively with a commercial feed to meet the nutrient requirements of birds. Lighting and feeding management were performed according to the instructions of Muscovy duck male breeder management guidelines. At the ending of feeding trial, the recovery performance standards and normal behaviors were done to evaluate the optimum welfare of the rest of breeders weekly.

During the 6-week experimental period, all eggs were collected from each replicate and recorded daily. Feed consumption and egg weight were measured weekly. Feed intake was calculated by dividing the total feed consumed by the number of ducks per replicate per day. The eggs were collected during the last week of the experimental period and then were stored in one storage room at a temperature of 15°C and a relative humidity of 70%. At the end of storage, all the eggs from one replicate (approximately 100 eggs) of the 3 dietary treatments were placed on the same egg tray (6 trays total) and then incubated in the same incubator (9TDJ-A, LanTianJiao Electronic Technology Company, Beijing, China). The eggs were incubated at a temperature of $37.5 \pm 0.5^\circ\text{C}$ and relative humidity of $55 \pm 5\%$ until E30 and then were transferred to hatchers. Eggs were candled on E7 and E28 to identify infertile, cracked, or nonviable

TABLE 1: Composition and nutrients levels of the basal diets for laying duck breeders during the experimental period (as-fed basis).

Item (%)	Laying period
Corn	51.67
Soybean meal	17.70
Corn gluten meal	7.75
Wheat middlings	8.97
Lard	1.84
Dicalcium phosphate	1.80
Limestone	8.50
Sodium chloride	0.30
DL-methionine	0.27
L-Lysine-HCl	0.20
Vitamin and mineral premix ¹	1.00
Total	100
Nutrient composition	
Calculated value (%)	
Metabolizable energy (MJ/kg)	11.63
Crude protein ²	18.51
Calcium ²	3.70
Total phosphate	0.60
Nonphytin phosphorus	0.44
Lysine	0.91
Methionine	0.57
Methionine+cysteine	0.84
Zinc ²	29.2

¹Provided per kilogram of diet without Zn addition: vitamin A, 5,000 IU; vitamin D₃, 800 IU; vitamin E, 20 IU; thiamine, 2.0 mg; riboflavin, 15 mg; pyridoxine, 4.0 mg; vitamin B₁₂, 0.02 mg; calcium pantothenate, 10 mg; folate, 0.15 mg; niacin, 60 mg; biotin, 0.20 mg; choline (choline chloride), 1,500 mg; Cu (CuSO₄·5H₂O), 8 mg; Fe (FeSO₄·7H₂O), 80 mg; Mn (MnSO₄·H₂O), 100 mg; Se (NaSeO₃), 0.3 mg; and I (KI), 0.4 mg.

²Analysed values based on triplicate determinations.

embryos. All removed eggs on E7 and E28 were counted, opened, and visually evaluated also to determine the actual embryonic mortality. Fertility was expressed as the percentage of fertile eggs in the total number of eggs set for each replicate per treatment. Hatchability and embryonic mortality were expressed as percentages of the hatched birds and dead embryos in the total number of fertile eggs of each replicate per treatment, respectively.

2.2. Sample Collections. The feed ingredients and diet samples from all the treatments were collected and analyzed for crude protein, calcium, and Zn contents. After fasting for 12 hours, blood samples were collected via a bronchial vein from the same two duck breeders in each replicate on the last day of weeks 2, 4, and 6 during the experimental period, respectively. Blood samples were separated into plasma and erythrocytes by centrifugation at 3000 × g for 15 min at 4°C. Erythrocyte samples were washed three times in cold isotonic saline (0.9%, v/w) and then haemolyzed with a ninefold volume of phosphate buffer (pH 7.4). The equal

volume of plasma and haemolyzed erythrocytes were pooled and stored at -20°C for further analysis.

Twelve eggs from each treatment (2 per replicate) were collected on the last day of week 6 of experimental period. The separated yolk from 2 eggs per replicate was pooled together and stored at -20°C for Zn analysis. On E29, 24 embryos (4 per replicate) from each treatment were killed by cervical dislocation. Equal weight subsamples of the livers from the 4 embryos in each replicate were pooled into one sample for analysis. Total one-gram liver sample of each replicate was homogenized at 8000 × g for 10 seconds in 9 mL of 0.9% sodium chloride buffer on ice and centrifuged at 3000 × g at 4°C for 15 min, and the resultant supernatant was used for the analyses of antioxidant ability. The liver samples from the embryos were immediately dissected and frozen in liquid nitrogen and then stored at -80°C for further investigation of the gene and protein expressions.

2.3. Determination of Zn Concentration. Zinc contents in samples including diets, breeder plasma, and egg yolk were measured using an inductively coupled plasma emission spectroscope (IRIS Intrepid II, Thermal Jarrell Ash, Waltham, MA) after wet digestions with HNO₃ and HClO₄ as described by Zhu et al. [11]. Validation of the mineral analysis was conducted using bovine liver powder (GBW (E) 080193, National Institute of Standards and Technology, Beijing, China) as a standard reference material. Calibrations for the Zn assay were conducted with a series of mixtures containing graded concentrations of standard solutions of Zn.

2.4. Determination of Zn Metalloenzyme Activities in Breeder Erythrocytes. ALP activity was measured using a HITACHI 7180 automatic biochemical analyzer (Hitachi Ltd., Tokyo, Japan) with a detection kit (A059-2-2, Nanjing Jiancheng Bioengineering Institute). CuZnSOD activity was determined by subtracting manganese superoxide dismutase (MnSOD) activity from total SOD (TSOD) activity according to the nitrite method [17]. 5'-NT activity was assayed by the determination of the P_i liberated from the substrate nucleotide as described previously [18]. Total protein concentration in erythrocytes was determined using a BCA Protein Assay Kit (23225, Pierce). All indices of erythrocytes were expressed as nitrite units per milligram protein.

2.5. Determination of Indices Related to Oxidative Damage. The activity of superoxide anion radical production was calculated and expressed as a percentage of control (vitamin C) based on the inhibition rate of superoxide anion radicals from the xanthine and xanthine oxygenase reaction following the instruction of a commercial assay (A052-1-1, Nanjing Jiancheng Institute of Bioengineering). The malondialdehyde (MDA) and protein carbonyl content (PCC) were determined by thiobarbituric acid colorimetric (A003, Nanjing Jiancheng Institute of Bioengineering) and 2,4-dinitrophenylhydrazine methods according to kits (A087, Nanjing Jiancheng Institute of Bioengineering), respectively. The 8-hydroxy-2-deoxyguanosine (8-OHdG) was determined with a commercially available ELISA test kit (H165, Nanjing Jiancheng Institute of Bioengineering). All indices

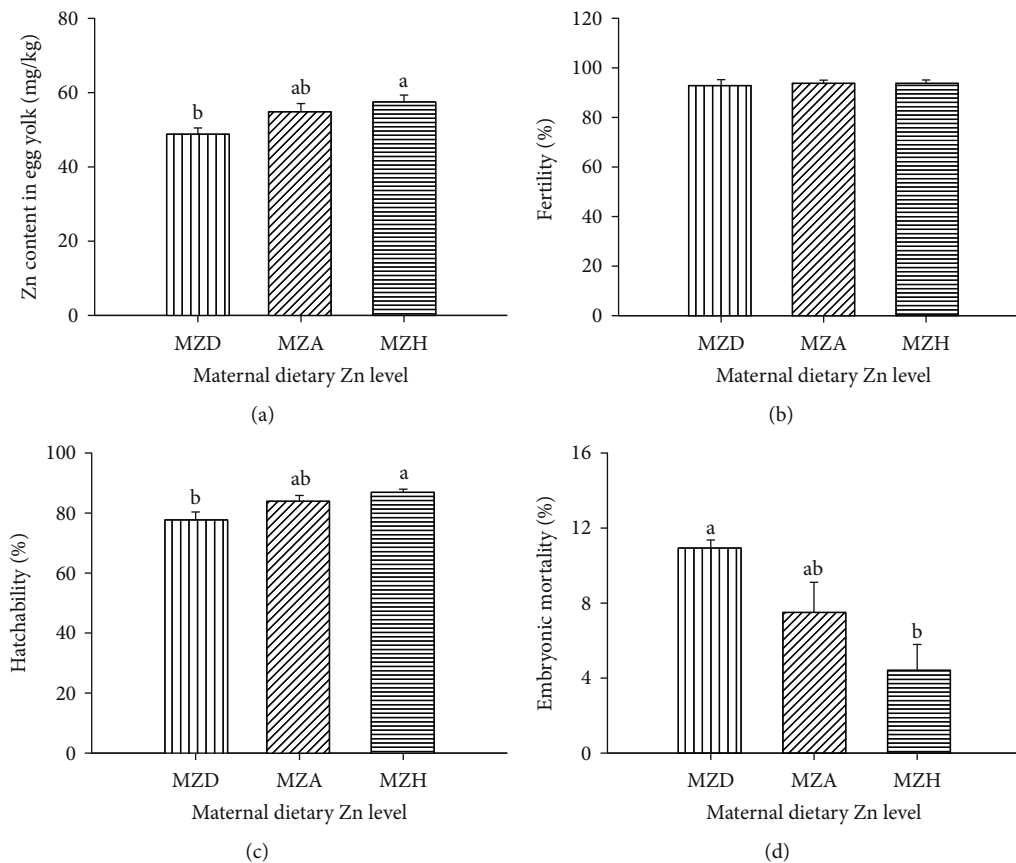


FIGURE 1: Effect of maternal dietary Zn on Zn concentration in (a) egg yolk, (b) fertility, (c) hatchability, and (d) embryonic mortality. The Zn content in egg yolk was measured on a fresh basis. All values are expressed as means \pm SE. Means with different letters (a and b) differ significantly ($P < 0.05$). Mean represented the average value of 6 replicates ($n = 6$). MZD: maternal Zn-deficient group (0 mg Zn/kg diet); MZA: maternal Zn-adequate group (60 mg Zn/kg diet); MZH: maternal Zn-high group (120 mg Zn/kg diet).

of supernatant were expressed as nitrite units per milligram protein.

2.6. Determination of Antioxidant Enzyme Activities. Supernatant of the liver homogenization solution was used to measure the activities of glutathione peroxidase (GSH-Px) and catalase (CAT) using the commercial kits (A005-1-2 and A007-1-1, Nanjing Jiancheng Bioengineering Institute) according to the instructions of the manufacturer. The total SOD (TSOD) and MnSOD activities were measured following the nitrite method described by Zhu et al., and CuZn-SOD activity was calculated by subtracting MnSOD activity from TSOD activity. MT content was determined using an ELISA kit for duck species (CG3309, Waltham).

2.7. RT-qPCR for Gene mRNA Expression. Total RNA was extracted from the embryonic liver tissues using Trizol reagent (15596018, Life Technologies), and then, reverse-transcription was performed using QuantiTech Reverse Transcription Kit (205311, Qiagen) following the manufacturer's protocols with genomic DNA wiping off. The protocol of two-step PCR using ABI Power SYBR Green PCR Master Mix was conducted as described previously [17]. The primer sequences are listed in Table S1. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was

used to normalize the expressions of the targeted genes. The $2^{-\Delta\Delta Ct}$ was used to calculate the mRNA level of each target gene using the MZD group as the reference group.

2.8. Western Blotting for Protein Expression. Total protein was extracted with ice-cold RIPA lysis buffer (P0013B, Beyotime Institute of Biotechnology). The procedure following the preparation of the protein sample and SDS-PAGE, blotting transfer, and detection of the protein-specific antibodies were performed as described previously [12]. The primary antibodies are listed in Table S2.

2.9. Statistical Analyses. All data were analyzed by one-way ANOVA using the PROC GLM procedure of the SAS (SAS Inst. Inc., Cary, NC). Additionally, the significant effect of dietary Zn on breeder plasma Zn concentration and erythrocytic Zn metalloenzyme activities was analyzed for each sampling time. All data were presented as mean \pm SEM. The cage served as the experimental unit for the indices of reproductive performance, while the pooled sample within a cage served as the experimental unit for other indices. Differences among means were tested by the Fisher's Least Significance Difference test method, and statistical significance was set at $P \leq 0.05$. The correlations of the parameters between the stages of breeder at week 6 during experimental

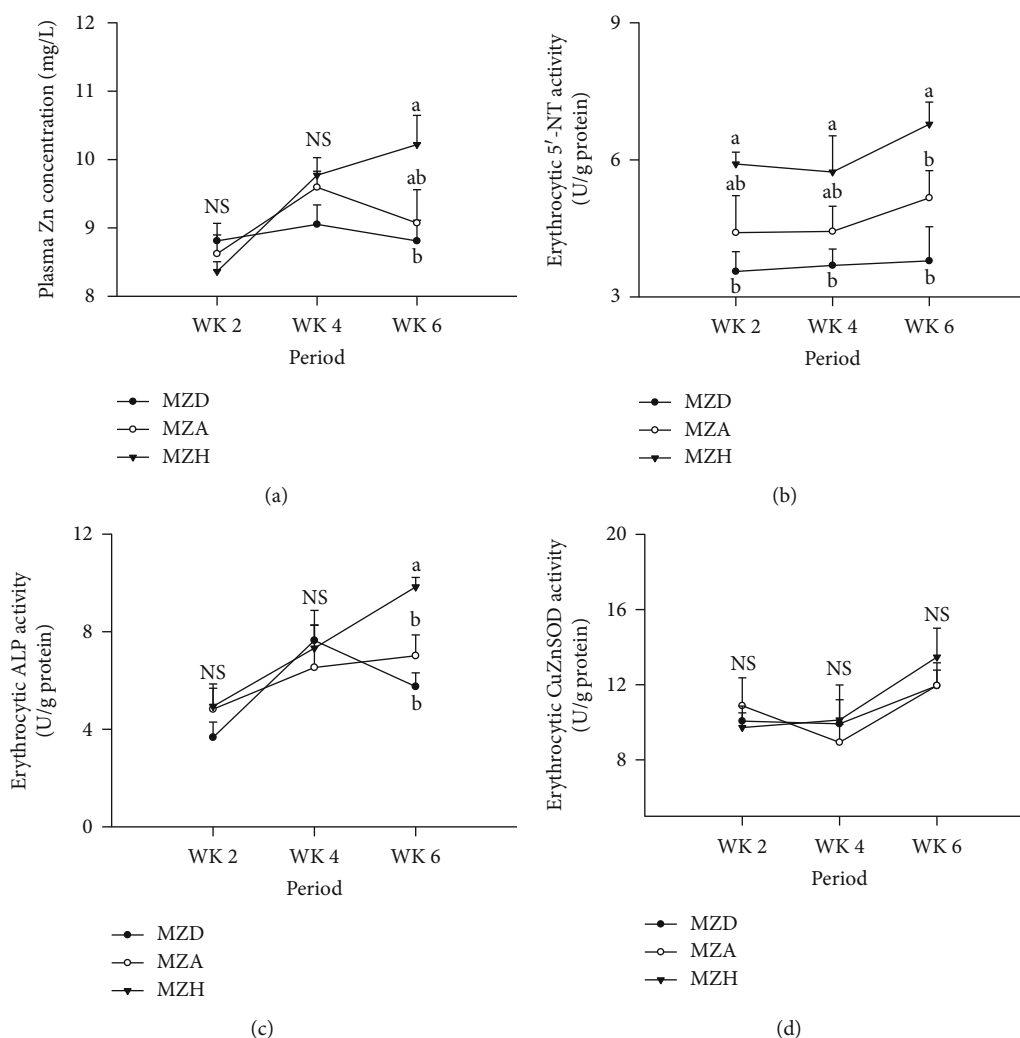


FIGURE 2: Effects of dietary Zn and age on (a) plasma Zn concentration and (b) erythrocytic 5'-NT, (c) ALP, and (d) CuZnSOD activities of duck breeders at weeks 2, 4, and 6 during experimental period. All values are expressed as means \pm SE. Means with different letters (a and b) differ significantly ($P < 0.05$) among dietary Zn groups at weeks 2, 4, and 6, respectively. Means with the letter (NS) showed no significant differences ($P > 0.05$) among dietary Zn groups at weeks 2, 4, and 6, respectively. Mean represented the average value of 6 replicates ($n = 6$). MZD: maternal marginal Zn-deficient group (0 mg Zn/kg diet); MZA: maternal Zn-adequate group (60 mg Zn/kg diet); MZH: maternal Zn-high group (120 mg Zn/kg diet); CuZnSOD: copper-zinc superoxide dismutase; ALP: alkaline phosphatase; 5'-NT: 5'-nucleotidase; WK: week; NS: no significant differences.

period and embryo on E29 were performed by Pearson correlation coefficients.

3. Results

3.1. Productive Performance and Zn Concentration in Egg Yolk. Dietary Zn levels did not affect ($P > 0.05$) egg weight (MZD 75.3 vs. MZA 76.1 vs. MZH 76.1 g), laying rate (MZD 83.9% vs. MZA 84.0% vs. MZH 83.2%), egg mass (MZD 63.2 vs. MZA 63.9 vs. MZH 63.3 g/bird/day), and feed: egg ratio (MZD 2.48 vs. MZA 2.44 vs. MZH 2.47) of duck breeders (Fig. S1). Maternal dietary Zn levels affected ($P < 0.05$) Zn concentration in egg yolk (Figure 1(a)), hatchability (Figure 1(c)), and embryonic mortality (Figure 1(d)), but did not influence fertility ($P > 0.05$, Figure 1(b)). The MZD group had a lower Zn content in egg yolk and hatchability as well as

higher embryonic mortality than the MZH group, with no differences between MZD and MZA groups.

3.2. Plasma Zn Concentration and Metalloenzyme Activities in Erythrocytes. Dietary Zn levels had no effect on CuZnSOD activity in erythrocytes of duck breeders at weeks 2, 4, and 6 ($P > 0.05$, Figure 2(a)). Compared to MZH, MZD decreased erythrocytic 5'-NT activity at weeks 2, 4, and 6 and did not differ from MZA at 2 and 4 weeks of age as well as neither MZA differed from MZH at weeks 2, 4, and 6. MZD decreased plasma Zn concentration and erythrocytic ALP activity of breeders at week 6, but did not affect those at weeks 2 and 4.

3.3. Oxidative Damage and Antioxidant Enzyme Activities in Embryonic Livers. Maternal dietary Zn levels influenced ($P < 0.05$) the contents of superoxide anion radical, MDA,

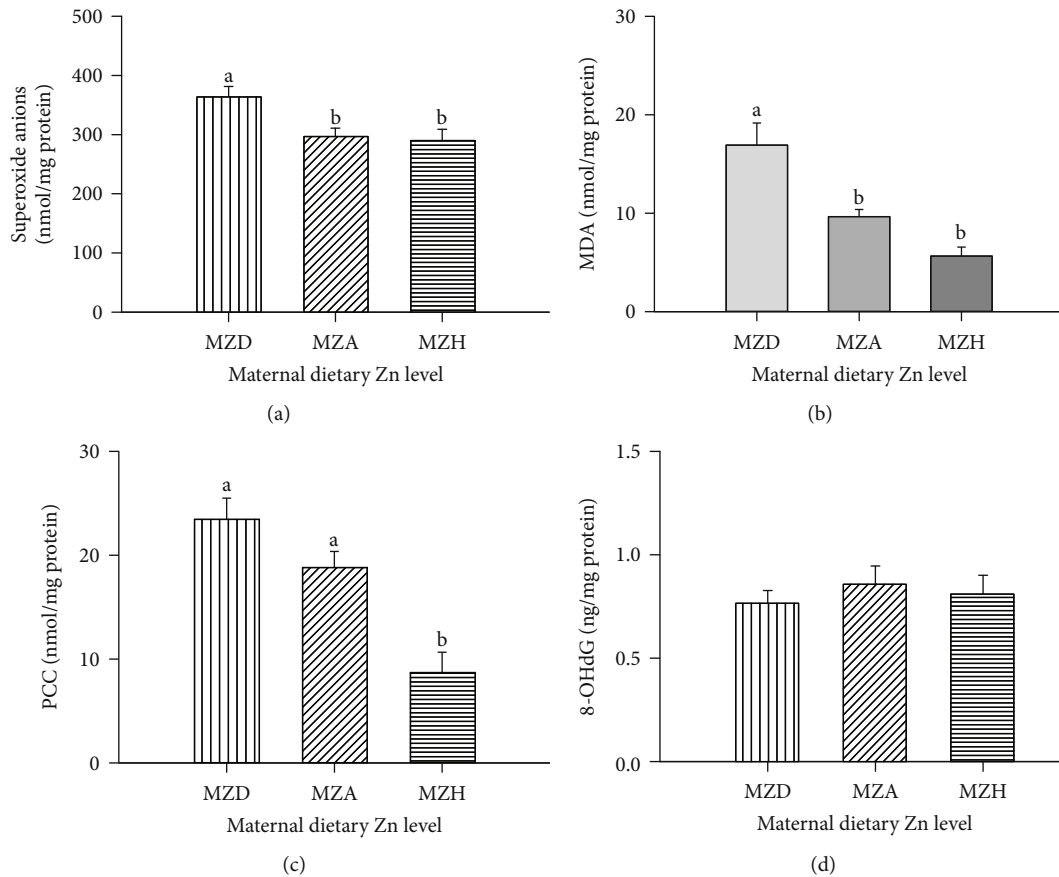


FIGURE 3: Effect of maternal dietary Zn on the contents of (a) superoxide anion radical, (b) MDA, (c) PCC, and (d) 8-OHdG in embryonic livers on E29. All values are expressed as means \pm SE. Means with different letters (a and b) differ significantly ($P < 0.05$). Mean represented the average value of 6 replicates ($n = 6$). MZD: maternal Zn-deficient group (0 mg Zn/kg diet); MZA: maternal Zn-adequate group (60 mg Zn/kg diet); MZH: maternal Zn-high group (120 mg Zn/kg diet); MDA: malondialdehyde; PCC: protein carbonyl content; 8-OHdG: 8-hydroxy-2-deoxyguanosine.

PCC (Figures 3(a)–3(c)), and MT (Figure 4(a)) and activities of GSH-Px (Figure 4(b)) and CuZnSOD (Figure 4(e)), but did not affect ($P > 0.05$) on the 8-OHdG content (Figure 3(d)) and CAT activity (Figure 4(c)) in embryonic liver on E29. Maternal dietary Zn deficiency increased the superoxide anion radical, MDA, and PPC contents and decreased GSH-Px activity in embryonic livers. The MT content was lower, and CuZnSOD activity was higher in embryonic liver in MZD than in MZH groups. There were no differences in MT content between MZD and MZA groups as well as CuZnSOD activity between MZA and MZH groups.

3.4. Target Gene and Protein Expressions in Embryonic Livers. As shown in Figure 5, embryonic liver had higher *MT1* and *BCL2* mRNA expression and lower *CAT*, *BAK1*, and *Caspase-9* mRNA expression in the MZH group than in the MZA group ($P < 0.05$), whereas there were no differences in these indexes between MZD and MZA groups. MZA group had lower *BAX* mRNA expression in embryonic liver than those from MZD and MZH groups ($P < 0.05$), with no differences between MZD and MZH groups. Compared to the MZD group, the MZH group had higher

AKT1 and *ERK1* protein expression of in embryonic liver ($P < 0.05$).

3.5. Correlation of Some Measured Parameters between Breeders and Embryos. As shown in Table 2, plasma Zn concentration was positively ($P < 0.01$) correlated with erythrocytic ALP and 5'-NT activities in breeders at week 6. Breeder plasma Zn concentration and erythrocytic ALP and 5'-NT activities were positively correlated with GSH-Px activity as well as *GPx*, *MT1*, and *BCL2* mRNA expression in embryonic liver on E29 ($P < 0.05$). The MDA content had positive correlation with embryonic mortality ($P < 0.01$), while the MDA content had negative correlation with GSH-Px activity and *MT1* mRNA expression in the embryonic liver ($P < 0.05$).

4. Discussion

The development and growth of poultry embryos are dependent upon the nutrient deposits in the eggs [19]. The yolk sac provides the chicken embryo with essential mineral nutrients for embryonic growth [20, 21]. Zinc as an essential nutrient is required in small amounts for normal growth and

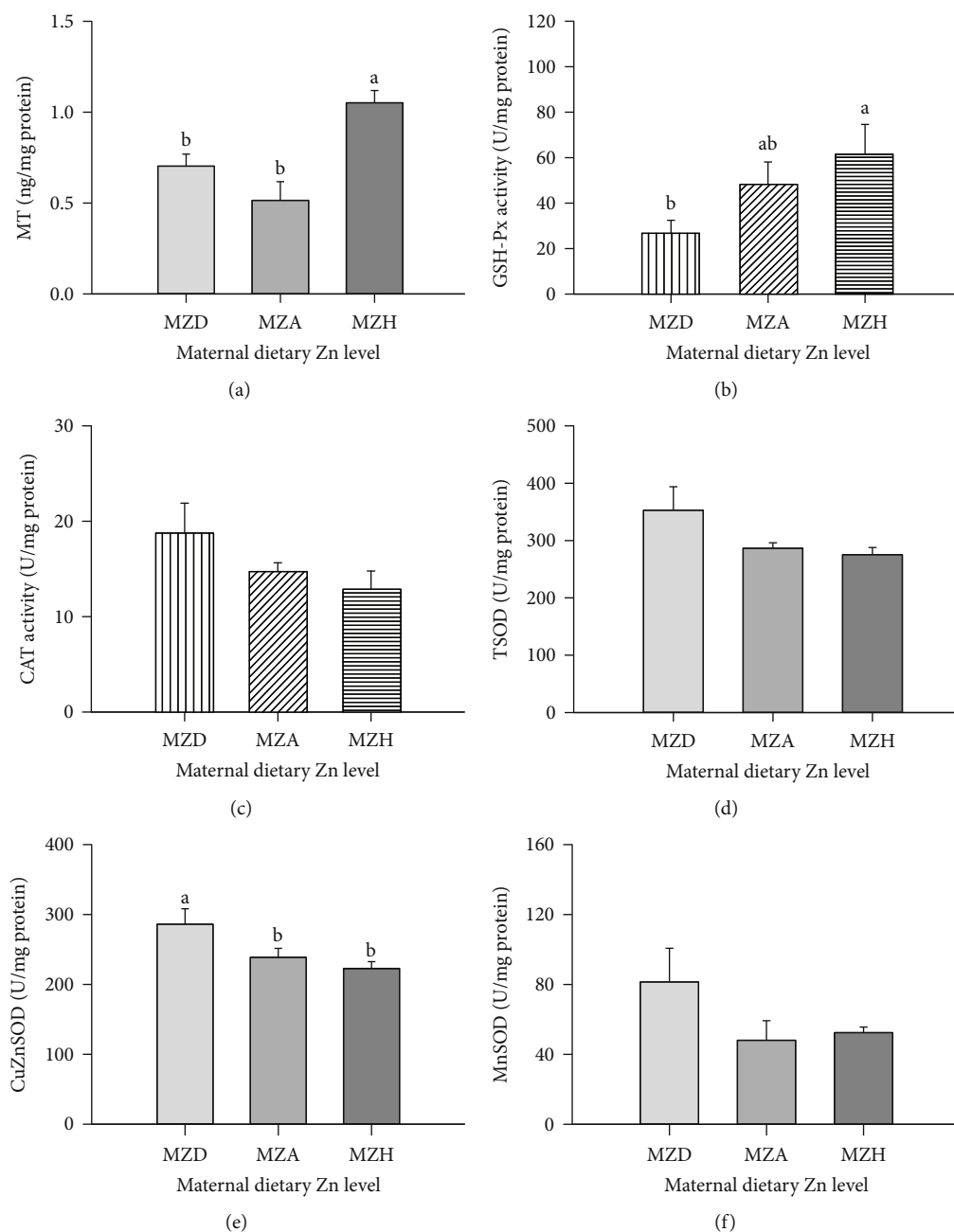


FIGURE 4: Effect of maternal dietary Zn on (a) MT content and (b) GSH-Px, (c) CAT, (d) TSOD, (e) CuZnSOD, and (f) MnSOD activities in embryonic livers at E29. All values are expressed as means \pm SE. Means with different letters (a–c) differ significantly ($P < 0.05$). Mean represented the average value of 6 replicates ($n = 6$). MZD: maternal Zn-deficient group (0 mg Zn/kg diet); MZA: maternal Zn-adequate group (60 mg Zn/kg diet); MZH: maternal Zn-high group (120 mg Zn/kg diet); MT: metallothionein; GSH-Px: glutathione peroxidase; CAT: catalase; TSOD: total superoxide dismutase; CuZnSOD: copper-zinc superoxide dismutase; MnSOD: manganese superoxide dismutase.

development of the avian embryo functioning as catalytic or structural cofactors in metal-containing enzymes [1]. Previous studies demonstrated that supplemental Zn in diets was essential to achieve normal reproductive performance in chickens [1] and rats [16]. However, the results from our study indicated that adding Zn to the diets had no effects on the characteristics of egg production performance of duck breeders, which was inconsistent with the positive results reported by laying hens [22]. The discrepancy

between the studies may depend on the differences in the genetic breeds (Muscovy duck breeder vs. Hisex Brown laying hen), supplemental Zn sources (Zn sulfate vs. Zn oxide), and Zn depletion periods (6 weeks vs. 12 weeks) of the birds. However, feeding Zn deficiency in duck breeder diets resulted in a lower hatchability due to an increase in the average day of embryonic mortality. The above results indicated that Zn requirement for laying performance might not be sufficient to maintain the hatchability and embryonic

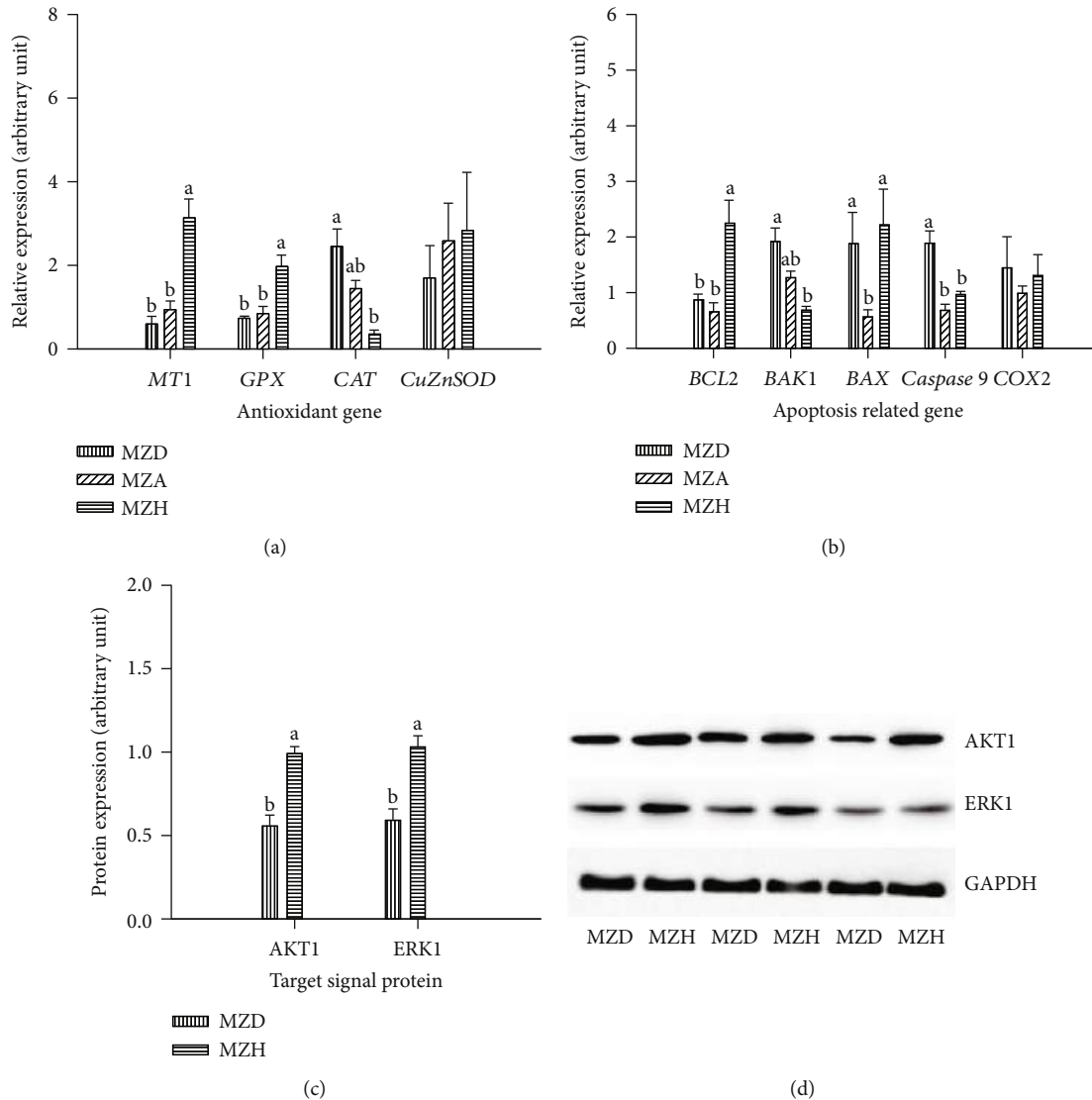


FIGURE 5: Effects of maternal dietary Zn levels on of (a, b) antioxidant genes (*MT1*, *GPx*, *CAT*, and *CuZnSOD*) and antiapoptotic gene (*BCL2*, *BAK1*, *BAX*, *Caspase-9*, and *COX2*) mRNA expressions as well as (c) signaling *AKT1* and *ERK1* protein expressions in the embryonic liver. (d) Representative immunoblots of the indicated proteins were listed. The *GAPDH* mRNA expression was used to normalize the expressions of the targeted genes. The *GAPDH* protein expression was selected to normalize target protein expressions. Means with different letters (a and b) differ significantly ($P < 0.05$). Mean represented the average value of 6 replicates ($n = 6$). MZD: maternal Zn-deficient group (0 mg Zn/kg diet); MZA: maternal Zn-adequate group (60 mg Zn/kg diet); MZH: maternal Zn-high group (120 mg Zn/kg diet); *MT1*: metallothionein 1; *GPx*: glutathione peroxidase; *CAT*: catalase; *CuZnSOD*: copper-zinc superoxide dismutase; *BCL2*: B-cell lymphoma-2; *BAK1*: *BCL2* antagonist/killer 1; *BAX*: *BCL2*-associated X protein; *COX2*: cyclooxygenase-2.

development of laying ducks, suggesting that embryonic development was much more sensitive to maternal marginal Zn deficiency than egg production. However, severe Zn deficiency in hen diets could impair both egg production and embryonic development [23], whereas maternal Zn supplementation or *in ovo* Zn injection in the yolk can eliminate these adverse effects [24]. Therefore, it is necessary to assess Zn status by measuring some specific sensitive indicators to prevent the marginal or severe Zn deficiency in breeder diets.

Some traditional and reliable biochemical or functional indicators (e.g., tissue Zn contents and bone mineralization) have been proposed for estimating Zn status in poultry

breeders [25]. In fact, only when Zn deficiency is relatively severe is it possible to detect changes in Zn concentrations in tissues. Some studies in rats and humans revealed that plasma Zn concentration and Zn metalloenzyme activities could be used as sensitive biomarkers to permit estimation of the prevalence of marginal Zn deficiency [4]. For example, plasma Zn concentration is approximately 50 times lower than that in tissues, and slight differences in uptake or release of Zn from these peripheral sites could profoundly affect the plasma Zn concentration [4]. Studies in the pregnancy of rats and humans also found a significant increase in the plasma Zn concentrations following supplementation [5, 6]. In this study, dietary Zn deficiency decreased the

TABLE 2: Correlation between the measured parameters of Zn supply in the breeders and embryos.

Item	Breeder stage ¹				Embryonic stage ²					
	Embryonic mortality	Plasma Zn concentration	Erythrocytic ALP activity	Erythrocytic 5'-NT activity	MDA content	MT content	GSH-Px activity	GPx mRNA	MT1 mRNA	BCL2 mRNA
Embryonic mortality	1.00									
Plasma Zn concentration	-0.30	1.00								
Erythrocytic ALP activity	-0.42	0.71**	1.00							
Erythrocytic 5'-NT activity	-0.50	0.75**	0.61*	1.00						
MDA content	0.81**	-0.55*	-0.36	-0.54*	1.00					
MT content	-0.50	0.26	0.63**	0.39	-0.36	1.00				
GSH-Px activity	-0.45	0.80**	0.60*	0.68**	-0.58*	0.22	1.00			
GPx mRNA	-0.39	0.63**	0.54*	0.69**	-0.43	0.44	0.70**	1.00		
MT1 mRNA	-0.40	0.75**	0.70**	0.65**	-0.49*	0.47*	0.74**	0.88**	1.00	
BCL2 mRNA	-0.37	0.60*	0.49	0.68**	-0.40	0.54*	0.67**	0.92**	0.87**	1.00

¹Embryonic mortality, plasma Zn concentration, erythrocytic ALP, and 5'-NT activity were measured in breeders at week 6 during experimental period. ²The MDA and MT contents, GSH-Px activity, and target gene mRNA expressions were determined in embryonic livers on E29. ALP: alkaline phosphatase; 5'-NT: 5'-nucleotidase; MDA: malondialdehyde; GSH-Px: glutathione peroxidase; GPx: glutathione peroxidase; MT1: metallothionein 1; BCL2: B-cell lymphoma-2. * $P < 0.05$; ** $P < 0.01$.

plasma Zn concentration of duck breeders at week 6 compared to other two groups, but did not occur at weeks 2 and 4. A similar change tendency responded to dietary Zn level was observed in erythrocytic ALP activity. Dietary Zn deficiency decreased erythrocytic 5'-NT activity at weeks 2, 4, and 6. The positive correlations between plasma Zn concentration and erythrocytic ALP and 5'-NT activities at week 6 implied that marginal Zn-deficient status occurred with the prolonged dietary Zn depletion. Moreover, erythrocytic 5'-NT activity responded to the more pronounced Zn-deficient status was more rapidly and reliably and consequently possessed the capacity to prevent the possible deleterious effects of severe Zn deficiency.

Maternal inadequate Zn decreased Zn deposition in the yolk, implied that Zn supply to target tissues of the developing embryos could decline. The liver is the most important organ for the storage and homeostatic regulation of Zn metabolism in the avian embryo [26]. Zinc as a cofactor of some distinct metalloenzymes [27], such as MT and CuZn-SOD, was thought to be particularly important for maintaining Zn-dependent functions of antioxidant ability during chick embryonic development [12]. Previous studies have been demonstrated that severe Zn deficiency in maternal diets resulted in growth retardation, abnormal development, and increased mortality of embryos [3, 11]. In the current study, the effect of maternal marginal Zn deficiency on embryonic development was studied. Maternal marginal Zn deficiency decreased the ability to scavenge superoxide anion radical production in association with the increased MDA and PPC contents. Previous studies have reported that the excessive ROS from oxidative stress led to the damage of lipid and protein and then could arrest the development of embryos in human [28]. Compared to the maternal high

Zn group, the maternal marginally Zn-deficient group decreased the MT content in livers of duck embryos. Similar findings were reported for the developing chick embryo showing the consistency between hepatic Zn levels and redox [26]. The positive correlation between MDA content and embryonic mortality also implied that the impaired antioxidant defense system induced by marginal Zn deficiency could contribute to more significant loss of the embryos. Furthermore, the parallel reduced MT1 mRNA expression was observed in embryonic livers from breeder fed a maternal marginal Zn deficient diet. Studies have demonstrated that MT expressions correlated with hepatic Zn accumulation during development could protect against the oxidative damage of Zn deficiency during pregnancy in transgenic and knockout mice [29, 30]. In chicken [12] and mouse [31], it is proved that maternal Zn deficiency suppressed MT mRNA expression of offspring embryos via epigenetic regulation by the DNA hypermethylation and histone hypoacetylation of the gene promoter. Zn deficiency has been shown to initiate apoptosis during development, altering embryogenesis [16]. In our study, the *BAK1*, *BAX*, and *Caspase-9* mRNA expressions in related to cell apoptosis were increased in the liver of marginal Zn-deficient duck embryos. Recent reports have been shown that the enhanced MT expressions presented more excellent antiapoptotic effects *in vivo* [32] and *in vitro* [33], which was confirmed in marginal Zn-deficient embryos in the present study. Maternal dietary Zn deficiency decreased antiapoptotic gene *BCL2* mRNA expression in the embryonic liver to promote apoptosis. The negative correlation between MDA content and embryonic MT1 and BCL2 mRNA expressions indicated that maternal marginal Zn deficiency might induce cell apoptosis due to the oxidative damage. In addition, a decrease of

the AKT1 and ERK1 protein levels in marginal Zn-deficient embryos suggests that Zn deficiency-induced apoptosis could be involved in growth factor signaling of AKT and ERK pathways by inhibiting cell cycle machinery [2].

5. Conclusions

In the present study, breeder erythrocytic 5'-NT activity could be developed as a sensitive biomarker to rapidly and reliably monitor the marginal and more pronounced Zn-deficient status. Maternal Zn deficiency impaired hatchability and increased embryonic mortality of duck embryos, which was positively correlated with embryonic liver MDA content. Maternal marginal Zn deficiency impaired antioxidant defense system and induced oxidative damage and apoptosis in embryonic liver. These deleterious effects possibly contributed to the greater loss of embryos during the developing stage.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

The paper has been submitted as the preprint posting (doi:10.21203/rs.3.rs-88546/v1 and URL link: <https://www.researchsquare.com/article/rs-88546/v1>). Upon acceptance of the article for publication, all authors agreed to the terms of the relevant journal's License to Publish or Copyright Assignment form. Authors also agreed that the preprint record is later updated with a DOI and a URL link to the published version of the article if the article is accepted.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Wei Gao and Liang Huang contributed equally to the present study. Yongwen Zhu and Lin Yang were responsible for all issues related to this paper. Wei Gao and Yongwen Zhu were responsible for the planning of the study, sample collections, and analyses, as well as the manuscript writing. Xiufen Zhang and Wence Wang were involved in the sample collections, biological analysis, and statistical analyses. Xinyan Ma, Wei Geng, and Chuang Liu were involved in the experimental design and data interpretations. All authors read and approved the final manuscript.

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Supplementary Materials

The data used to support the findings of this study are included within the article. Fig. S1: effect of dietary Zn on egg weight, laying rate, egg production, feed intake, and feed/egg ratio. Supplemental Table 1: nucleotide sequences of specific primers for RT-qPCR. Supplemental Table 2: summary of the antibodies used for western blotting. (*Supplementary Materials*)

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Improving the quality of Napier grass silage with pyroligneous acid: Fermentation, aerobic stability, and microbial communities

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The presence of undesirable microorganisms in silage always leads to poor fermentation quality and low aerobic stability. Pyroligneous acid (PA), a by-product of biochar production, is known to have strong antimicrobial and antioxidant activities. To investigate the effects of PA on fermentation characteristics, aerobic stability, and microbial communities, Napier grass was ensiled with or without 1 and 2% PA for 30 days and then aerobically stored for 5 days. The results showed that PA application decreased ($P < 0.01$) the pH value, ammonia nitrogen content, and number of undesirable microorganisms (coliform bacteria, yeasts, and molds) after 30 days of ensiling and 5 days of exposure to air. The temperature of the PA-treated group was stable during the 5-day aerobic test, which did not exceed room temperature more than 2°C. The addition of PA also enhanced the relative abundance of *Lactobacillus* and reduced that of *Klebsiella* and *Kosakonia*. The relative abundance of *Candida* was higher in PA-treated silage than in untreated silage. The addition of PA decreased the relative abundance of *Kodamaea* and increased that of *Monascus* after 5 days of exposure to air. The abundances of *Cladosporium* and *Neurospora* were relatively high in 2% PA-treated NG, while these genera were not observed in the control group. These results suggested that the addition of PA could improve fermentation characteristics and aerobic stability, and alter microbial communities of silage.

KEYWORDS

pyroligneous acid, fermentation quality, microbial communities, aerobic stability, Napier grass

Introduction

Ensiling has become a universal method for preserving fresh forage and supplying moist feedstock all year round (Wang et al., 2021). With the increasing demand for livestock products, more attention has been paid to silage production, especially in developing countries. Napier grass (*Pennisetum purpureum* Schum) is an important source for manufacturing biofuel and animal feed. It is widely cultivated for ruminant feed in tropical and subtropical regions because of its short growth cycle, high biomass, and strong adaptability (Tao et al., 2021). In these regions, the main constraint restricting long-term feed supply is humidity and rain. Ensiling might be a better choice for preservation of forage to constantly provide highly palatable and nutritious feed for livestock. In general, fermentation of silage mainly depends on lactic acid bacteria (LAB), which produce organic acids (mainly lactic acid and acetic acid), for creating an acidic environment and suppress the microbial metabolism and reproduction, through which the original quality of fresh forage is preserved as much as possible (He et al., 2020a). However, the inadequate epiphytic LAB count and the low-water soluble carbohydrate (WSC) content of raw material would suppress the success of ensiling under natural fermentation conditions, thus failing to meet the daily nutritional demand of livestock (Wu et al., 2020). Consequently, *Clostridia* and *Enterobacter* would grow vigorously, causing abundant proteolysis and butyric acid accumulation. After exposure to air, aerobic spoilage of silage is also frequently observed. Yeast and *Acetobacter* would compete with LAB for substance, causing dry matter loss and spoilage (Kung et al., 2021). Undesirable microorganisms might also produce numerous secondary metabolites, including mycotoxins, which would affect the health and production of animals (Zong et al., 2022). Silage with potential safety hazards would also incur huge economic losses to farmers. Therefore, it is necessary to take some measures for improving fermentation quality and aerobic stability of silage.

Pyrolygneous acid (PA), also called wood vinegar, is a brown liquid by-product of pyrolysis of biomass, which is transformed into biofuel and biochar (Zhang et al., 2019a). PA is a complex mixture of compounds and contains over 200 kinds of natural organic compounds, including organic acids, phenols, aldehydes, alcohols, esters, ketones, furan and pyran derivatives, hydrocarbons, and nitrogen compounds (Zhu et al., 2021). Due to the presence of these chemical ingredients, PA is considered organic wastewater and would impose an extreme burden on ecological environment stability (Cezary, 2015). Therefore, the effective application of PA is one of the methods of waste recycling and achieving green and sustainable development. Organic acids and phenolic compounds are the dominant compounds of PA, which have exhibited remarkable antimicrobial property. In general, organic acids account for around 30–70% of total organic compounds present in PA,

TABLE 1 Characteristics of the fresh Napier grass before ensiling (\pm SD, $n = 3$).

Item	Means \pm SD
Dry Matter (g/kg)	210 \pm 10.21
Crude protein (g/kg DM)	139 \pm 1.93
Neutral detergent fiber (g/kg DM)	350 \pm 17.2
Acid detergent fiber (g/kg DM)	122 \pm 6.83
Water-soluble carbohydrates (g/kg DM)	57.1 \pm 2.47
Lactic acid bacteria (\log_{10} CFU/g FM)	3.92 \pm 0.14
Coliform bacteria (\log_{10} CFU/g FM)	5.15 \pm 0.26
Yeast (\log_{10} CFU/g FM)	3.78 \pm 0.16
Molds (\log_{10} CFU/g FM)	<2.00

FM, fresh matter; DM, dry matter; CFU, colony-forming units; SD, standard deviation.

90% of which is acetic acid (Fan et al., 2022). PA with a high concentration of organic acids might inhibit the fermentation of microorganisms and reduce the nutrient loss of silage. Simultaneously, previous studies indicated that acetic acid can also improve the aerobic stability of silage. However, there are few studies on the effect of PA on fermentation quality and aerobic stability of silage.

Therefore, we hypothesized that the addition of PA could improve the fermentation quality and aerobic stability of Napier grass by inhibiting undesirable microorganisms. In the present study, Napier grass was harvested randomly and ensiled with or without 1 or 2% PA for 30-day fermentation and then aerobically stored for 5 days. Following that, the fermentation characteristics and aerobic stability were analyzed. Moreover, the change of microbial community is determined to help us comprehend the effect of PA on silage fermentation.

Materials and methods

Silage preparation

Dwarf Napier grass was collected from the experimental plot of South China Agricultural University, which was cultivated at a row width of 60 cm and an intrarow spacing of 40 cm for 90 days. Dwarf Napier grass was harvested in May 2021, which was immediately chopped to a length of around 2 cm using a grass cutter. After sufficient mixing, chemical compositions and microbial populations of the fresh material (FM) were determined, which were performed in triplicate. Different concentrations of PA were applied to the fresh Napier grass (approximately 200 g) and then assigned to one of the following treatments: (1) no additive (CK), (2) 1% pyrolygneous acid (fresh matter basis, 1% PA), and (3) 2% pyrolygneous acid (fresh matter basis, 2% PA). A total of 42 samples (3 treatments \times 2 days \times 7 replicates) were packed in polyethylene bags (20 \times 30 cm;

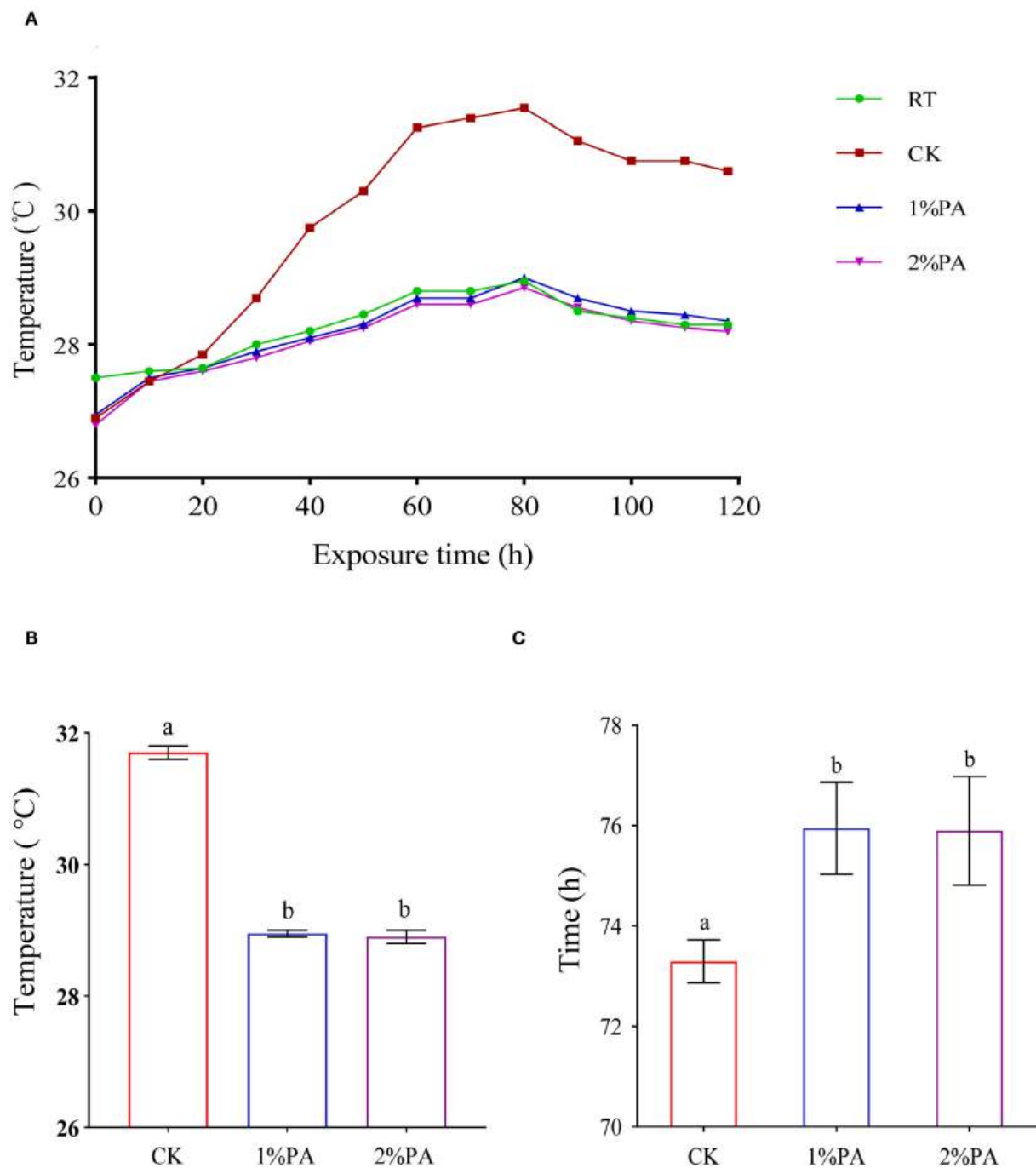


FIGURE 1

(A) Aerobic stability of Napier grass silages with or without 1% and 2% pyroligneous acid during the 5-day aerobic test. (B) Maximum temperature attained within 5 days of aerobic exposure and (C) the time required.

Dongguan Bojia Packaging; China). Subsequently, the samples were sealed in bags using a vacuum sealing machine (Lvye DZ280; Dongguan Yijian Packaging Machinery, Dongguan, China) to reach anaerobic conditions and were placed in a

room with ambient temperature (25–30°C). After 30 days of ensiling, the bags were opened, and fermentation quality and microbial communities were measured on day 0 and day 5 of aerobic exposure.

Assessing aerobic stability

The determination methods were similar to those used in our earlier study (He et al., 2020b). After 30 days of fermentation, seven bags of each treatment groups were opened, thoroughly mixed, and separated into three repetitions. Following that, 400 g of silage from each treatment groups was placed loosely into 1,000-mL plastic buckets to estimate aerobic stability, with nine buckets in total. A layer of cheesecloth was covered around the barrel to reduce moisture volatilization and potential contamination but to permit air penetration. In addition, all the barrels were placed in cartons. A layer of polystyrene foam was laid between the barrels and cartons to prevent rapid thermal loss. A thermograph was used to measure room temperature and the temperature of silage at 10-min intervals during 5 days of aerobic exposure (SMOWO MDL-1048A, Shanghai Tianhe Automation Instrument Co., Ltd. (Shanghai, China). In general, if the silage temperature exceeds the room temperature above two degrees, it is considered that the silage had underwent aerobic deterioration.

Determination of fermentation characteristics and chemical compositions

According to Wang et al. (2021), fermentation characteristics and chemical compositions of the silage sample were determined on days 0 and 5 of aerobic exposure, respectively. A measure of 20 g of the sample was added to with 180 mL of normal sterile saline and mixed with shaking. The supernatant was gradient-diluted from 10^{-1} to 10^{-6} . Serial dilutions of 1 mL were, respectively, inoculated in Man, Rogosa, and Sharpe (MRS) agar and Violet Red Bile agar to culture lactic acid bacteria (LAB) and coliform bacteria under a temperature of 30°C for 2 days (Chen et al., 2021). Meanwhile, 100 μ L of the diluent was added to Rose Bengal agar and cultured for 3 days under 28°C to obtain yeasts and molds (Guo et al., 2021). Another 20 g of the sample was mixed with 180 mL distilled water and stored overnight at 4°C. Subsequently, it was filtered, pH value was measured, and organic acids and ammonia nitrogen ($\text{NH}_3\text{-N}$) were analyzed. pH was determined using a glass electrode pH meter (PHS-3C, INESA Scientific Instrument, Shanghai, China). The $\text{NH}_3\text{-N}$ content was analyzed by using the phenol-hypochlorite colorimetric method (Broderick and Kang, 1980). Organic acids (mainly lactic acid, acetic acid, propionic acid, and butyric acid) were determined by high-performance liquid chromatography (HPLC) (column, Shodex RSpak KC-811S-DVB gel C (8.0 mm 930 cm; Shimadzu, Tokyo, Japan) under

the following conditions: oven temperature: 50°C, mobile phase: 3 mmol/L HClO_4 , flow rate: 1.0 mL/min, injection volume: 5 μ L, and detector: SPD-M10AVP (Bai et al., 2020). The remaining silage sample was dried at 65°C for 2 days to measure the content of dry matter and protein components [true protein (TP) and crude protein (CP)] (Ke et al., 2017). The CP content was measured using an automatic Kjeldahl apparatus (Kjeltec 2300 Auto Analyzer, FOSS Analytical AB, Hoganas, Sweden) according to the method of the Association of Official Analytical Chemists. At the same time, the contents of neutral detergent fiber (NDF), detergent fiber (ADF), and WSC in the fresh material samples were also determined as mentioned by Wang et al. (2019). The contents of NDF and ADF were measured using an A220 Fiber Analyzer (ANKOM Technology Corp., Macedon, NY, USA), while WSC concentration was analyzed by 3,5-dinitrosalicylic acid colorimetry.

Microbial diversity analysis

A DNA kit (Omega Biotek, Norcross, GA, U.S.) was used for total DNA extraction in accordance with the manufacturer's instructions. The primers of 341F (CCTACGGGNGGCWGCAG) and 806R (GGACTACHVGGGTATCTAAT) were used to amplify the V3-V4 region of 16S rDNA. For fungi, the ITS region was targeted using primers ITS3_KYO2F (GATGAAGAACGYAGYRAA) and ITS4R (TCCTCCGCTTATTGATATGC). The purified polymerase chain reaction (PCR) products were sequenced using the Illumina HiSeq 2500 system. In addition, the analysis of raw sequences was performed as described in Wang et al. (2019). Finally, microbial communities were analyzed by the free online platform (<http://www.omicshare.com/tools>), which included alpha diversity, β -diversity, and relative abundance.

Statistical analysis

In the present study, IBM SPSS20.0 software was used to evaluate the effects of PA and exposure time on fermentation characteristics with two-way analysis of variance (ANOVA). Duncan's test was used to compare the degree of difference between different treatments. If the *P*-value was lower than 0.05, it would be inferred to have a significant effect. The relevant figures of microbial communities were obtained by using the Omicsmart online platform, and the aerobic stability assessment diagrams were constructed by GraphPad prism 8 software. Furthermore, all of them were enhanced by Adobe Illustrator CS 6.0 software.

Results

Characteristics of the fresh Napier grass

The chemical compositions and microbial populations of Napier grass before silage are summarized in Table 1. The DM content was 210 g/kg FM, and the contents of CP, NDF, ADF, and WSC were 139 g/kg DM, 350 g/kg DM, 122 g/kg DM, and 57.1 g/kg DM, respectively. For microorganisms, the LAB count was $3.92 \log_{10}$ CFU/g FM. Coliform bacteria and yeast counts were $5.15 \log_{10}$ CFU/g FM and $3.78 \log_{10}$ CFU/g FM, respectively. The count of molds was less than $2.00 \log_{10}$ CFU/g FM.

Aerobic stability of Napier grass silage

Aerobic stability, the maximum temperature attained within 5 days of aerobic exposure, and the time required are listed in Figure 1. The untreated Napier grass deteriorated after aerobic exposure of 58 h. PA-treated Napier grass showed higher aerobic stability than the untreated silage. The temperatures in PA treatments did not exceed the room temperature more than 2°C during the 5-day aerobic test and were lower than those of the control group ($P < 0.05$), which were below 29°C.

Fermentation quality of Napier grass silage

Fermentation characteristics are shown in Table 2. PA markedly reduced the pH value ($P < 0.01$) when compared with the control group. On day 5 compared with day 0, the pH value of silage increased from 4.52 to 6.97 in the untreated silage, was unchanged in 1% PA-treated silage, and even prominently decreased in 2% PA-treated silage ($P < 0.01$). The addition of PA resulted in the increase in lactic acid and acetic acid contents ($P > 0.01$) on days 0 and 5, respectively. The numbers of yeasts and molds in all treatments were less than the detectable levels on day 0. The number of coliform bacteria was decreased by PA ($P < 0.01$), while it was relatively high in the control group. On day 5 of aerobic exposure, the numbers of yeasts and molds were significantly reduced in PA-treated silage, compared with the control group. Meanwhile the LAB count was significantly increased in the 2% PA-treated group ($P < 0.05$). The addition of PA reduced the $\text{NH}_3\text{-N}$ proportion ($P < 0.01$), when compared with the control group. The TP content increased from day 0 to day 5 in the untreated silage. In addition, on day 5, the TP content was higher in the control group than that in PA-treated silage.

Microbial diversity of Napier grass silage

Alpha diversity of microbial communities is shown in Table 3. Good's coverage values of all treatments were greater than 0.99. For bacterial communities, Sobs, Chao1, and Ace indices were higher in PA-treated silage than in the untreated silage, while the Simpson index was opposite. The different treatments resulted in the variation of fungal communities. The addition of PA led to the decrease in Chao1, Ace, and Simpson indices, compared with the control group. The Sobs index was also decreased by 2% PA treatment. Moreover, all indices of microbial communities reduced after 5 days of exposure to air. The β -diversity of microbial communities is shown in Figures 2, 3, respectively. For bacterial communities, distinct segregation was observed between PA-treated and untreated silage, as well as in the control group on different days of aerobic exposure. However, the PA-treated samples only had a little shift in the bacterial community. For fungal communities, 1% PA-treated samples were slightly separated from the untreated samples after 30 days of ensiling. However, 2% PA-treated and untreated silage were separated from each other. After 5 days of exposure to air, clear segregation was observed among all treatment groups.

Microbial abundance of Napier grass silage

The relative abundance of microbial communities after 30 days of ensiling and 5 days of exposure to air is shown in Figures 4, 5, respectively. At the phylum level, the dominant bacteria were *Cyanobacteria*, *Proteobacteria*, and *Firmicutes* in the fresh Napier grass material, and the relative abundances of were 71.45, 24.80, and 3.04%, respectively. After ensiling, the relative abundance of *Cyanobacteria* decreased, while that of *Firmicutes* increased in all treatment groups. The addition of PA decreased the relative abundance of *Proteobacteria*, when compared with the control. After 30 days of ensiling, the relative abundances of *Lactobacillus*, *Lactococcus*, *Kosakonia*, and *Klebsiella* were 8.36, 18.11, 22.40, and 8.10%, respectively, in the untreated silage. The addition of PA increased the relative abundance of *Lactobacillus* and decreased that of *Kosakonia* and *Klebsiella*. After 5 days of exposure to air, PA-treated silage had greater relative abundances of *Lactobacillus* and *Lactococcus* and lower relative abundances of *Lactobacillus*, *Klebsiella*, *Paenibacillus*, and *Bacillus* than those in the untreated silage. For fungal communities, *Ascomycota* was the most predominant phylum, followed by *Basidiomycota* before ensiling (Figure 5). After exposure to air, the relative abundance of *Ascomycota* increased, while the relative abundance of *Basidiomycota* decreased in all treatment groups. The addition of PA lowered the abundance of *Mortierellomycota*. On the genus level, the relative abundance of *Candida* increased, while the relative

TABLE 2 Chemical compositions and fermentation characteristics of Napier grass with or without PA treatment under aerobic exposure.

Items	Days	Treatment			SEM	P-value		
		CK	1%PA	2%PA		D	T	D×T
Dry Matter (g/kg FM)	D0	194	193	191	0.181	0.60	0.72	0.55
	D5	191	199	195				
pH	D0	4.52 ^{aB}	4.03 ^b	4.06 ^{bA}	0.077	<0.01	<0.01	<0.01
	D5	6.97 ^{aA}	4.00 ^b	3.83 ^{bB}				
Lactic acid (g/kg DM)	D0	0.26	0.45	0.93	0.16	0.45	0.14	0.78
	D5	0.20	0.93	1.27				
Acetic acid (g/kg DM)	D0	0.06	0.09	0.21	0.07	0.037	0.55	0.847
	D5	0.30	0.52	0.54				
Lactic acid bacteria (log ₁₀ CFU/g FM)	D0	8.19	7.90	8.09 ^B	0.12	0.02	0.34	0.84
	D5	9.07	8.43	8.76 ^A				
Coliform bacteria (log ₁₀ CFU/g FM)	D0	4.15 ^{aB}	<2.00 ^{bB}	<2.00 ^{bB}	0.502	0.55	<0.01	0.07
	D5	8.58 ^{aA}	5.52 ^{bA}	6.00 ^{bA}				
Yeast (log ₁₀ CFU/g FM)	D0	<2.00 ^B	<2.00 ^B	<2.00 ^B	0.41	<0.01	-	-
	D5	7.43 ^{aA}	4.56 ^{cA}	5.41 ^{abA}				
Molds (log ₁₀ CFU/g FM)	D0	<2.00 ^B	<2.00	<2.00	-	-	-	-
	D5	6.06 ^{aA}	<2.00 ^b	<2.00 ^b				
Crude protein (%DM)	D0	11.3	11.7	11.9	0.13	0.18	0.04	0.54
	D5	10.6	11.3	11.9				
True protein (%DM)	D0	6.35 ^B	6.27	6.79	0.12	0.34	0.02	0.01
	D5	7.88 ^{aA}	5.84 ^b	6.44 ^b				
Ammonia nitrogen (%DM)	D0	0.121 ^a	0.047 ^b	0.033 ^c	0.01	0.04	<0.01	0.06
	D5	0.230 ^a	0.055 ^b	0.041 ^b				

Means in the same column (A–B) or row (a–c) followed by different letters differ ($P < 0.05$). SEM, standard error of means; DM, dry matter; CFU, colony-forming units; FM, fresh matter; D, aerobic exposure days effect; T, treatments effect; D×T, the interaction effect of treatments and aerobic exposure days; D0, aerobic exposure of day 0; D5, aerobic exposure of day 5.

TABLE 3 Alpha diversity of microbial communities of Napier grass silage during aerobic exposure.

α diversity	Item	Treatment	Sobs	Chao1	Ace	Simpson	Coverage
Bacteria	D0	CK	208.66	230.32	224.98	0.82	1.00
		1%PA	217.00	252.57	261.22	0.74	0.99
		2%PA	220.33	257.96	261.90	0.74	1.00
	D5	CK	165.00	198.12	205.93	0.80	0.99
		1%PA	180.67	210.20	221.16	0.66	0.99
		2%PA	192.33	229.29	241.73	0.63	1.00
Fungi	D0	CK	495.67	576.33	570.65	0.93	1.00
		1%PA	482.00	544.73	538.04	0.94	1.00
		2%PA	382.33	447.77	449.99	0.79	0.99
	D5	CK	266.33	331.73	348.81	0.57	1.00
		1%PA	273.00	324.68	336.70	0.56	0.99
		2%PA	251.33	312.07	311.92	0.56	1.00

CK, control; D0, aerobic exposure of day 0; D5, aerobic exposure of day 5; 1% PA, 1% FM pyroligneous acid added; 2% PA, 2% FM pyroligneous acid added.

abundance of *Pseudozyma* decreased after 30 days of ensiling, compared with the fresh material. PA application decreased the relative abundance of *Mortierella*. A higher abundance of *Cladosporium* was observed in 2% PA-treated silage than in

the control group. After 5 days of exposure to air, the relative abundance of *Kodamaea* decreased, while that of *Monascus* increased with PA addition. Moreover, the high abundance of *Neurospora* was observed in 2% PA-treated silage.

Discussion

Characteristics of the fresh Napier grass

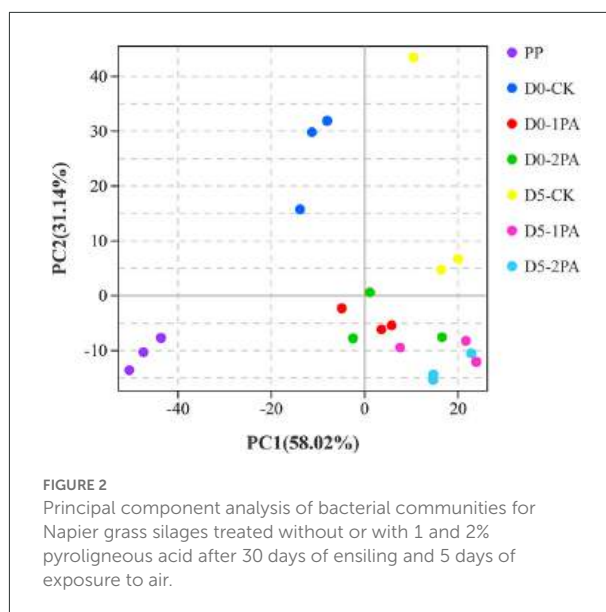
In the present study, the DM content was lower than the ideal value (30–35%) of ensiling (Guyader et al., 2018). As Wang et al. (2021) reported, the suitable DM content of the fresh material is necessary to inhibit the fermentation of undesirable microorganisms, mainly *Clostridium*, which would lead to the nutrient loss and the generation of high proportion of $\text{NH}_3\text{-N}$ during ensiling. The relatively high number of *Clostridium* might be due to the inadequate DM content. The CP content of Napier grass observed in this study was higher than that determined by Du et al. (2022). The difference might be due to the factors such as climate, location, varieties, harvest time, and plant conditions (Wang et al., 2020). The WSC content and LAB count of the fresh material are two decisive factors to obtain well-preserved silage. In general, 60–70 g/kg of the DM WSC content is required to provide the sufficient fermentation substance (Wang et al., 2019). As dominant bacteria, the LAB count should reach the theoretical requirement ($>5.00 \log_{10}$ CFU/g FM) (Wang et al., 2018). But both the WSC content and LAB count did not meet conditions of good fermentation. The numbers of undesirable microorganisms were also relatively high. Therefore, measures should be taken to improve the quality of Napier grass silage.

Aerobic stability of Napier grass silage

The changing temperature was recognized as a key indicator to estimate the aerobic stability of silage (Drouin et al., 2021). After exposure to air, aerobic fungi grow abundantly and release massive heat in the process of metabolizing and consuming nutrients. In particular, yeasts are regarded as the promoter of aerobic deterioration, the number of which can partly reflect the increased temperature of silage (He et al., 2020b). Therefore, the improvement in aerobic stability of PA-treated silage could be indirectly explained by a reduction in the number of yeasts. With the extension of aerobic exposure time, the temperature of silage could achieve a peak value during the period of the vigorous growth of aerobic fungi (Da Silva et al., 2018). The addition of PA markedly decreased the maximum temperature of silage, which indicates that PA has an important influence on inhibiting the activity and growth of aerobic fungi and improving aerobic stability of silage.

Fermentation quality of Napier grass silage

The pH value less than 4.2 is the standard for well-preserved silage, which was greatly affected by acid concentration and



buffer capacity of the material (Kung et al., 2021). In the present study, the decreased pH value might be due to accumulated lactic acid and acetic acid in PA-treated silage, which effectively inhibited the growth of undesirable microorganisms (coliform bacteria, yeasts, and molds). In the process of fermentation, abundant enzymatic reactions and microbial activities occur (He et al., 2020a). Among these reactions, protein hydrolysis is one of the most important reactions, where TP is converted to non-protein nitrogen (such as small peptides and amino acids free nitrogen) and $\text{NH}_3\text{-N}$ (Wang et al., 2021). $\text{NH}_3\text{-N}$, an alkaline substance, is produced by the respiration of plant cells and the metabolism of microorganisms (mainly the metabolism of microorganisms such as coliform bacteria). PA application significantly reduced the $\text{NH}_3\text{-N}$ content, indicating that it could effectively decrease proteolysis by direct acidification.

Microbial diversity of Napier grass silage

The next-generation sequencing technique has been extensively used to detect the composition and abundance of microbial communities in silage (Ni et al., 2017). In the present study, Good's coverage values indicated that most microorganisms were sufficiently captured by sequencing. In addition, the microbial α -diversity of each treatment was evaluated by OTUs (Sobs), richness (Chao1 and Ace indexes), and diversity (Simpson). The addition of PA resulted in the increase in Sobs, Chao1, and Ace values and a decrease in the Simpson value in Napier grass silage, indicating an increase in the richness of bacterial communities in Napier grass silage but a decrease in its diversity. This might show that the higher the abundance of dominant bacteria, the lower the diversity

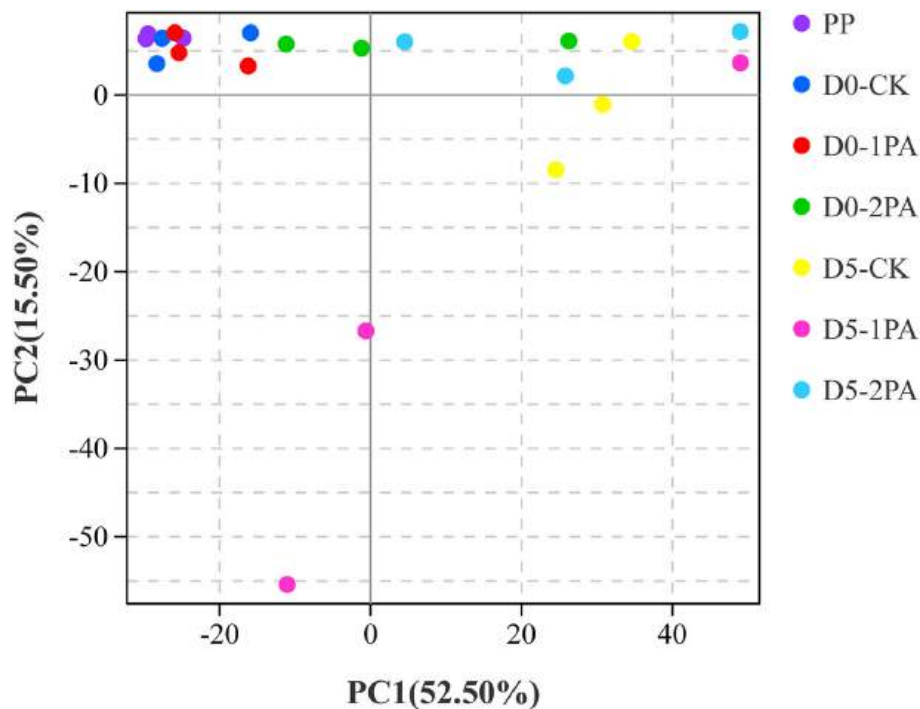


FIGURE 3

Principal component analysis of fungal communities for Napier grass silages treated without or with 1 and 2% pyroligneous acid after 30 days of ensiling and 5 days of exposure to air.

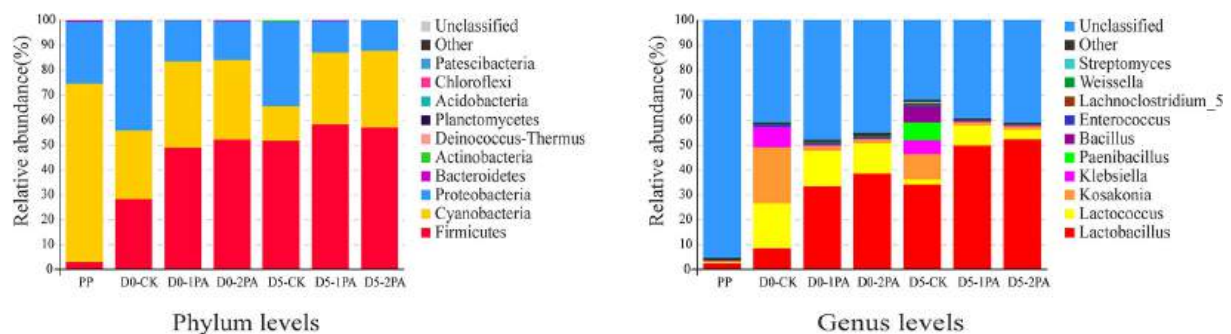


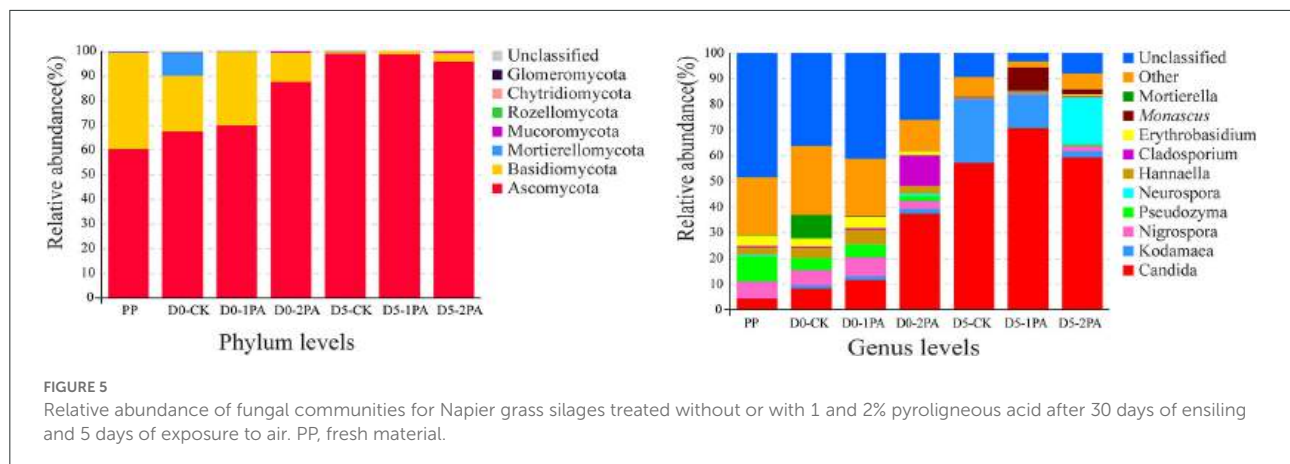
FIGURE 4

Relative abundance of bacterial communities for Napier grass silages treated without or with 1 and 2% pyroligneous acid after 30 days of ensiling and 5 days of exposure to air. PP, fresh material.

of the bacterial community (Ogunade et al., 2018). Moreover, PA application showed strong anti-fungal property, thus decreasing the richness and diversity of fungal communities of Napier grass, especially in 2% PA treatment. With the increase in the duration of aerobic exposure, the microbial α -diversity of each treatment reduced. Similarly, Zhang et al. (2019b) also found that the α -diversity of fungal communities decreased from day 0 to day 3 of aerobic exposure. However, the change in bacterial α -diversity found in this study was

inconsistent with that of our research, and its diversity did not decrease.

The β -diversity of microbial communities was analyzed to compare the difference in the flora structure and species composition among the samples using PCA, a specific analysis tool. A clear separation between PA-treated and untreated silage showed that PA exerted an apparent effect on microbial communities. Moreover, the increase in the PA concentration might promote the variance of the fungal community, whereby



resulting in clear segregation between 2% PA-treated and untreated silage. The extended duration of aerobic storage might also influence the β -diversity of fungal communities, which increased the discreteness of all samples.

Microbial abundance of Napier grass silage

In the process of aerobic storage, the relative abundance of microbial communities changed, which might cause the variation of the chemical composition (Zhang et al., 2021). *Cyanobacteria*, *Firmicutes*, and *Proteobacteria* were the most prevalent bacterial phyla in silage (Liu et al., 2019). Among these phyla, *Firmicutes* was the prominent bacterium in most grass silage samples, which has a positive effect on hydrolysis and acidogenesis (St-Pierre and Wright, 2014). In the present study, *Cyanobacteria* was the most abundant bacterium detected before ensiling. However, the relative abundance of *Firmicutes* increased after ensiling; especially, the dominant phylum shifted from *Cyanobacteria* to *Firmicutes* in PA-treated silage. Moreover, *Proteobacteria* might have low acid-tolerant ability, and its growth was affected by PA application. Ridwan et al. (2015) reported that *Proteobacteria* could use lactic acid and cause nutrient loss. Thus, PA application might be beneficial to preserve forage nutrition. *Lactobacillus* and *Lactococcus* are commonly used as silage additives, which can quickly occupy the dominant position after competing with undesirable microorganisms at the early ensiling stage and produce organic acids to ensure good fermentation (Yang et al., 2016). Their high relative abundance might lead to a decreased pH value and improved silage fermentation quality in PA-treated silage. On the contrary, *Bacillus* and *Paenibacillus* are aerobic bacteria, which can rapidly consume organic acids and sugar, and increase the pH value (Graf et al., 2016). The low relative abundances of *Bacillus* and *Paenibacillus* would expectedly improve fermentation quality and aerobic stability in PA-treated

silage after 5 days of exposure to air. *Kosakonia*, belonging to *Enterobacteriaceae* family, possesses the characteristics of promoting plant growth, such as nitrogen fixation (Quintas-Nunes et al., 2022), and can also decrease the conversion of molecular nitrogen to NH_3 and mainly synthesize proteins (Gao et al., 2020). The growth of *Kosakonia* might explain the phenomenon that the untreated silage had a high TP content after 5 days of exposure to air. The content of true protein was higher in the control than in 1% PA- and 2% PA-treated silage. However, *Kosakonia* might have weak acid-resistant ability, and its abundance was reduced in PA-treated Napier grass silage. *Klebsiella* is a Gram-negative facultative anaerobe and is regarded as a harmful bacterium in silage, which can cause inflammation and aerobic spoilage of feed (Lin et al., 2021). The high relative abundance of *Klebsiella* may be one of the reasons for aerobic deterioration in the untreated Napier grass.

Fungi are considered as the main promoters of aerobic deterioration of silage. Understanding the dynamics of fungal composition and their relative abundances is conducive to analyzing the role of different fungal communities and the effects of PA. In the present study, *Ascomycota* and *Basidiomycota* were the most dominant phyla present in the silage samples. Similarly, Romero et al. (2017) found that *Ascomycota* was the predominant fungal phylum before and after ensiling. *Mortierellomycota* is often associated with the increase in the pH value of soil (Shi et al., 2020). It might also explain the higher pH value in the untreated silage after 30 days of ensiling. *Candida* was one of the colonizers in Napier grass, the relative abundance of which was increased after ensiling and aerobic exposure. Khunnamwong et al. (2020) previously reported that three *Candida* species played a major role in the inhibition of *Aspergillus fumigatus* growth due to the fungistatic effect. However, the relevant report also indicated that *Candida* was an undesirable microorganism and could assimilate lactic acid to accelerate the aerobic decay of silage (Liu et al., 2019). But in the present study,

the high abundance of *Candida* in PA-treated silage did not accelerate the spoilage of Napier grass silage. Perhaps more research is needed to understand the effect of *Candida* during the aerobic exposure of silage. *Cladosporium* is a prevailing dominant endophytic genus in many plants, which can produce secondary metabolites with antioxidant, antimicrobial, and growth-promoting properties (Chen et al., 2022). Therefore, *Cladosporium* with a high abundance in 2% PA-treated silage might have a positive effect on improving fermentation quality and aerobic stability of silage. *Mortierella* is often detected in over-heated and rotting plant material with pH values of 8–9, which is hardly isolated from good-quality silage or hay (Austwick, 1976). In the present study, *Mortierella* was effectively inhibited by PA after 30 days of ensiling, the relative abundance of which was far below 0.15%. After 5 days of exposure to air, the addition of PA increased the relative abundance of *Monascus*. According to the report of Liu et al. (2022), *Monascus* can produce a variety of nutritional or functional molecules (including small molecular peptides, free amino acids, and ergosterol), which can inhibit other microorganisms. Therefore, *Monascus* might be a beneficial fungus for silage preservation. *Kodamaea*, belonging to the *Ascomycota* phylum, is reported to cause life-threatening infections in humans (Diallo et al., 2019). The high abundance of *Kodamaea* might be undesirable in silage. *Neurospora* can produce xylanase (Liu et al., 2020). The high abundance of *Neurospora* might degrade lignocellulose and provide sufficient fermentation substrate for LAB during ensiling. It might promote LAB fermentation and obtain a lower pH value in 2% PA-treated NG.

Conclusion

In the present study, PA application decreased the numbers of coliform bacteria, yeasts, and molds; pH value; and $\text{NH}_3\text{-N}$ content. PA-treated silage samples were stable during the 5-day aerobic test. The addition of PA increased the relative abundance of *Lactobacillus* and reduced that of *Klebsiella*, and *Kosakonia*. For fungal communities, it also increased the relative abundance of *Candida*. After 5 days of exposure to air, PA application decreased the relative abundance of *Kodamaea* and increased that of *Monascus*. The abundances of *Cladosporium* and *Neurospora* were relatively high in 2% PA-treated NG, while these genera were invisible in the control group. In sum, PA application could improve fermentation characteristics and aerobic stability, as well as alter microbial communities of silage. The addition of 2% PA showed a better effect.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://submit.ncbi.nlm.nih.gov/subs/bioproject/SUB11801185/overview>; PRJNA827708 <https://www.ncbi.nlm.nih.gov/sra/PRJNA827708>, PRJNA858920.

Author contributions

DC and MZ: investigation, software, data curation, formal analysis, and writing—original draft. YZho: investigation, methodology, visualization, and validation. LG and WZ: investigation, methodology, visualization, and validation. WX: revision and validation. MW: conceptualization, data curation, project administration, supervision, and validation. YZhu: conceptualization, funding acquisition, project administration, resources, funding acquisition, and validation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author WZ was employed by company Zhengzhi Poultry Industry Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Change of zinc mobilization and gene expression of key zinc transport proteins between the yolk sac membrane and liver of duck embryonic developing

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ABSTRACT Zinc (Zn) deposition in egg yolk is essential for the rapid growth and complete development of the avian embryo. Thus, it is crucial to obtain maximal Zn mobilization at an appropriate time during development in favor of the survival of avian embryos. The aim of this study was to study the developmental change of Zn mobilization and gene expression related to key Zn transport proteins between the yolk sac membrane and embryonic liver from the incubation d 17 (E17) to d 32 (E32) during duck embryonic developing. The weights of duck embryo, embryo without yolk sac, and embryonic liver increased as well as the yolk sac weight decreased linearly ($P < 0.0001$) when incubation day increased. The Zn concentration in the yolk sac did not change from E17 to E29 and only declined significantly from E29 to E32 of duck embryos, while hepatic Zn level decreased linearly as with the increased incubation time ($P < 0.01$). When the incubation day increased, the decreased Zn amount in the yolk sac and the increased

Zn amount in the embryonic liver were observed ($P < 0.0001$). The calculated transfer-out rate of Zn in the yolk sac and transfer-in rate of Zn in livers were both increased from E23-26 to E29-32 ($P < 0.01$). Among E17, E23 and E29, the *solute carrier family 39 member (ZIP)* of *ZIP10*, *ZIP13*, and *ZIP14* genes mRNA expressions were increased in yolk sac membrane but were decreased in the embryonic liver, while *metallothionein 1* mRNA expression was increased both in the yolk sac membrane and liver ($P < 0.05$). In conclusion, yolk sac membrane and embryonic liver tissues displayed the similar developmental patterns of Zn mobilization and *metallothionein 1* mRNA expression from E17 to E32 during duck embryonic developing. The appropriate time of the maximal rate of Zn mobilization were observed between E29 and E32 of duck embryo, associated with the significant changes of gene expression related to some key Zn transport proteins on E29 in yolk sac membrane and liver tissues.

Key words: zinc mobilization, developmental change, zinc transport protein, duck embryo

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INTRODUCTION

Embryonic growth and development are dependent on the nutrients deposited in the egg (van der Wagt et al., 2020). Zinc (Zn) as an essential trace mineral was required in a small amount for the rapid growth and complete development of the avian embryo

(Richards, 1997). Severe Zn deficiency resulted in the abnormal embryonic development and poor performing offspring in both hens (Blamberg et al., 1960) and broiler breeders (Zhu et al., 2017b). The disruption of embryonic development was associated with Zn function either as catalytic or structural cofactors in metal-containing enzymes and proteins as a function of development (Huang et al., 2019). Maternal Zn supply or in ovo Zn injection increased Zn deposition in egg yolk and then was transferred to the developing avian embryo from storage sites to promote the growth and development (Zhu et al., 2017a; Sun et al., 2018). Thus, it is crucial to study the developmental change of Zn mobilization to obtain an appropriate time during development in favor

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of the survival of avian embryos. A number of reports have been reported on the transfer of trace minerals (Zn, copper, manganese, etc) from the egg to the developing avian embryos (Ramsay, 1951; Sandrock et al., 1983; McCormick, 1991). Richards (1991b) showed that the Zn concentration declined in yolk sac of the chicken embryo and liver tissue of turkey embryo between d 14 and 28 of incubation (Richards, 1991), indicating that an active Zn mobilization occurred in the interorgan transport during development. However, it was unclear whether there was the parallel developmental change of Zn mobilization between yolk sac and liver tissues in avian species. The mobilization and uptake of yolk Zn stores is mediated by the yolk sac membrane principally; while the transferred Zn was transported and stored in embryonic liver, which is the most important organ for the storage and homeostatic regulation of trace mineral metabolism (Richards, 1997). The Zn homeostasis is regulated by the large number of transport proteins that are potentially dedicated to Zn^{2+} transport and storage, including members of the ZnT (Zn^{2+} transporter) family, members of the ZIP (i.e., Zn^{2+} -regulated metal transporter) family and metallothionein isoforms (Sekler et al., 2007). However, limited information was available for the gene expression of key Zn-binding protein on Zn homeostasis regulation. Hence, the aims of the present study were to determine the developmental change of Zn mobilization and gene expression related to key Zn transport proteins between yolk sac membrane and liver tissues from incubation d 17 to 32 during duck embryonic developing.

METHODS AND MATERIALS

Animals and Incubation

All animal protocols used in the present study were approved by the South China Agricultural University Institutional Animal Care and Use Committee.

Eighty 33-wk-old laying duck breeders were selected from a commercial breeder farm (WENS Group, Yunfu, Guangdong, China) and were fed restrictively (160 g/d/bird) with a commercial feed at the nutritional level (11.82 MJ ME/kg, 180 g CP/kg, 8.0 g lysine/kg, 7.2 g methionine + cysteine/kg, 24.0 g calcium/kg, 3.8 g available phosphorus/kg, 87.4 mg zinc/kg). All birds were reared in the caged system under a temperature of $22 \pm 2^\circ\text{C}$ and a humidity of $55 \pm 5\%$. Water was available ad libitum and a lighting program 16L: 8D was provided. On the last 2 d of 33 wk of age, a total of 80 hatched eggs were selected to study the developmental changes of weights and Zn mobilizations in embryos. All eggs were placed in 2 trays at the middle of the trolley, which were maintained at an incubation temperature of $37.5 \pm 0.5^\circ\text{C}$ and a relative humidity of $55 \pm 5\%$ until embryonic d 30 (E30). Eggs were turned at an angle of 90° every hour from the start of incubation until E30. Then, all eggs were transferred to hatching crates and moved to hatchers. The hatcher was set at a temperature of $37.0 \pm 0.5^\circ\text{C}$, which declined to $36.0 \pm 0.5^\circ\text{C}$ at

the end of incubation. Male duck breeders were fed the same diet formulated to meet the nutritional requirements throughout the experimental period. The practice of semen collection started at 25 wk of age, and the quality of semen was determined by the volume and numbers of semen and sperm motility. During the experimental feeding period, semen was collected and mixed from male duck breeders at 33 wk of age. Artificial insemination was performed every twice one week.

Sample Collections and Analyses

Ten egg embryos, representing the weight distribution of the eggs at set, were selected on E17, E20, E23, E26, E29, and E32, respectively. Two embryos were killed by cervical dislocation and then the egg embryos, yolk sac, yolk sac-free embryo and liver were weighed to calculate the average values per replicate from each incubation time. The yolk sac content was separated from the yolk sac and then homogenized. The yolk sac and liver samples were stored at -20°C for Zn analyses. Small pieces of yolk sac membrane and embryonic liver samples on E17, E23, and E29 were rinsed in a 0.9% autoclaved-saline solution and placed in microcentrifuge tubes at -80°C for analysis of mRNA expression of genes related to Zn absorption and transport. Equal weight subsamples from the 2 embryos in each replicate were pooled into one sample for the measurements of Zn content and gene expression.

Zinc contents in yolk sac and embryonic liver samples were measured using an inductively coupled plasma emission spectroscope (IRIS Intrepid II, Thermal Jarrell Ash, Waltham, MA) after wet digestions with HNO_3 and HClO_4 as described by (Zhu et al., 2017b). The total Zn contents of the yolk sac and embryonic liver were calculated by multiplying Zn concentration and weight. The relative Zn mobilization rate was calculated as the ratio of Zn content change expressed as the absolute values in yolk sac or embryonic liver per day between E17-20, E20-23, E23-26, E26-29, and E29-32, respectively. For determination of gene mRNA expression, total RNA extraction, reverse-transcription, and RT-qPCR for gene mRNA expression were conducted as described previously (Zhu et al., 2015). The primer sequences are listed as following: *GAPDH* (forward) GGTGCTAAGC GTGTCATCATCTC, (reverse) CCCCTCAGCTGATGCTCCCATGA; *metallothionein 1* (forward) AAAGGCTGCTGCTCCTGCT, (reverse) AGCTGCACTTGGCGGAGG; ZIP6 (forward) ACGCAGATCATCAGCAGAACTTGG, (reverse) GACCTAACC GAGCAACCGACTTG; ZIP8 (forward) AACCACCATCATCCAGCAACG-G, (reverse) ACGGCATCACTCAGTGTTACCATC; ZIP10 (forward) GCCA CAACCACAGCCACCAC, (reverse) AATGCCTCCAAGTGCCACAAGAC; ZIP13 (forward) TGCAGT GCAACAACGGA GAAGG, (reverse) TCTAGCGTCAGGAAGGTCAGGAAG; ZIP14 (forward) TGCTA CTGGCTGAAGGAGGTGAG, (reverse) TGGAA-GACGGAGACGGTGAAGG. The *GAPDH* was used

to normalize the expressions of the targeted genes. The $2^{-\Delta\Delta C_t}$ was used to calculate the mRNA level of each target gene.

Statistical Analyses

Data were analyzed by one-way ANOVA using the PROC GLM procedure of the SAS (SAS Inst. Inc., Cary, NC). Orthogonal polynomials were applied for linear and quadratic effects on the parameters of both embryonic development and tissue Zn mobilization in response of incubation time. All data were presented as mean \pm SEM. The replicate served as the experimental unit. Differences among means were tested by the Fisher's Least Significance Difference test method, and statistical significance was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

The development and growth of poultry embryos are dependent upon the trace minerals deposits in the eggs (Torres and Korver, 2018; Hopcroft et al., 2019). Zinc is an essential nutrient required in small amounts for normal growth and development of the avian embryo functioning as catalytic or structural cofactors in metal-containing enzymes (Huang et al., 2019). When Zn level was increased in the egg and yolk sac, the hatchability was increased primarily due to the decrease incidence of middle and latter embryonic mortality (Zhu et al., 2017a). Therefore, it is crucial to achieving an appropriate time of Zn mobilization exerting its biological functions to promote embryonic growth and development (Richards and Steele, 1987). The developmental processes and Zn mobilization between the yolk sac and liver tissues have been studied in duck embryos from E17 to E32. When incubation day increased, the weights of duck embryo, embryo without yolk sac, and embryonic liver increased linearly but the yolk sac weight decreased linearly ($P < 0.0001$; Figure 1). The weights of the embryo, embryo without yolk sac, and embryonic liver increased

from 32.84 g, 6.88 g, and 0.057 g on E17 to 49.17 g, 45.67 g, and 1.188 g on E32, increased by 15.33 g, 38.79 g, and 1.13 g between E17 and E32, respectively. The weight of the yolk sac decreased from 26.7 g to 3.51 g from E17 to E32. A similar pattern was observed in chick embryos reported previously (Yadgary et al., 2010; Yadgary and Uni, 2012). The Zn concentration in the yolk sac did not change from E17 to E29 and only declined significantly from E29 to E32 of duck embryos ($P > 0.05$, Figure 2). It was not agreed with the declined Zn concentration in yolk sac between incubation reported in the developing chick embryo (Dewar et al., 1974). Hepatic Zn level decreased linearly as with the increased incubation time, which was consistent with that observed in turkey embryo during the incubation period (Richards and Weinland, 1985). The Zn amount in the yolk sac decreased linearly when the incubation day increased, while the Zn amount in the embryonic liver increased linearly as with the increased incubation time ($P < 0.0001$; Figure 2). Also, our results showed that the calculated transfer-out rate of Zn in the yolk sac and transfer-in rate of Zn in livers were both increased from E23–26 to E29–32. The parallel developmental changes of Zn mobilization rate in the interorgan transport during the latter half incubation implied that the maximal rate of Zn mobilization during E29 to E32 could be conducted to serve a wide range of biological reactions, such as functioning as an antioxidant defense system. It was indicated that close to hatching, the greater Zn demand may be required from the yolk sac to the target tissues to maintain the rapid growth and development (Richards, 1997). Because that the yolk sac and liver tissues were developing to its full function during incubation, it is possible that the transfer of Zn to the embryo is upregulated by the yolk sac during the latter time of incubation. Additionally, the developmental change of Zn mobilization suggested that transfer mechanisms might be involved in some key Zn transport proteins in duck embryos at the different developmental stage.

The complexity and importance of Zn homeostasis is regulated by the large number of proteins that are

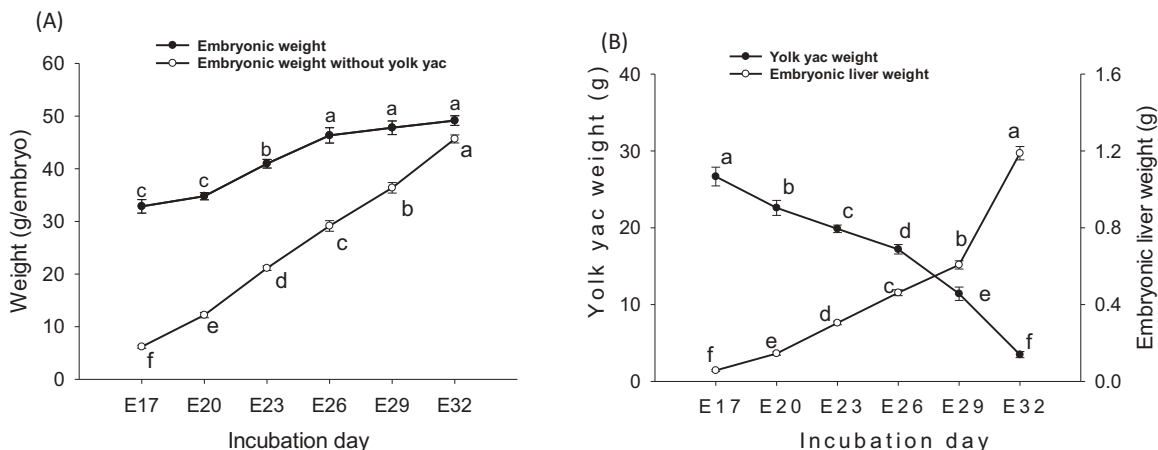


Figure 1. Developmental changes of weights in the embryo, embryo without yolk sac, yolk sac, and embryonic liver from E17 to E32. All values are expressed as means \pm SE. Means with different letters (A-F) differ significantly ($P < 0.05$) between incubation days. Mean represented the average value of 5 replicates ($n = 5$).

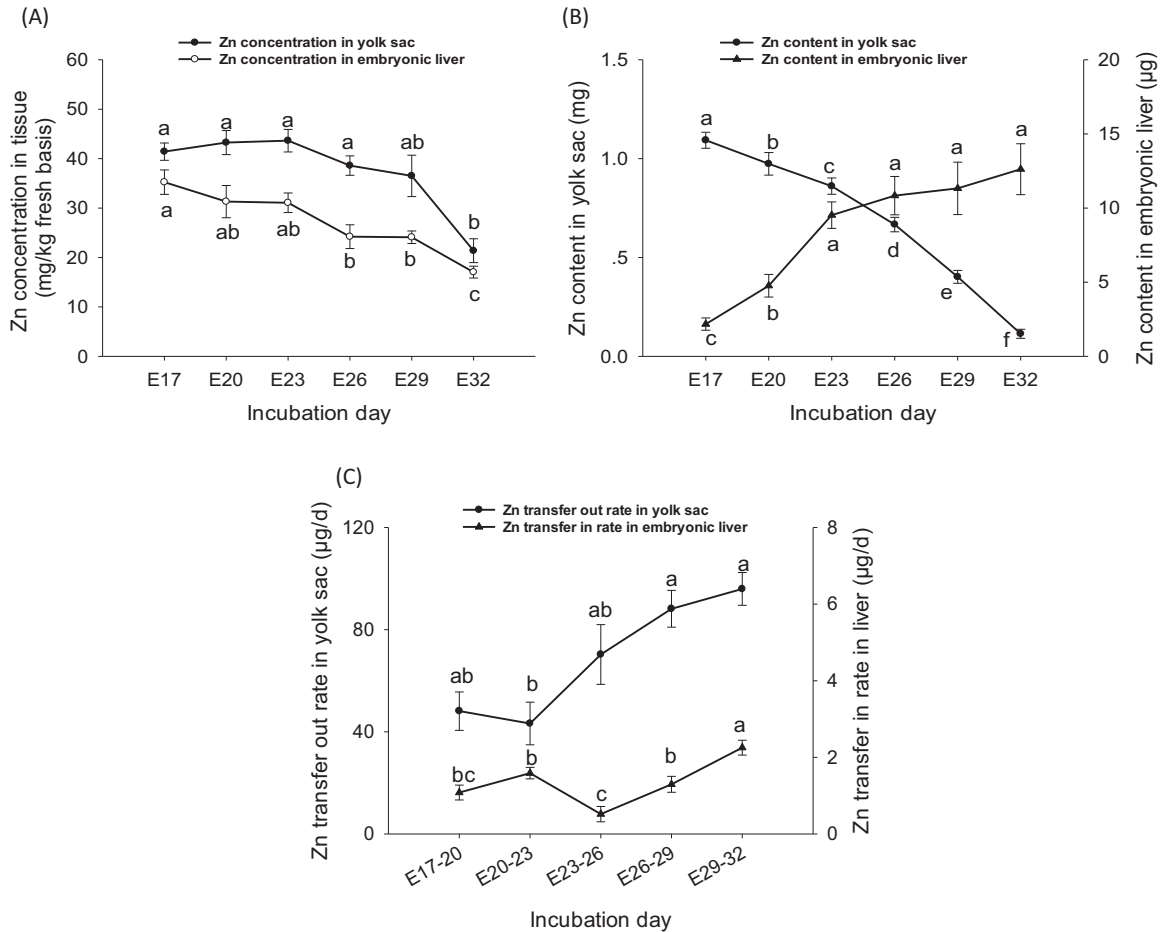


Figure 2. The developmental changes of Zn concentration (A) and Zn amount (B) in yolk sac as well as Zn mobilization rates (C) in yolk sac and embryonic liver. All values are expressed as means \pm SE. Means with different letters (A-F) differ significantly ($P < 0.05$) between incubation days. Mean represented the average value of 5 replicates ($n = 5$). The total Zn contents in yolk sac and embryonic liver were calculated by multiplying Zn concentration and weight. Then, the relative Zn mobilization rates were calculated as the ratio of Zn content change in yolk sac or embryonic liver per day during E17–20, E20–23, E23–26, E26–29, and E29–32, respectively.

potentially dedicated to Zn^{2+} transport and buffering, such as ZIP member family and metallothionein isoforms (Sekler et al., 2007). In mammals, ZIP transporters increase intracellular cytoplasmic Zn by promoting extracellular and, perhaps, vesicular Zn transport into cytoplasm. The expressions of Zn transporter protein genes have been proved to be sensitive to the changes of Zn status during embryonic development (Andrews et al., 2004; Dufner-Beattie et al., 2006). However, the role of ZIP transporter has not been fully revealed in avian species. In response to the increased incubation day, *ZIP10*, *ZIP13*, and *ZIP14* mRNA expressions were increased in yolk sac membrane but were decreased in the embryonic liver ($P < 0.05$), while *ZIP8* mRNA expression was increased in yolk sac membrane from E17 to E23 and then decreased from E23 to E29 ($P < 0.01$; Table 1). It is assumed that the different synthetic abilities of specific Zn-binding protein between yolk Zn mobilization and hepatic Zn transportation and storage. Between E17 and E29, *metallothionein 1* mRNA expression in both the yolk sac membrane and embryonic liver was increased with the increased incubation time ($P < 0.05$; Table 1). Similar findings were confirmed by the greater hepatic *metallothionein 1* mRNA expression in the chick embryo at the latter incubation

stage. As reported previously, hepatic Zn level of cytoplasmic Zn could induce metallothionein expression as a function of development in embryo. Moreover, maternal Zn supply or *in ovo* Zn injection could induce *metallothionein* mRNA expression in chick embryos during the latter half of incubation (Zhu et al., 2017a; Sun et al.,

Table 1. The developmental change of gene expressions related to key Zn transport proteins in yolk sac membrane and embryonic liver tissues.

Item ^{1,2}	Incubation day	MT1	ZIP6	ZIP8	ZIP10	ZIP13	ZIP14
YCM	E17	0.41 ^b	1.40	1.31	0.68 ^b	0.75 ^b	0.68 ^b
	E23	2.78 ^a	1.20	1.07	1.43 ^a	0.76 ^b	1.00 ^b
	E29	3.56 ^a	1.00	1.08	1.41 ^a	1.44 ^a	1.56 ^a
	SEM	0.67	0.24	0.17	0.13	0.15	0.16
	P value	0.01	0.54	0.55	0.002	0.001	0.008
Liver	E17	0.44 ^b	1.27	0.56 ^b	1.85 ^a	2.25 ^a	3.41 ^a
	E23	1.81 ^{ab}	1.04	1.59 ^a	1.19 ^b	1.14 ^b	0.59 ^b
	E29	4.86 ^a	1.02	0.63 ^b	0.76 ^b	0.73 ^b	0.74 ^b
	SEM	0.87	0.21	0.17	0.18	0.31	0.43
	P value	0.04	0.68	0.003	0.006	0.03	0.002

YCM = yolk sac membrane; *GAPDH* = glyceraldehyde 3-phosphate dehydrogenase; MT1 = metallothionein 1; ZIP 6, 8, 10, 13, 14 = solute carrier family 39 member 6, 8, 10, 13, 14.

The *GAPDH* expression was used to normalize the expressions of the targeted genes.

¹Mean represented the average value of 5 replicates ($n = 5$).

²Lacking common letters (a or b) significant differences at $P < 0.05$.

2018). De et al. (1991) reported that primary cultures of chick embryo hepatocytes and fibroblasts in response to Zn supplementation of culture medium by upregulation of *metallothionein* mRNA expression. The significant changes of *metallothionein 1* and *ZIP10*, *ZIP13*, and *ZIP14* mRNA expressions in tissues on E29 confirmed that the mobilization and uptake of Zn homeostasis was strengthened between the yolk sac membrane and target embryonic liver at this appropriate time.

In conclusion, yolk sac membrane and embryonic liver tissues displayed the similar developmental patterns of Zn mobilization and *metallothionein 1* mRNA expression of duck embryo. The appropriate time of the maximal rate of Zn mobilization were observed between E29 and E32 of duck embryo, associated with the significant changes of genes expression related to some key Zn transport proteins on E29 in yolk sac membrane and embryonic liver tissues.

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DISCLOSURES

The authors declare that there is no conflict of interest related to the preparation and publication of this paper.

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The developmental pattern related to fatty acid uptake and oxidation in the yolk sac membrane and jejunum during embryogenesis in Muscovy duck

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ABSTRACT This study aimed to investigate the developmental change of body growth and gene expression related to fatty acid uptake and oxidation in the yolk sac membrane (YSM) and jejunum during embryogenesis in Muscovy ducks. The weights of embryos and yolk sac (YS) (5 embryos per replicate, $n = 6$) were recorded on embryonic days (E)16, E19, E22, E25, E28, E31, and the day of hatch (DOH). The fat and fatty acid contents in YSM, jejunal histology, and gene expression related to fatty acid metabolism in YSM and jejunum were determined in each sampling time. Among the nonlinear models, the maximum growth is estimated at 2.83 (E22.5), 2.67 (E22.1), and 2.60 (E21.3) g/d using logistic, Gompertz, and Von Bertalanffy models, respectively. The weight of YS, and ether extract-free YS as well as the amounts of fat and fatty acids in YS decreased ($P < 0.05$) linearly, whereas the villus height, crypt depth, villus height/crypt depth,

and musculature thickness in jejunum increased ($P < 0.05$) linearly during embryogenesis. The mRNA expression of *CD36*, *SLC27A4*, and *FABP1* related to fatty acid uptake as well as the mRNA and protein expressions of *PPAR α* and *CPT1* related to fatty acid oxidation increased in a quadratic manner ($P < 0.05$) in both YS and jejunum, and the maximum values were achieved during E25 to E28. In conclusion, the maximum growth rate of Muscovy duck embryos was estimated at 2.60 to 2.83 g/d on E21.3 to E23.5, while the accumulations of lipid and fatty acid in YS were decreased in association with the increased absorptive area of morphological structures in jejunum. The gene and protein expression involved in fatty acid metabolism displayed a similar enhancement pattern between YSM and jejunum during E25 to E28, suggesting that fatty acid utilization could be strengthened to meet the energy demand for embryonic development.

Key words: growth curve, yolk sac membrane, jejunum, fatty acid, embryo

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INTRODUCTION

The selection for increased growth in potential breeders adversely affects the embryonic survival of their offspring (Nestor and Noble, 1995). As the growth of embryos increases, the alteration of nutrients metabolism may not supply enough nutrient to support their growth and development, which can result in a decline in the viability of embryos (Reséndiz-Infante and Gauthier, 2020). The nutritional requirements of avian embryos during incubation are derived from the

albumen and yolk (van der Wagt et al., 2020). The yolk sac (YS) is a membranous structure attached to the developing embryo that acts to support hematopoiesis, metabolism, and coagulation (Goh et al., 2023). The distribution of lipids within the YS undergoes dynamic changes across various embryonic stages and is transferred to embryos directly into the intestine via the YS stalk or through the highly vascularized yolk sac membrane (YSM) (Ding and Lilburn, 1996; Meng et al., 2021). As the energy demand increases during embryogenesis, fatty acids (FA) in the yolk can be mobilized more dramatically to support embryonic growth via enhancing mitochondrial β -oxidation for ATP generation (Ornoy and Miller, 2023; Zhang et al., 2023). Additionally, the YS generates the first blood and immune cells, while the jejunum serves as the primary absorption site (Goh et al., 2023). The specialized surface area of the jejunum, such as villi or microvilli, is increased to

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maximize nutrient absorption to maintain the development and growth of avian embryos (Ginzel et al., 2021; Fish et al., 2024). So far, the developmental change of nutrient utilization has been extensively studied in chick embryos (Tong et al., 2013; Nasri et al., 2020; Petit et al., 2022; Yin et al., 2023), but rarely in Muscovy duck embryos. Moreover, most studies have focused on the role of amino acids (Wang et al., 2023; Lugata et al., 2024), carbohydrates (Retes et al., 2018), and minerals (Halgrain et al., 2022; Kpodo and Proszkowiec-Weglarz, 2023) on improving embryonic viability, but the nutritional role of either lipid or fatty acid on embryonic development is relatively limited. It is hypothesized that understanding the mobilizations of lipid and fatty acids between YSM and jejunum could be crucial for the growth and viability of avian embryo development. Therefore, the growth curve and developmental pattern of gene expression related to fatty acid uptake and oxidation between YSM and jejunum have been investigated in the present study, which could provide new insight into understanding the fatty acid utilization in the pre-hatch period to improve embryonic growth of Muscovy duck.

MATERIALS AND METHODS

Ethics Statement

All animal protocols used in the present study were approved by the South China Agricultural University Institutional Animal Care and Use Committee (SCAU-10564).

Eggs Incubation

All hatched eggs were purchased from the same batch of 33-wk-old laying duck breeders in a commercial breeder farm (WENS Group, Yunfu, Guangdong, China). After fumigation with 37% formalin and potassium permanganate (2:1), a total of 300 eggs were randomly distributed among 6 trays of an automatic incubator (Dezhou Keyu Hatching Equipment Co., Ltd, Dezhou, China) and incubated at a temperature of $37.5 \pm 0.5^\circ\text{C}$ and $55 \pm 5\%$ humidity until embryonic d 31 (E31). Next, all eggs were transferred to hatching crates and moved to hatchers, which was set at $37.0 \pm 0.5^\circ\text{C}$ and then dropped to $36.0 \pm 0.5^\circ\text{C}$ on the day of hatch (DOH). Infertile eggs and nonviable embryos were discarded on E15 after candling identification. Then, 30 fertilized eggs (75.2 ± 1.8 g) were selected for sample collection on E16, E19, E22, E25, E28, E31, and DOH (5 embryos per replicate, $n = 6$).

Sample Collection

Five egg embryos per replicate, represented the weight distribution of the eggs at the set, were selected on E16, E19, E22, E25, E28, E31, and DOH, respectively. The whole embryo and YS were weighed individually. The YS from 5 embryos each replicate with equal weight were pooled together for ether extract and fatty acid analysis. A 1 cm mid-jejunum sample from one of 5

embryos was fixed in 4% formaldehyde for histological analysis, while the YSM and the rest of the jejunum samples were rinsed with ice-cold phosphate-buffered saline frozen in liquid nitrogen and stored at -80°C for relative expression of gene mRNA and protein analysis.

Ether Extract and Fatty Acid Analysis

Lipid extraction and fatty acid analysis of YS samples were determined as described previously (Zhang et al., 2023). The YS samples were vacuumed frozen and dried (FD8-5, Gold Sim, CA) and ether extract analysis was conducted according to procedures from AOAC International 2007.01 (Lehotay et al., 2007) using a solvent extraction system (ST 255 Soxtec, FOSS, Hillerod, Denmark). Once the lipids were extracted, they were converted into fatty acid methyl esters (FAMES) through a process called transesterification. The FAMES were then analyzed using gas chromatography (GC). The separated fatty acids were then detected using a flame ionization detector (FID) or mass spectrometer. The total amount of either lipids or fatty acid in YS at each sampling time were calculated by multiplying the nutrient concentration in the YC by the weight of the YC.

Histological Analysis

After fixing over 24 h, the jejunum samples were dehydrated, embedded in paraffin, cut into sections, and stained with standard hematoxylin-eosin (H & E) solution. The slices were observed and photographed using a microscope (Eclipse E100 and DS-U3, Nikon, Tokyo, Japan). The villus height, crypt depth, and musculature thickness in H & E sections were calculated by the software Image J (National Institutes of Health, MD).

Gene mRNA Relative Expression Analysis

Total RNA extraction and cDNA synthesis in YSM and jejunum samples were performed according to reagent protocols using Trizol reagent (Invitrogen, Carlsbad, CA) and Primer Script RT Reagent Kits (TaKaRa, Dalian, China). The primer sequences were listed as following: *CD36* molecule (CD36), TATCGTTTCGCAGTTCCTCGTGAAG (forward), AGTTCTGGGATATGACCTCCTCTGTAC (reverse); *solute carrier family 27 member 4* (SLC27A4), GCCTGATGACGTGATGTACGAC TG (forward), AGAACTTCTTGCGGATGACGATGG (reverse); *peroxisome proliferator-activated receptor alpha* (PPAR α) ACCATCCTGATGATACCTTCCTCTTCC (forward), AAGTTGAGCATGTTCTGTGACAAGTTG (reverse); *retinoid X receptor alpha* (RXR α) TGCAGCCATTGTCC TCTTCAAC (forward), GATGCGTACACCTTCTCC CGTAAC (reverse); *fatty acid-binding protein 1* (FABP1) TGGGAATAAGTTCAAGGTTACCGTCAC (forward), GGTCTTGGCTTTCTCTCCT GTCAG (reverse); *carnitine palmitoyltransferase 1* (CPT1)

CCGCCATCTGTTCTGCCTCTATG (forward), TGTGTTGCTGTGGTGTCTGACTTG (reverse); *acyl-CoA oxidase 3 (ACOX 3)* GAAGGAGAAG-CAGTCAGGGCAAAG (forward), GCAATGGC-TAGTGACCGACAGTAG (reverse), *long-chain-acyl-CoA dehydrogenase (ACADL)*, TGGTGCCATTGC-CATGACAGAAC (forward), CTCCCGTTAA-GAATCCAGTCACTTCC (reverse); β -actin, TACGCCAACACGGTGCTG (forward), GATTCAT-CATACTCCTGCTTG (reverse). Real-time quantitative PCR (**RT-qPCR**) was performed via SYBR Green Realtime PCR Master Mix kit (QPK-201, TOYOBO, Osaka, Japan) on the detection system (Applied Biosystems QuantiStudio 7 Flex, Life Technologies, Carlsbad, CA) following the program: 95 °C for 1 min, followed by 40 cycles each at 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 45 s. The specificity of the amplification was validated by melting curve analysis at the end of the PCR run. The quantification of the mRNA expression was calculated by the comparative CT method ($2^{-\Delta\Delta CT}$) (Livak and Schmittgen, 2001).

Western Blotting Analysis

The YSM and jejunum samples collected on E16, E22, E28, and DOH were homogenized in tissue lysis (AWB0136, Abiowell, Changsha, China). After measurement of concentration via the bicinchoninic acid (BCA) assay kit (Beyotime Biotechnology, Shanghai, China), proteins were separated by SDS-PAGE and transferred to nitrocellulose filter membranes. Immunoreactivity was developed with an enhanced chemiluminescence (ECL) solution (AWB0005, Abiowell). Primary antibodies used were FASN (10624-2-AP, Proteintech, Chicago, IL), PPAR α (ab24509, Abcam, Cambridge, England), CPT1 (15184-1-AP, Proteintech), and β -actin (66009-1-Ig, Proteintech). Primary antibodies used were HRP goat anti-mouse IgG (SA00001-1, Proteintech) or HRP goat anti-rabbit IgG (SA00001-2, Proteintech). The bands were examined using a chemiluminescence imaging system (ChemiScope6100, Qinxian, Shanghai, China) and their density was determined using Image J software.

Statistical Analysis

The non-linear regression models of Logistic, Gompertz, and Bertalanffy (Aggrey, 2002) were applied for the growth curve using the PROC NLIN procedure of the SAS v9.2 (SAS Inst. Inc., Cary, NC). The form of the equation was: where W is the weight corresponding to age (t) with 3 parameters: A = asymptotic or maximum growth response, B = intercept or weight when age (t) = 0, and K = rate constant. Those analysis data were analyzed with a one-way analysis of variance (ANOVA) using the PROC GLM procedure of the SAS v9.2. Data were deemed significant at P -values < 0.05, and the notable differences between groups were identified by Duncan's multiple comparisons test.

Orthogonal polynomial contrasts were used to identify linear and quadratic effects on the indices responded to sampling time (incubation days).

RESULTS AND DISCUSSION

Growth traits played a crucial role in avian embryo development, influencing the overall size, shape, and physiological characteristics of the developing bird. The growth curve regressions were applied for the description of embryonic asymptotic weight and the maximum growth rate at the appropriate time (Makgopa et al., 2023), which had a significant impact on the health, survival, and future performance of offspring birds. In the present study, the maximum growth of Muscovy duck embryos was estimated at 2.83 (E22.5), 2.67 (E22.1), and 2.60 (E21.3) g/d used Logistic, Gompertz, and Von Bertalanffy models ($R^2 > 0.98$; Table 1), respectively. As reported in Pekin duck embryos (Onbasilar et al., 2014; Wei et al., 2024), the relative embryo weights increased dramatically during the period of E16 to E20 (Figures 1A and 1B). This knowledge of the rapid growth period of duck embryos could be applied in the duck industry to evaluate the effect of preincubation handling on embryo development. Embryonic growth and development of poultry embryos were dependent on the availability of nutrients (e.g., proteins, fat, vitamins, and minerals) deposited in the fertile eggs (van der Wagt et al., 2020). YS was a crucial organ for maternal-fetal nutrient exchange, and one of its functions was that the epithelial cells of the YS tissue secreted digestive enzymes and mediated the transport of various nutrients, which partly served as intestine when the organs were developing and maturing (Wong and Uni, 2021; Keuls et al., 2023). The weights of avian YS and lipids gradually decreased as their utilization with the gradual growth of avian embryos (Yadgary et al., 2013). In this study, the weights of YS and ether extract-free YS were decreased ($P < 0.05$) linearly during the embryonic period from E16 to DOH (Figure 1C), which was in line with the previous studies of the embryos of Muscovy duck (Bai et al., 2022), Pekin duck (Onbasilar et al., 2014) and broiler chick (Elibol et al., 2023). It was inferred that the lipid and fatty acids were transferred out to support various aspects of growth, included the

Table 1. Growth curve parameters in non-linear regression models for Muscovy duck embryos (n = 30).

Model	Logistic	Gompertz	Von Bertalanffy
A	43.8	50.2	55.6
B	439.8	24.4	3.13
K	0.26	0.14	0.11
Wpi (g)	21.9	18.5	16.5
Ti (d)	23.5	22.1	21.3
Wmax (g/d)	2.83	2.67	2.60
MSR	10.8	10.4	10.5
R ²	0.98	0.98	0.98

Note: MSR, mean squared residue, calculated by dividing the sum of the squares of the residue by the number of observations; R², adjusted coefficient of determination.

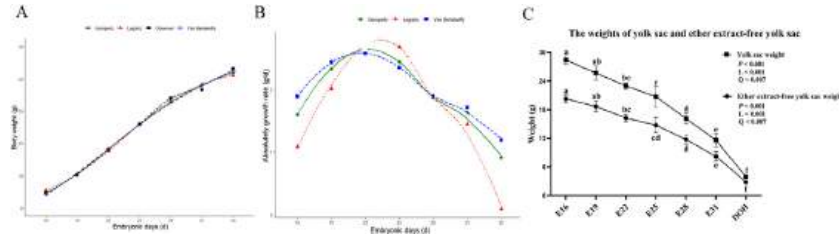


Figure 1. Developmental changes of body growth and yolk sac (YS) weight in the Muscovy duck embryogenesis. Growth curves (A) and absolute growth rate (B) were estimated by the logistic, Gompertz, and Von Bertalanffy models ($n = 30$). (C) The weights of YS and ether extract-free YS were decreased linearly as with the process of incubation.

rate of cell division, organ development, and overall body size (Liu et al., 2022; Bai et al., 2023). There was a linear decline ($P < 0.05$) in the amounts of ether extract and fatty acid of YS along with the process of incubation (Table 2). A similar pattern was observed in several studies of embryonic development of chick (Yadgary et al., 2010; Liu et al., 2020) and turkey (Ding and Lilburn, 2000). As reported previously, several functional FAs (n-6 PUFAs, n-3 PUFAs) declined significantly during the mid-later incubation period, mainly through yolk translocation to the liver or muscle (Cherian et al., 1997; Whelan and Fritsche, 2013). The jejunum was critical for the proper development of the avian embryo by facilitated the absorption of nutrients and supported the growth and maturation of the digestive system (Hu et al., 2024). The jejunum structural changes showed that the villus height, crypt depth, the ratio of villus height to crypt depth, and musculature thickness increased ($P < 0.05$) linearly or quadratically during embryogenesis (Figure 2). This pattern was consistent with previous reports in the Muscovy duck that the jejunum developed rapidly during incubation and increased significantly in weight and length (Ding et al., 2011). Furthermore, similar developmental changes in the intestine were observed in the avian embryos of chick (Reicher and

Uni, 2021), quail (Cruz et al., 2019), and turkey (Ding and Lilburn, 2000). It was implied that the capacity for nutrient digestion could be enhanced to maintain the growth potential of duck embryos during the mid-later incubation period. This enhancement might be associated with the increase in the absorptive area of the jejunum.

The energy supply during the mid-later incubation was mainly generated from fatty acid β -oxidation, which was important to maintain the growth and development of poultry embryos (Noble and Cocchi, 1990). Both YSM and the intestine were responsible for fatty acid metabolism in embryogenesis. Previous studies have shown that a large amount of lipid was absorbed and transferred to the avian embryos through the YSM (Shibata et al., 2023; Babacanoglu, 2024). As the lipid of the yolk was absorbed into the developing embryo's digestive system, the jejunum was responsible for broke down the complex lipids into simpler forms that could be absorbed and utilized for energy production, membrane formation, and other essential functions (Speier et al., 2012; Schneider, 2016). In our study, the mRNA expressions of *CD36*, *SLC27A4*, and *FABP1* related to fatty acid uptake and *PPAR α* , *CPT1*, *RXR*, *ACOX*, and *ACAD* related to fatty acid oxidation were increased in

Table 2. Developmental changes in the amounts of ether extract and fatty acid in the yolk sac of Muscovy duck (g).

Item	E16	E19	E22	E25	E28	E31	DOH	SEM	<i>P</i>	Linear	Quadratic
Ether extract	20.28 ¹	18.65 ^{1,2}	16.25 ^{2,3}	14.78 ^{3,4}	11.75 ⁴	8.23 ⁵	2.82 ⁶	0.954	< 0.001	< 0.001	0.007
C14:0	0.050 ¹	0.048 ¹	0.040 ^{1,2}	0.032 ^{2,3}	0.028 ^{3,4}	0.018 ^{4,5}	0.007 ⁵	0.003	< 0.001	< 0.001	0.040
C16:0	2.298 ¹	2.128 ^{1,2}	1.920 ^{1,2}	1.838 ²	1.250 ³	0.948 ³	0.295 ⁴	0.118	< 0.001	< 0.001	0.012
C16:1	0.183 ¹	0.167 ¹	0.163 ¹	0.148 ^{1,2}	0.116 ²	0.063 ³	0.027 ³	0.010	< 0.001	< 0.001	0.006
C18:0	0.600 ¹	0.523 ^{1,2}	0.465 ^{2,3}	0.407 ^{3,4}	0.335 ⁴	0.203 ⁵	0.082 ⁶	0.029	< 0.001	< 0.001	0.019
C18:1n-9	3.530 ¹	2.858 ²	2.947 ²	2.417 ²	1.877 ³	1.193 ⁴	0.490 ⁵	0.170	< 0.001	< 0.001	0.068
C18:2n-6	0.895 ¹	0.827 ¹	0.765 ¹	0.720 ¹	0.500 ²	0.295 ^{2,3}	0.118 ³	0.050	< 0.001	< 0.001	0.009
C18:3n-3	0.003 ^{1,2}	0.040 ¹	0.028 ¹⁻³	0.037 ^{1,2}	0.025 ^{2,3}	0.015 ^{3,4}	0.005 ⁴	0.002	< 0.001	0.593	0.038
C20:3n-6	0.023 ^{1,2}	0.028 ¹	0.020 ^{1,2}	0.018 ²	0.022 ^{1,2}	0.008 ³	0.003 ³	0.002	< 0.001	0.014	0.150
C20:3n-3	0.252 ¹	0.225 ¹	0.208 ^{1,2}	0.170 ^{2,3}	0.128 ³	0.075 ⁴	0.032 ⁴	0.132	< 0.001	< 0.001	0.005
C24:1n-9	0.023 ¹	0.025 ¹	0.020 ¹	0.183 ^{1,2}	0.021 ^{2,3}	0.010 ³	0.002 ⁴	0.002	< 0.001	0.794	0.319
C22:6n-3	0.052 ¹	0.043 ²	0.038 ²	0.025 ³	0.020 ³	0.010 ⁴	0.002 ⁴	0.003	< 0.001	< 0.001	0.821
MUFAs	3.737 ¹	3.050 ²	3.130 ^{1,2}	2.583 ^{2,3}	1.985 ³	0.843 ⁴	0.518 ⁴	0.190	< 0.001	< 0.001	0.147
PUFAs	1.255 ¹	1.163 ^{1,2}	1.060 ^{1,2}	0.970 ^{2,3}	0.695 ³	0.268 ⁴	0.160 ⁴	0.071	< 0.001	< 0.001	0.062
n-6 PUFAs	0.918 ¹	0.853 ¹	0.783 ¹	0.737 ¹	0.522 ²	0.308 ^{2,3}	0.123 ³	0.051	< 0.001	< 0.001	0.006
n-3 PUFAs	0.333 ¹	0.310 ^{1,2}	0.273 ^{2,3}	0.228 ³	0.170 ⁴	0.103 ⁵	0.042 ⁵	0.018	< 0.001	< 0.001	0.001
UFAs	4.988 ¹	4.218 ^{1,2}	4.187 ^{1,2}	3.552 ²	2.680 ³	1.668 ⁴	0.683 ⁵	0.247	< 0.001	< 0.001	0.022
SFAs	2.950 ¹	2.698 ^{1,2}	2.427 ²	2.280 ²	1.615 ³	1.168 ³	0.380 ⁴	0.149	< 0.001	< 0.001	0.007

Note: MUFAs are the sum of monounsaturated fatty acids that include C16:1, C18:1n-9, and C24:1n-9. PUFAs are the sum of polyunsaturated fatty acids that include C18:2n-6, C20:3n-6, C18:3n-3, C20:3n-3, and C22:6n-3. N-6 PUFAs are the sum of n-6 polyunsaturated fatty acids that include C18:2n-6 and C20:3n-6. N-3 PUFAs are the sum of n-3 polyunsaturated fatty acids that include C18:3n-3, C20:3n-3, and C22:6n-3. UFAs are the sum of unsaturated fatty acids that include MUFAs and PUFAs. SFAs are the sum of saturated fatty acids that include C14:0, C16:0, and C18:0.

¹⁻⁶Data were expressed as mean \pm SEM ($n = 6$), and data at the same line with different lowercase letters indicated statistically significant differences (one-way ANOVA, $P < 0.05$).

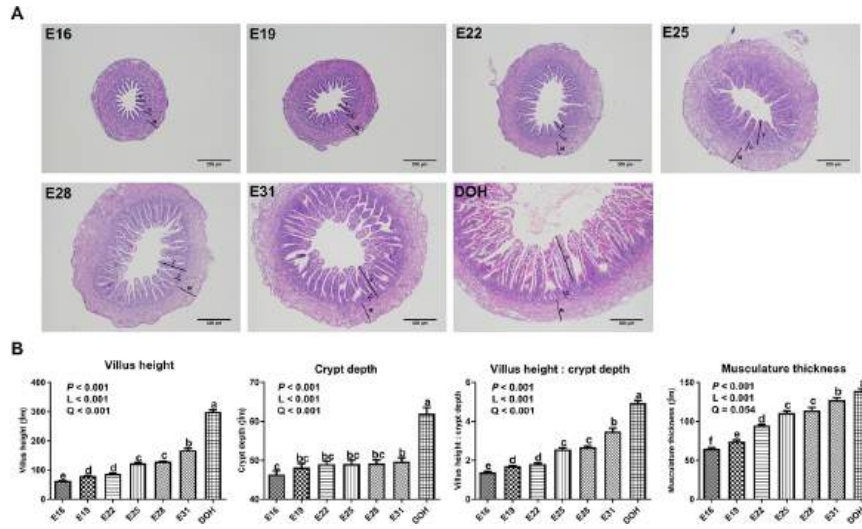


Figure 2. Representative jejunum H & E stained sections in the Muscovy duck embryogenesis. (A) Representative jejunum H & E stained sections and the magnification is 10×20 and the ruler in the lower right corner of the picture is $200 \mu\text{m}$. V, villus height; C, crypt depth; M, musculature thickness. (B) Villus height; (C) crypt depth; (D) villus height: crypt depth; (E) musculature thickness; (B–E) the lengths of villus height, crypt depth, and musculature thickness were determined using Image J software. Data were expressed as mean \pm SEM ($n = 6$), and bars with different lowercase letters indicated statistically significant differences (one-way ANOVA, $P < 0.05$). L, linear; Q, quadratic.

a quadratic manner in both YS and jejunum and were achieved the maximum value on E28 (Figure 3A). This pattern was consistent with the results reported in previous studies that the FAs uptake between the YSM and jejunum was significantly increased to support embryonic growth (Dube et al., 2012; Pepino et al., 2014). Furthermore, the protein expression of PPAR α and CPT1 increased ($P < 0.05$) linearly or quadratic manner both in YSM and jejunum, reached a plateau period of E22–E31 (Figures 3B–3E), which was highly coordinated with the decrease of the amounts of fat and fatty acid in

YS in this study (Table 2) and the increase of the ratio of fat uptake to poultry embryo weight as reported previously (Yadgary et al., 2010; Lešić et al., 2017; Kuchar-ska-Gaca et al., 2023). Several studies have found that the expression levels of genes associated with fatty acid oxidation (PPAR α and CPT1) were significantly up-regulated in the YSM and jejunum during the growth of chick embryos (Fu et al., 2020; Fu et al., 2022). Overall, there was a similar developmental pattern of gene and protein expressions related to fatty acid uptake and oxidation between YSM and jejunum during embryonic

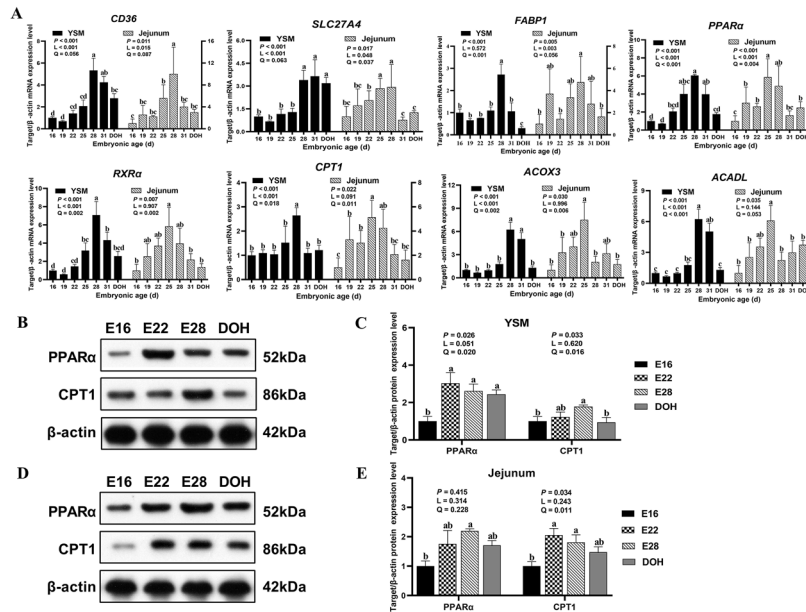


Figure 3. The gene and protein relative expression related to fatty acid metabolism in the Yolk sac membrane (YSM) and jejunum during Muscovy ducks' embryogenesis. (A) Targeted gene relative expression of YSM and jejunum ($n = 6$); (B, C) targeted protein relative expression of YSM ($n = 3$); (D, E) targeted protein relative expression of jejunum ($n = 3$). Abbreviations: CD36, CD36 molecule; SLC27A4, solute carrier family 27 member 4; FABP1, fatty acid-binding protein 1; PPAR α : peroxisome proliferator-activated receptor alpha; RXR α : retinoid X receptor alpha; CPT1: carnitine palmitoyltransferase 1; ACOX3: acyl-CoA oxidase 3; ACADL: long-chain-acyl-CoA dehydrogenase. Data were expressed as mean \pm SEM, and bars with different lowercase letters indicated statistically significant differences (one-way ANOVA, $P < 0.05$). L, linear; Q, quadratic.

development. In the future study, the administration of functional fatty acids in perinatal nutrition by either maternal diet fed or in ovo injection could improve embryonic growth and post-hatch performance of ducks, which provide a new way to optimize duck production.

In conclusion, the maximum growth rate of Muscovy duck embryos was estimated at 2.60 to 2.83 g/d on E21.3 to E23.5, while the accumulations of lipid and fatty acid in YS were decreased in association with the increased absorptive area of morphological structures in jejunum as the incubation days increased. The gene and protein expression involved in fatty acid metabolism displayed a similar enhancement pattern between YSM and jejunum during E25 to E28, suggesting that fatty acid utilization could be strengthened to meet the energy demand for embryonic development.

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Author Contributions: HL, Xiufen Zhang, and XW designed this study, carried out the experiments and measurements, and drafted the manuscript. QW, WZ, CL, and SW helped to analyze the experiment traits. WZ, Xin Zuo, and WX assisted with the incubation trial. HL, HY, XW, and WW helped with the data analysis. YZ participated in the study's design, coordination, and manuscript writing. All authors read and approved the final version of the manuscript.

DISCLOSURES

The authors declare no conflicts of interest.

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Effects of fermented cottonseed meal inclusions on growth performance, serum biochemical parameters and hepatic lipid metabolism of geese during 28–70 d of age

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ABSTRACT The aim of this study was to investigate the effects of solid-state fermented cottonseed meal (FCSM) inclusion levels on the growth performance, serum biochemical parameters and hepatic lipid metabolism in geese from 28 to 70 d of age. A total of 288 twenty-eight-d-old male geese were randomly divided into 4 treatments with FCSM levels of 0, 5, 15 and 25% including 0, 22.74, 67.33, 111.27 mg FG/kg diet, respectively. Each treatment contained 6 replicates and 12 birds per replicate. Treatments of FCSM inclusions from 0 to 25% had no effect on growth rate and feed intake in geese during d 28 to 70. The F/G ratio was increased ($P < 0.05$) in geese fed the diet with 25% FCSM compared with birds fed the diet with 0% FCSM. Treatment with 25% FCSM levels had no effect on the contents of TC, TG, HDL-C, LDL-C, but increased ($P < 0.05$) AST and ALT activities in serum of

geese at d 70. Treatment with 25% FCSM increased the contents of FG, HDL-C, TC, C18:2n6, C20:4n6 and PUFA and decreased ($P < 0.05$) the contents of NEFA, SFA, MUFA in liver compared with treatment of 0% FCSM inclusion. Additionally, treatment with 25% FCSM decreased ($P < 0.05$) the *PPAR α* , *AMPK*, and *LXR* mRNA expression related to lipid deposition, and increased ($P < 0.05$) *PPAR γ* and *ACC* mRNA expression related to lipolysis in liver compared with birds fed the diet with 0% FCSM. Overall, treatment with 0 to 15% FCSM (≤ 67.33 mg FG/kg diet) had no adverse effects on the growth performance and lipid metabolism of geese. However, treatment fed 25% FCSM (111.27 mg FG/kg diet) decreased feed efficiency and promoted hepatic lipid deposition associated with the alteration of related gene expression in geese at 28 to 70 d of age.

Key words: goose, fermented cottonseed meal, growth performance, serum biochemical parameters, hepatic lipid metabolism

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INTRODUCTION

The growth of the poultry industry globally can be limited by the scarcity and high cost of conventional feedstuffs (Diarra, 2021), leading us to find alternative feedstuffs for poultry. Soybean meal (SBM) was widely used in poultry diets due to the good nutritional value with high digestibility of crude protein (CP) and amino acids (AA). However, as the growing demand and fluctuating price of SBM, the competition with humans for the limited protein resources will intensify in China (Selaledi et al., 2020). Therefore, there was an urgent

need to explore some alternative and cost-effective protein feedstuffs as a replacement of SBM for poultry production. Cottonseed meal (CSM) as an alternative feedstuff has been widely used to replace SBM partially in poultry diets to reduce the feed cost (Świątkiewicz et al., 2016). The use of CSM in poultry diets has been mainly restricted in poultry diets due to the limitations on the imbalance of AA-profile and the presence of anti-nutritional factors (ANF) (Yu et al., 2020).

Free gossypol (FG) as the main ANF in CSM was a phenolic compound produced by pigment glands in cotton stems, leaves, seeds, and flower buds. High FG intake from CSM can induce some toxic effects on growth performance and physiological metabolism in poultry, such as growth depression, liver damage, lipid metabolism disorder and even mortality (Gadelha et al., 2014). Previous studies showed that feeding the increase of FG concentration in CSM diets reduced growth rate and increased hepatic lipid deposition in broilers (Henry

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et al., 2001; Blevins et al., 2010). Additionally, some researchers reported that excessive FG accumulation from CSM intake impaired growth performance and induced toxic damage in liver, associated with elevated levels of serum aspartate aminotransferase (**AST**) and alanine aminotransferase (**ALT**) of ducks at the starter period (Zeng et al., 2014; Zhu et al., 2017). However, Yu et al., (2019) reported that the use of 75% CSM containing 183 mg FG/kg diet had no adverse effect on growth performance, but altered the fatty acids (**FA**) composition in liver of geese from 28 to 63 d of age. Therefore, the differences in the tolerance of FG toxicity remain unclear among poultry breeds at different growth periods.

As a promising microbial fermentation technique, the solid-state fermentation (**SSF**) has been employed to reduce FG residues of CSM to improve its nutritional value (Olukomaiya et al., 2019). It has been demonstrated that the FG content in CSM was decreased remarkably from 820 to 210 mg/kg with *Bacillus subtilis* BJ-1 (Tang et al., 2012) and from 583.40 to 191.70 mg/kg with *Candida utilis* (Xiong et al., 2016), respectively. However, a previous study found that there was no beneficial effect on FG degradation in CSM after SSF with *L. plantarum* and *B. clausii* (Yusuf et al., 2022). These various effects on FG degradation of CSM after fermentation could be due to the differences of the CSM sources, fermentation strains and fermentation conditions. In addition, in vivo studies have reported that the use of fermented cottonseed meal (**FCSM**) as a substitute in SBM had no effects on growth performance and lipid metabolism in broilers (Wang et al., 2017) and in ducks (Liu et al., 2017). However, there was less information on the effect of FCSM in geese compared to broilers and ducks. Therefore, the objective of this study was to investigate the nutritional value of FCSM after the fermentation using *Monascus*, *Bacillus subtilis*, and *Candida utilis*, and the effects of FCSM inclusion levels on growth performance, serum biochemical parameters and hepatic lipid metabolism of geese from 28 to 72 d of age.

MATERIALS AND METHODS

Fermentation of CSM

Fermented CSM was produced by mixing SSF with *Monascus*, *Bacillus subtilis* and *Candida utilis* provided by the Feed Science Institute of Zhejiang University (Hangzhou, China). Dried CSM was soaked with distilled water to maintain a moisture content of 500 g/kg. The CSM was inoculated with *Monascus*, *Bacillus subtilis* and *Candida utilis* (80 mL, 1.0×10^8 cells/mL, the ratio of 2:3:5) in a tank. The CSM was blended evenly and incubated at 30°C for 72 h in an incubator. After fermentation, the contents of FG, dry matter (**DM**), CP, crude ash (**Ash**), ether extract (**EE**) and crude fiber (**CF**) were measured in both CSM and FCSM, respectively.

Birds, Management, Dietary Treatments

The animal care and use protocol was approved by the Animal Care and Use Committee of South China Agricultural University (SCAU-10564). A total of 288 one-day-old male geese were obtained from Shantou Baisha Animal Husbandry Co. Ltd. (Shantou, China) and then kept in the floor pens in a building with a controlled environment, equipped with central heating at 30°C. The geese were fed the same corn-soybean meal diet (12.13 MJ/kg metabolizable energy, 200 g/kg CP, 10 g/kg Lys, 3.8 g/kg Met, 6.5 g calcium/kg, and 3 g/kg nonphytate phosphorus) from 1 to 27 d of age. At d 28, based on the similar average body weight (**BW**) of geese in each replicate pen, the birds were weighed individually and divided into 4 groups with 6 replicate pens of 12 birds per replicate. The water and feed were provided ad libitum. Four experimental diets were formulated with inclusion levels of 0, 5, 15, or 25% FCSM in the corn-SBM basal diet, containing 0, 22.74, 67.33 and 111.27 mg FG/kg diet based on the analyzed values, respectively. The experimental diets were formulated to be isocaloric and isonitrogenous, with CP and ME levels as well as other nutrient levels meeting or exceeding the **NRC (1994)** for geese. The extra synthetic AAs were added to achieve the same Lys, Met and Thr level across the experimental diets with the inclusion of FCSM as the substitute for SBM. The ingredients and nutrient compositions of experimental diets were presented in **Table 1**. The calculated values of fatty acid (**FA**) composition in feedstuffs were provided in **supplemental Table S1**. The feed intake and final BW of the geese in each replicate were weighed individually at d 70. The average daily gain (**ADG**), average daily feed intake (**ADFI**), and the feed/gain ratio (**F/G**) were calculated for geese at the experimental period from 28 to 70 d of age.

Sample Collection and Preparations

At d 70, 2 geese in each replicate were randomly selected and fasted for 12 h for sampling via the bronchial vein (10 mL/bird) before euthanasia. Serum samples were obtained by centrifuging blood samples at $3,000 \times g$ for 20 min at 4°C, and samples were stored at -20°C for analysis of biochemical indices. Then, the birds were euthanized by CO₂ inhalation and were immediately bled. A piece of liver sample of bird was collected and frozen in liquid nitrogen and then stored at -80°C for relative expression of gene mRNA analysis. while the rest of the liver samples were collected for the analysis of FA composition.

Sample Analyses

The CSM and FCSM were sampled and analyzed for the DM, Ash, CP, EE, and CF contents by using the standard methods of the Association of Official Analytical Chemists (**AOAC, 2005**). All analyses were performed in duplicate. The contents of FG in CSM, FCSM

Table 1. Ingredients and nutrient compositions of experimental diets (as-fed basis).

Items	Dietary FCSM inclusion			
	0%	5%	15%	25%
Ingredient (%)				
Corn	57.78	58.64	61.11	62.59
Soybean meal	23.97	19.37	9.33	0.00
FCSM	0.00	5.00	15.00	25.00
Wheat bran	5.00	5.00	5.00	5.00
Distillers dried grains with solubles	0.61	0.61	0.61	0.61
Rice husk powder	6.06	4.82	2.45	0.24
Soybean oil	3.00	3.00	2.85	2.84
Dicalcium phosphate	1.39	1.40	1.42	1.46
Limestone	0.76	0.72	0.66	0.58
DL-Met (98%)	0.12	0.11	0.11	0.11
Threonine (98.5%)	0.11	0.12	0.16	0.20
L-lysine hydrochloride (78%)	0.00	0.01	0.10	0.17
Premix ¹	1.00	1.00	1.00	1.00
Salt	0.20	0.20	0.20	0.20
Nutrient composition ²				
ME (MJ/kg) ³	11.82	11.84	11.84	11.85
Crude protein ²	17.06	17.52	17.44	17.56
Crude fat ²	2.74	2.68	2.44	2.25
Crude fiber ²	5.63	5.74	5.77	5.58
Calcium ²	0.83	0.80	0.85	0.88
Available phosphorus ²	0.41	0.44	0.43	0.43
Total phosphorus ²	0.74	0.71	0.75	0.77
Digestible lysine ³	0.64	0.63	0.62	0.63
Digestible methionine ³	0.26	0.27	0.26	0.27
Digestible arginine ³	0.72	0.71	0.74	0.71
Digestible threonine ³	0.51	0.52	0.52	0.53
Free gossypol ² (mg/kg)	n/d	22.74	67.33	111.27

Abbreviations: ME, metabolizable energy; FCSM, fermented cottonseed meal.

¹Provided per kilogram of diet: 9,000 IU of vitamin A, 1,500 IU of vitamin D₃, 7.5 IU of vitamin E, 4.8 mg of vitamin B₂, 0.6 mg of vitamin B₁, 0.9 mg of vitamin B₁₂, 20 mg of niacin, 0.15 mg of folic acid, 1.5 mg of biotin, 500 mg of choline chloride, 7.5 mg of calcium pantothenate, 90 mg Zn (ZnSO₄), 70 mg of Mn (MnSO₄), 80 mg of Fe (FeSO₄ · 7 H₂O), 8 mg of Cu (CuSO₄ · 5H₂O), 0.4 mg of I (KI) and 0.3 mg of Se (Na₂SeO₃ · 5H₂O).

²Analyzed values.

³Calculated value.

and liver samples were determined by HPLC as described previously (Ricci et al., 2015). The concentration of TG (A110-1-1), and total cholesterol (TC, A111-1-1), high-density lipoprotein (HDL-C, A112-1-1), low-density lipoprotein (LDL-C, A113-1-1), and non-esterified fatty acid (NEFA, A042-2-1), as well as the activities of AST (C010-2-1) and ALT (C009-2-1) in serum and liver were measured using commercial kits obtained from the Nanjing Jiancheng Institute of Bioengineering (Jiangsu, China). Fatty acid composition of the liver was determined as described previously (Smink et al., 2010). Briefly, lipids from the liver tissues were extracted with chloroform and methanol (2:1, vol/vol) and FA methyl esters were prepared by transesterification with boron trifluoride etherate. Fatty acid profiles of the liver were determined by gas chromatography (GC-7890A, Agilent Technologies, Santa Clara, CA).

Fatty acid profiles of the liver tissues were reported as percentages of total FA. The gene mRNA expression was performed using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) method as described previously (Zhang et al., 2023). Total RNA was extracted using Trizol (Invitrogen, CA) and reverse transcription of the RNA was conducted using the Primer Script RT Reagent Kit (TaKaRa, Dalian, China). The samples were analyzed in duplicate by qRT-PCR performed on a detection system (Applied Biosystems QuantiStudio 7 Flex, Life Technologies, Carlsbad, CA).

Statistical Analysis

All values were subjected to one-way ANOVA by using the general linear model procedure of SAS (SAS Institute, Cary, NC). The treatment comparisons for significant differences were tested by the LSD method. Orthogonal polynomials were applied for linear and quadratic effects of dependent variables to independent variables. Each replicate served as the experimental unit for all statistical analyses. Significant differences were set at $P \leq 0.05$.

RESULTS

Nutrient Composition

The nutrient composition of CSM and FCSM was presented in Table 2. After fermentation, there was a slight decrease in the contents of CP from 45.67% (CSM) to 44.26% (FCSM) and Ash from 13.15% (CSM) to 12.40% (FCSM). There was an increase in the contents of DM from 92.71% (CSM) to 93.19% (FCSM), CF from 13.03% (CSM) to 15.52% (FCSM), and EE from 1.07% (CSM) to 1.65% (FCSM). The content of FG was decreased by 53.2% from 940 mg/kg (CSM) to 440 mg/kg (FCSM).

Growth Performance

Dietary FCSM inclusions affected F/G ($P < 0.05$) and did not influence ($P > 0.05$) final BW, ADG and ADFI of geese from d 28 to d 70 (Table 3). The F/G of geese increased linearly ($P < 0.01$) as dietary FCSM inclusions increased. Geese fed the diet with 25% FCSM had greater F/G ratio ($P < 0.05$) than birds fed the diet with 0% FCSM, and no differences ($P > 0.05$) were observed among other groups.

Table 2. Analyzed values of nutrient composition of CSM and FCSM (% of dry matter basis).

Component	DM (%)	CP (%)	EE (%)	CF (%)	Ash (%)	FG (mg/kg)
CSM	92.71	45.67	1.07	13.03	13.15	940
FCSM	93.19	44.26	1.65	15.52	12.40	440

Abbreviations: CSM, cottonseed meal; FCSM, fermented cottonseed meal; DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fiber; FG, free gossypol.

Table 3. Effects of dietary FCSM inclusions on the growth performance parameters of geese at d 70.¹

Dietary FCSM inclusion	0%	5%	15%	25%	SEM	<i>p</i> -value	Linear	Quadratic
Initial BW (g/bird)	1,130	1,120	1,130	1,140	0.020	0.994	n/d	n/d
Final BW (g/bird)	3,220	3,160	3,150	3,010	0.119	0.830	n/d	n/d
ADFI (g/d/bird)	229.21	229.94	239.55	232.38	7.229	0.511	n/d	n/d
ADG (g/d/bird)	49.84	49.68	49.08	45.38	2.219	0.719	n/d	n/d
F/G	4.53 ^b	4.64 ^{ab}	4.84 ^{ab}	5.04 ^a	0.120	0.035	0.003	0.603

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; F/G, feed/gain ratio; SEM, standard error of mean.

Means with no common superscript within each row are significantly different ($P < 0.05$).

¹Each value represents the mean of six replicates.

Serum Parameters

Treatment of FCSM inclusions affected ($P < 0.05$) ALT and AST activities and had no differences ($P > 0.05$) on the contents of TC, TG, HDL-C, LDL-C in serum of geese at d 70 (Table 4). There was a linear increase ($P < 0.01$) in ALT activity and a quadratical increase ($P < 0.01$) in AST activity in serum as dietary FCSM inclusions increased. Geese fed the diet with 25% FCSM had higher ALT and AST activities in serum than the birds fed the diet with 0% FCSM ($P < 0.05$).

Lipid Metabolism and FG Content of Liver

Treatment of FCSM inclusions affected ($P < 0.05$) the contents of HDL-C, TC, NEFA and FG, but had no effects on the contents of LDL-C and TG in liver of geese at 70 d (Table 5). The contents of HDL-C and FG in liver was increased ($P < 0.01$) linearly and quadratically, while the TC was increased ($P < 0.01$) linearly as the FCSM inclusions increased. Treatment of 25% FCSM had higher ($P < 0.05$) hepatic HDL-C and FG contents than other treatments and higher ($P < 0.05$) hepatic TC

and FG contents than treatment of 0% FCSM. The hepatic NEFA content of geese from the treatments of 5% to 25% FCSM was lower ($P < 0.01$) than the treatment with 0% FCSM.

Fatty Acids Composition

Treatment of FCSM inclusions affected ($P < 0.05$) the concentrations of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and C14:0, C18:2n6, C20:4n6, C18:1n9, but had no effect ($P > 0.05$) on hepatic C17:0, C18:0, C20:1, C20:2 and C22:6n3 in geese at 70 d (Table 6). Total amount of SFA in geese fed the diet with 5% FCSM was higher ($P < 0.05$) than other groups, while geese fed 25% FCSM had the higher ($P < 0.05$) contents of C14:0, C18:2n6, and C20:4n6 and the lower ($P < 0.05$) content of C18:1n9 than other FCSM treatments. Geese fed the diet with 25% FCSM exhibited lower ($P < 0.05$) MUFA levels and higher ($P < 0.05$) PUFA levels in liver compared to those fed the diet with 0% FCSM.

Table 4. Effects of dietary FCSM inclusions in serum biochemical parameters of geese at d 70.¹

Dietary FCSM inclusion	0%	5%	15%	25%	SEM	<i>p</i> -value	Linear	Quadratic
ALT (mIU/mL)	1.10 ^b	3.10 ^a	2.76 ^a	3.69 ^a	0.436	0.007	0.004	0.302
AST (mIU/mL)	1.81 ^b	2.38 ^b	2.37 ^b	3.96 ^a	0.445	0.019	0.165	0.007
TC (μmol/mL)	4.83	4.48	4.38	4.36	0.283	0.642	n/d	n/d
TG (μmol/mL)	1.81	2.15	2.32	2.26	0.160	0.148	n/d	n/d
HDL-C (μmol/mL)	2.77	1.67	2.45	2.31	0.287	0.095	n/d	n/d
LDL-C (μmol/mL)	0.83	0.93	1.08	0.96	0.114	0.491	n/d	n/d

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; HDL, high-density lipoprotein; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; SEM, Standard error of mean; TC, cholesterol; TG, triglyceride.

Means with no common superscript within each row are significantly different ($P < 0.05$).

¹Each value represents the mean of 6 replicates.

Table 5. Effects of dietary FCSM inclusions on the hepatic lipid and FG content in liver of geese at d 70.¹

Dietary FCSM inclusion	0%	5%	15%	25%	SEM	<i>p</i> -value	Linear	Quadratic
FG (μg/mg)	n/d	0.36 ^{ab}	0.32 ^b	0.48 ^a	0.047	0.035	0.04	0.042
HDL-C (μg/mg protein)	2.50 ^b	2.40 ^b	2.16 ^b	4.42 ^a	0.391	0.002	0.003	0.008
LDL-C (μg/mg protein)	7.56	6.48	7.33	7.95	1.280	0.872	n/d	n/d
TC (μg/mg protein)	0.20 ^b	0.25 ^b	0.27 ^{ab}	0.33 ^a	0.026	0.016	0.003	0.872
TG (μg/mg protein)	0.12	0.13	0.14	0.13	0.013	0.681	n/d	n/d
NEFA (μg/mg protein)	60.59 ^a	45.84 ^b	36.36 ^b	39.16 ^b	3.263	0.001	<0.001	0.007

Abbreviations: FG, free gossypol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SEM, Standard error of mean; TC, cholesterol; TG, triglyceride; NEFA, non-esterified fatty acid.

Means with no common superscript within each row are significantly different ($P < 0.05$).

¹Each value represents the mean of six replicates.

Table 6. Effects of dietary FCSM inclusions on hepatic fatty acid composition in geese at d 70¹ (%).

Dietary FCSM inclusion	0%	5%	15%	25%	SEM	p-value
C14:0	0.22 ^b	0.30 ^{ab}	0.29 ^{ab}	0.39 ^a	0.08	0.014
C15:0	2.13 ^b	2.59 ^a	2.07 ^b	2.17 ^b	0.16	0.009
C16:0	20.86 ^{ab}	22.36 ^a	18.73 ^b	18.71 ^b	0.87	0.037
C17:0	0.17	0.21	0.19	0.19	0.02	0.775
C18:0	17.50	18.09	18.64	17.89	0.26	0.824
C18:1n9	45.41 ^a	40.59 ^{ab}	42.05 ^{ab}	37.66 ^b	0.17	0.012
C18:2n6	10.46 ^b	12.57 ^b	13.64 ^b	19.39 ^a	1.32	0.011
C20:1	0.44	0.51	0.46	0.43	0.07	0.442
C18:3n3	1.27 ^b	1.07 ^b	1.95 ^a	1.42 ^{ab}	0.25	0.007
C20:2	0.46	0.55	0.59	0.51	0.03	0.083
C20:4n6	0.21 ^b	0.35 ^a	0.39 ^a	0.42 ^a	0.06	0.007
C20:5n3	0.28 ^b	0.29 ^{ab}	0.37 ^a	0.25 ^b	0.05	0.032
C22:6n3	0.59	0.52	0.63	0.57	0.09	0.288
SFA	40.88 ^b	43.55 ^a	39.92 ^b	39.35 ^b	4.22	0.031
MUFA	45.85 ^a	41.10 ^b	42.51 ^{ab}	38.09 ^b	1.35	0.012
PUFA	13.27 ^b	15.35 ^b	17.57 ^a	22.56 ^a	2.01	0.044

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Means with no common superscript within each row are significantly different ($P < 0.05$).

¹Each value represents the mean of 6 replicates.

Lipid Metabolism-Related Gene Expression in Liver

Treatment of FCSM inclusions affected ($P < 0.01$) *PPARα*, *AMPKα*, *LXR* and *ACC* mRNA expression, but had no effect ($P > 0.05$) on *PPARβ* and *ChREBP* mRNA expression in geese at 70 d (Table 7). The *PPARα* was decreased linearly ($P < 0.01$) as the FCSM inclusions increased. The *AMPKα* and *LXR* mRNA expressions were decreased linearly ($P < 0.05$) and quadratically ($P < 0.01$ and *PPARγ* and *ACC* mRNA expressions were increased linearly ($P < 0.05$), when the treatment of FCSM levels increased. Treatment of 25% FCSM decreased ($P < 0.05$) the hepatic *PPARα*, *AMPKα* and *LXR* mRNA expression and increased ($P < 0.05$) hepatic *PPARγ* mRNA expression in geese compared with geese fed the diets with 0% FCSM. The hepatic *ACC* mRNA expression of geese fed the diet with 25% FCSM was higher ($P < 0.01$) than that of geese fed the diets with 0% FCSM and 15% FCSM.

Table 7. Effect of dietary FCSM inclusion on lipid metabolism controlling genes in liver of geese at d 70.¹

Dietary FCSM inclusion	PPARα	PPARβ	PPARγ	ChREBP	AMPKα	FAS	ACC	LXR
0%	0.90 ^a	1.00	1.00 ^b	1.14	1.00 ^a	1.00 ^a	1.00 ^b	1.00 ^a
5%	0.57 ^b	1.06	1.37 ^{ab}	0.97	0.61 ^b	0.54 ^{bc}	2.93 ^a	0.51 ^b
15%	0.51 ^b	0.85	1.42 ^a	0.80	0.46 ^b	0.44 ^c	1.67 ^b	0.50 ^b
25%	0.36 ^b	0.67	1.63 ^a	0.92	0.56 ^b	0.67 ^b	2.73 ^a	0.67 ^b
SEM	0.072	0.105	0.121	0.113	0.071	0.074	0.320	0.073
P-value	<0.001	0.067	0.021	0.360	<0.001	<0.001	<0.001	<0.001
Linear	<0.001	n/d	0.006	n/d	<0.001	0.015	0.023	0.027
Quadratic	0.090	n/d	0.425	n/d	0.001	<0.001	0.491	<0.001

Abbreviations: *PPARα*, peroxisome proliferator activated receptor alpha; *PPARβ*, peroxisome proliferator activated receptor beta; *PPARγ*, peroxisome proliferator activated receptor gamma; *ChREBP*, carbohydrate responsive element binding protein; *AMPKα*, adenosine monophosphate activated protein kinase α; *FAS*, fatty acid synthase; *ACC*, acetyl-CoA carboxylase; *LXR*, liver X-activated receptor.

Means with no common superscript within each row are significantly different ($P < 0.05$).

¹Each value represents the mean of 6 replicates.

DISCUSSION

During fermentation, microbes can degrade the macromolecular proteins into micromolecular peptides and free AAs by secretion considerable amounts of proteases (Li et al., 2022). In this study, there was a decline of CP content from 45.67% (CSM) to 44.26% (FCSM) and slight increases of DM, CF and EE of FCSM on DM basis after fermentation. The loss in CP may be due to the deamination of AAs into ammonia gas by microorganisms during the fermentation process. It was not agreed with the result that there was an increase in CP and a decrease in CF of CSM after fermentation (Wang et al., 2017). The contradiction in the alteration of nutrient composition of FCSM possibly due to the differences in the used CSM sources, micro-organisms and fermentation conditions (Olukomaiya et al., 2019). In addition, Olukomaiya et al (2019) reported that microbial fermentation was currently considered and effective way to reduce the contents of anti-nutritional factor in CSM as reported previously, including FG. The dramatic change in the FG content was declined from 940 mg FG/kg CSM to 440 mg FG/kg FCSM by mixing SSF with *Monascus*, *Bacillus subtilis* and *Candida utilis*. The positive effect of FG degradation was agreed with previous studies in CSM by the fermentation using *Bacillus subtilis* BJ-1 (from 820 mg/kg to 210 mg/kg) (Tang et al., 2012) and *Bacillus coagulans* (from 923.80 to 167.90 mg/kg) (Zhang et al., 2022). For one reason, the decrease in FG could be degraded by the related clastic enzymes secreted by microorganisms. For the other reason, the FG combined with either CP or AAs to form bound gossypol, which cannot be absorbed into digestive tract and had no harmful effects on the body health.

The use of CSM in poultry diets has been mainly restricted in poultry diets due to the presence of FG. In the present study, treatment of FCSM levels from 0% to 25% had no effect on growth rate and feed intake of geese during 28 to 70 d of age, which was consistent with previous studies about the usage of CSM in broilers (Nie et al., 2015; Niu et al., 2021) and geese (Yu et al., 2023). However, dietary with inclusion of 20.18% CSM (30.27 mg FG/kg diet) decreased growth rate in Jiangnan White geese during d 42 to 70 (Yu et al., 2019),

which was in line with the results that the inclusion of 9% FCSM (19.3 mg FG/kg diet) decreased ADG and ADFI in Cobb broiler chickens during d 29 to 35 (Niu et al., 2021). These inconsistent results on growth performance could be due to the differences of the experimental time, strain and age of birds, and FG content in the used CSM sources, etc. In our study, there was a linear increase in F/G as the FCSM inclusions increased, and the F/G of geese fed the diet with 25% FCSM (111.27 mg FG/kg diet) remarkably increased compared with the usage of 0% FCSM. This decline in feed efficiency was line with the results about Jiangnan White geese fed the diet with inclusion of 13.46% CSM (Yu et al., 2020). Similar results were confirmed in Arbor Acres broilers fed a diet with 15.1% FCSM (52.25 mg FG/kg diet) during d 1 to 42 (Wang et al., 2017) and in Cherry Valley ducks fed the diets with 5.83%–23.3% FCSM (36–153 mg FG/kg diet) during d 15 to 35 (Zeng et al., 2015). It was speculated that the decreased feed efficiency of geese might be due to the lower digestibility of AAs when the SBM was replaced by the increased levels of FCSM (D. Nagalakshmi, 2007). In addition, it was presumably due to that FG binds with free epsilon amino groups of essential AAs especially lysine resulting in digestibility of protein and AA decreased, as the FG intake increased with the FCSM inclusions increased (Cater and Lyman, 1969). However, some studies also reported that there were no effects on feed efficiency in broilers feeding the maximum 300 mg FG/kg diet during d 21–42 (Lordelo et al., 2005) and maximum 183 mg FG/kg diet from CSM in geese during d 28 to 63 (Yu et al., 2023). The above results implied that the maximum tolerance of FG could be different among the different digestive physiology among poultry breeds. As reported previously (Fang et al., 2023), the special microbiomes in caecum can actively ferment and effectively reduce FG content by production of degrading enzyme, which could be different between broilers and geese.

It has been proven that FG had a strong tendency to accumulate in liver and induce hepatotoxicity in broilers (Blevins et al., 2010) and ducks (Zhu et al., 2019). There was a linear increase in the FG accumulation in liver as the increasing FCSM inclusion and the maximum value was observed in liver of geese fed 25% FCSM containing 111.27 mg FG/kg diet. Previous studies have shown that excessive FG accumulation from CSM affected hepatic lipid metabolism in poultry (Blevins et al., 2010; Zeng et al., 2015). Pervious study reported that feeding a diet containing 16.99% CSM (136 mg FG/kg diet) increased TC concentration in serum and liver of geese (Yu et al., 2023). In addition, it was reported that feeding a diet containing 18.5% CSM (700 mg FG/kg diet) increased TC concentration in serum and liver of cows (Hawkins et al., 1985). Our result showed that the usage of 25% FCSM (110 mg/kg FG) increased ALT and AST activities in serum and hepatic TC and HDL-C levels in liver of geese. Zhu et al. (2017) found that liver damage was induced in the ducklings when received a diet with 320 mg FG/kg in association with the elevated serum ALT and AST activities. Zeng et al. (2014,2015)329

demonstrated that the degree of the liver damage increased with the increase in the dietary CSM and FG level via histopathology analysis. The above results suggested that the high FG intake could induce hepatic damage and lipid metabolic disorder in poultry. Additionally, treatment with 25% FCSM (111.27 mg FG/kg diet) increased the contents of C14:0 and C18:2n6, C20:4n6 and n-6 PUFA ratios and decreased content of C18:1n9 in liver of geese at d 70 compared with other groups. In addition, geese fed the diet with 25% FCSM exhibited lower MUFA levels and higher PUFA levels in liver compared to those fed the diet with 0% FCSM. However, Yu et al (2023) demonstrated that the levels of FG in diets with 5.66 to 22.65% CSM (44–183 mg FG/kg diet) had no effect on C14:0 and C18:1n9, C18:2n6, C20:4n6 and n-6 PUFA ratios of geese at d 63. For one reason, the alteration of FA compositions of liver of geese could due to the differences in FA composition of experimental diets as presented in Table S1. For the other hand, the cyclopropane fatty acid as one of the main ANFs in CSM could effectively interfere with FA metabolism and change FA distribution in tissues as reported previously (Evans et al., 1963).

It had been demonstrated that the activation of *PPARα* and *ACC* expressions were involved in the stimulation of lipid lipolysis and synthesis in liver of animals (Han et al., 2020; Xiao et al., 2022). In the present study, the up-regulation of *ACC* mRNA expression and down-regulation of *PPARα* mRNA expression were observed in liver of geese fed the diet with 25% FCSM, suggesting that the lipid deposition was enhanced and then contributed to the increased TC content in liver. It was speculated that the alteration of *ACC* and *PPARα* mRNA expression might be associated with the increased n-6 PUFA accumulation in liver of geese fed 25% FCSM. As reported previously, dietary supplemented with 7.5 to 9% n-6 PUFA increased *ACC* mRNA expression and decreased *PPARα* mRNA expression in liver of fishes (Jin et al., 2019; Dong et al., 2023). Additionally, the key genes of *AMPK* and *LXR* played an important role on the regulation of lipid catabolism in broilers (Zhou et al., 2020). In the present study, there were decrease in *AMPK* and *LXR*s mRNA expression in liver of geese fed the diet with 25% FCSM (111.27 mg FG/kg diet) compared with the birds fed the diet with 0% FCSM. Previous studies have shown that *AMPK* activation leads to the phosphorylation of *ACC*, resulting in *ACC* inactivation, a reduction in acetyl-CoA levels, and thereby inhibiting hepatic fat synthesis while stimulating FA oxidation in broilers (Zhang et al., 2024). However, it was unclear whether the alterations of gene expression related to lipid metabolism were involved in FG accumulations in liver of geese.

CONCLUSIONS

In the present study, treatment with 0 to 15% FCSM inclusions (≤ 67.33 mg/kg FG diet) had no effect on growth performance rate in geese at 28 to 70 d of age,

while treatment with 25% FCSM inclusion (111.27 mg/kg FG diet) increased F/G in geese during d 28 to 70. Moreover, treatment with 25% FCSM level promoted lipid deposition in the liver associated with the alterations of gene expressions related to lipid metabolism.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.psj.2024.103702](https://doi.org/10.1016/j.psj.2024.103702).

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Exogenous Linoleic Acid Intervention Alters Hepatic Glucose Metabolism in an Avian Embryo Model

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In the present study, developmental changes of gluconeogenesis and glycolysis in an avian model were measured, and then the intervention effects of *in ovo* feeding (IOF) linoleic acid (LA) on hepatic glucose metabolism were evaluated. In Experiment 1, thirty fertilized eggs were sampled on embryonic days (E) of 16, 19, 22, 25, 28, 31, and thirty newly-hatched ducklings at hatch (E34 and E35). In Experiment 2, a total of 120 fertilized eggs (60 eggs for each group) were injected into the yolk sac with PBS as the control group and LA as the IOF LA group on E25. Twelve eggs were selected for sample collection on E28 and E31. Serum contents of glucose, pyruvate, and lactate increased ($p < 0.05$) linearly or quadratically from E16 to hatch, as well as hepatic glycogen and pyruvate contents. Hepatic mRNA expression related to energy homeostasis, gluconeogenesis, and glycolysis increased ($p < 0.05$) in embryogenesis, and the plateau period was presented on E25–E31. IOF LA decreased ($p < 0.05$) serum contents of glucose, triacylglycerol, cholesterol, and hepatic oleic acid, unsaturated fatty acids on E28, as well as the gene expression relative to gluconeogenesis. IOF LA increased ($p < 0.05$) pyruvate content in serum and liver, and hepatic gene expression relative to glycolysis on E31. In summary, hepatic gluconeogenesis and glycolysis were enhanced to meet the increasing energy demands of embryonic development during E25 – hatch. Exogenous LA intervention on E25 could inhibit hepatic gluconeogenesis and enhance glycolysis during the later developmental period, disrupting glucose embryonic homeostasis and energy status.

Keywords: avian embryo model, glucose homeostasis, linoleic acid, *in ovo* feeding, energy status

INTRODUCTION

The avian embryo derives all its nutrient requirements from the nutrient deposits in the fertile egg during incubation. Avian embryos make use of O₂ accession for fatty acid (FA) oxidation to get energy with the vascular system and pulmonary development (Tazawa et al., 1983; Moran, 2007). However, energy metabolism shifts from yolk lipids to the predominant carbohydrate substrate when the embryo reaches the oxygen consumption plateau (Donaldson et al., 1991; De Oliveira et al., 2008). Thus, gluconeogenesis and glycolysis are enhanced during the late-hatch stage to maintain the energy homeostasis as well as normal physiological status for embryonic growth and development (Hu et al., 2017; Wan et al., 2018). For example, glycolysis

reduces glucose to pyruvate and adenosine triphosphate (ATP), and pyruvate is converted to lactate that can be recycled back to glucose in the liver *via* the Cori cycle once the oxygen is available (De Oliveira et al., 2008; Bolaños et al., 2010). In addition, avian embryos are much easier to maintain and manipulate than most other vertebrate species. Therefore, it is speculated that an avian embryo during the last phase of incubation could be considered as an ideal model to understand vertebrate development *via* altering glucose homeostasis.

In ovo feeding (IOF) is an efficient technique to evaluate the effects of the exogenous nutrients on embryonic development using an avian model (Givisiez et al., 2020). IOF can deliver early nutrients and additives to embryos *via* yolk sac, albumen, air sac, and amnion et al. (Das et al., 2021). Previous studies have shown the effects of IOF carbohydrates, amino acids, minerals, and vitamins on embryonic development, and these exogenous nutrients can change blood histology, modify the regulation of transcription of different genes, and improve hatchability and perinatal growth in embryos and neonates (Tangara et al., 2010; Yair et al., 2015; Chen et al., 2021). In addition, IOF supplements like probiotics, prebiotics, and synbiotics protect against harmful gut microbes through competitive exclusion and maintain intestinal immune homeostasis (Shehata et al., 2021). There are limited reports on the effects of FA interventions. Linoleic acid (LA) has been proved to be involved in the regulation of glucose homeostasis *via* the alteration of FA and glucose metabolism *in vivo* and *in vitro* (Hamilton and Klett, 2021). Dietary LA could decrease blood glucose in obese rats (Matravadia et al., 2016; Holmäng et al., 2018). Glucose acts as an important fuel source for embryonic development (Minhas and Khan, 2016). Therefore, the developmental changes of serum glucose and hepatic glycogen concentrations in embryogenesis were measured to determine the critical period of glucose metabolism in the current study. We hypothesized that LA may decrease embryonic serum glucose and the adverse effects of IOF LA on the alteration of glucose metabolism to regulate embryonic development was evaluated using the avian embryo model.

MATERIALS AND METHODS

Ethics Statement

The protocol was reviewed and approved by the Animal Care and Use Committee of South China Agricultural University with the following reference number: No. 20110107–1.

Abbreviations: ADP, Adenosine diphosphate; AMP, Adenosine monophosphate; AMPK, Adenosine monophosphate-activated protein kinase; ATP, Adenosine triphosphate; CHO, Cholesterol; CoA, Coenzyme A; FA, Fatty acid; FAMES, Fatty acid methyl esters; HKDC, Hexokinase; IOF, *In ovo* feeding; LA, Linoleic acid; NAD, Nicotinamide adenine dinucleotide; PBS, Phosphate-buffered saline; PC, Pyruvate carboxylase; PCK, Phosphoenolpyruvate carboxykinase; PFKFB2, 6-Phosphofructo-2-kinase; PFKL, Phosphofructokinase; PKM, Pyruvate kinase M1/2; PRKAA1, Protein kinase AMP-activated catalytic subunit alpha 1; RT-qPCR, Real-time quantitative PCR; SFAs, Saturated fatty acids; TG, Triacylglycerol; UFAs, Unsaturated fatty acids.

Incubation and *in ovo* Feeding

Fertilized Muscovy duck eggs were obtained from a commercial breeder farm (Wen's Food Group Co., Ltd., Yunfu, Guangdong, China), and all the eggs were collected from hens at 33 week of age belonging to the same breeder flock. The eggs were incubated in the automatic-controlled incubator (Dezhou Keyu Hatching Equipment Co., Ltd., Dezhou, China) according to standard hatchery practices ($37.5 \pm 0.5^\circ\text{C}$), relative humidity ($55 \pm 5\%$) until embryonic day 31 (E31). Next, all eggs were transferred to hatching crates and moved to hatchers which were set at a temperature of $37.0 \pm 0.5^\circ\text{C}$ and declined to $36.0 \pm 0.5^\circ\text{C}$ at the end of incubation.

In Experiment 1, a total of 350 Muscovy duck eggs were collected for incubation, and unfertilized and unviable eggs were discarded after candling on E15. A total of 180 viable eggs ($75.2 \pm 1.8\text{g}$) and 30 hatchlings ($43.19 \pm 0.7\text{g}$) were applied for the test (five embryos per replicate, $n=6$). Thirty viable eggs were selected for samples collection on E16, E19, E22, E25, E28, and E31, and 30 ducklings at hatch (E34 and E35). In Experiment 2, a total of 200 eggs were collected for incubation, and 120 viable eggs ($79.6 \pm 1.9\text{g}$) were divided into two groups on E25, the control group and the treatment group, with 60 eggs each. The eggs in the control group or the treatment group were injected into the yolk sac with a volume of $100\mu\text{l}$ of phosphate-buffered saline (PBS, G4202, Servicebio, Wuhan, China) or LA ($\geq 98\%$, 62230, Sigma-Aldrich, Wyoming, United States), respectively. The IOF procedure was conducted as previously reported (Chen et al., 2020). In brief, the site for injection was found by illumination (about one-third from the sharp end of the egg) and disinfected with 75% ethanol before injection. A small hole (0.8 mm in diameter) was drilled on the eggshell by a hand-held pearl drill. The sterile disposable $25.0 \times 0.6\text{mm}$ needle was attached to a 1.0 ml syringe, which was replaced after each egg injection. The hole was sealed with medical adhesive tape ($1.0 \times 1.0\text{cm}^2$) immediately after injection, and the egg was transferred to the incubator. All eggs were kept outside the incubator for less than 30 min during the injection process. Twelve eggs were selected to collect samples (two embryos per replicate, $n=6$) on E28 and E31.

Sample Collection

Samples of serum and liver were pooled together for each replicate in Experiment 1 (five embryos) and Experiment 2 (two embryos). Blood samples were collected from the umbilical vein of the embryos using glass Pasteur pipettes ($7 \times 150\text{mm}$). The tip of a glass Pasteur pipette was melted on the outer flame of an alcohol lamp and then withdrawn with a forcep to make a needle $< 0.3\text{mm}$ in diameter. Blood samples were collected from the jugular vein of ducklings using disposable syringes at hatch. The serum was separated by centrifugation ($1-14$, Sigma, Germany) at $664 \times g$ for 10 min at room temperature and then stored at -20°C for biochemical index analysis. The liver samples were cleaned of extraneous tissues, rinsed with ice-cold PBS, and then frozen in liquid nitrogen and stored

at -80°C for analysis of biochemical index and relative expression of gene mRNA and protein.

Biochemical Analysis

The serum glucose was measured using an automatic biochemical analyzer (Roche Cobas C702, Basel, Switzerland). The contents of glycogen, pyruvate, lactate, triacylglycerol (TG), and cholesterol (CHO) in serum or liver were measured according to the manufacturer's instructions for each assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The serum contents of adenosine phosphate and coenzyme were measured using the ELISA assay kits as follows (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China): ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP), acetyl coenzyme A (acetyl CoA), and malonyl coenzyme A (malonyl CoA). Hepatic nicotinamide adenine dinucleotide (NAD) contents were measured using the assay kit (Beyotime Biotechnology, Shanghai, China) including NADH (reduced form) and NAD^+ (oxidized form).

Fatty Acids Analysis

FA profiles were determined by using gas chromatography (7890A, Agilent Technologies, Santa Clara, CA, United States). Lipids from liver tissues (Experiment 2) were extracted with chloroform and methanol (2:1, vol/vol), and the separated lipid fraction was converted to FA methyl esters (FAMES) by saponification using 0.5M KOH-methanol, followed by methylation with 200 μl boron trifluoride diethyl etherate (B104430, Sigma-Aldrich). Next, the FAMES were mixed with N-hexane and saturated sodium chloride solution, and the mixture was shaken for 10 min and centrifuged at $1,200 \times g$ for 10 min. The upper phase was collected and dried with sodium sulfate for gas chromatographic analysis. The FAMES were identified by comparing retention times to FAME standards (CRM47885, Sigma-Aldrich). The results of FA composition were reported as the percentage of total FA.

Gene mRNA Relative Expression Analysis

The total RNA isolation and the real-time quantitative procedure were conducted as previously reported (Kang et al., 2017; Madkour et al., 2021). Total RNA was extracted from the liver samples according to reagent protocols using a Trizol reagent (Invitrogen, Carlsbad, USA) followed by purification of total RNA using the kit with DNase treatment (Magen, Guangzhou, China). The RNA quality and quantity were determined using agarose gel electrophoresis (4.5%) and NanoDrop 2000 (Thermo Scientific, Wilmington, DE, USA). One μg of total RNA was converted into cDNA by reverse transcription as described in Primer Script RT Reagent Kits (TaKaRa, Dalian, China). Primer sequences were obtained from GenBank (Table 1) and designed and synthesized by Sangon Biotechnology Co., LTD (Shanghai, China). The PCR products were analyzed by using agarose gel electrophoresis (4.5%). Each qPCR reaction had a final volume of 10 μl of the reaction mixture, which consisted of 5 μl SYBR Green Realtime PCR Master Mix kit (QPK-201, TOYOBO, Osaka, Japan) with 3.2 μl DNase/RNase-Free water, 0.4 μl forward and reverse specific primers for each gene and 1 μl of cDNA template. Real-time quantitative PCR was performed using the detection

system (Applied Biosystems QuantiStudio 7 Flex, Life Technologies, Carlsbad, CA, United States). The following cycling conditions were used: 95°C for 1 min, followed by 40 cycles each at 95°C for 15 s, 60°C for 15 s, and 72°C for 45 s. Melt-curve analysis was performed to verify the specificity of qPCR-amplified products. The quantification of the mRNA expression was calculated by the comparative CT method ($2^{-\Delta\Delta\text{CT}}$; Livak and Schmittgen, 2001).

Western Blotting Analysis

The liver samples collected on E16, E22, E28, and hatch were used for western blotting analysis. The tissues were lysed with ice-cold RIPA lysis buffer containing 1 mmol/l phenylmethylsulfonyl fluoride. The lysates were placed on ice for 10 min and the homogenate was centrifuged at $12,000 \times g$ for 5 min at 4°C . The protein concentration in the collected supernatant was measured using the bicinchoninic acid (BCA) assay kit (Beyotime Biotechnology, Shanghai, China). The protein supernatant was mixed with loading buffer, denatured for 5 min by heat shock at 95°C , and then stored at 4°C for western blotting analysis. Proteins were separated *via* an electrophoretic SDS-PAGE on 10% gel. Next, proteins were transferred to a nitrocellulose membrane with Trans-Blot, and the membrane was blocked with a buffer containing 5% skim milk. Membranes were incubated overnight at 4°C with rabbit monoclonal antibodies for adenosine monophosphate-activated protein kinase alpha 1 (AMPK α 1, ab32047, Abcam, Cambridge, England), glucose-6-phosphatase (G6PC1, ab243319, Abcam), and β -actin (66009-1-Ig, Proteintech, Chicago, United States). Then, membranes were incubated with HRP goat anti-mouse IgG (SA00001-1, Proteintech) or HRP goat anti-rabbit IgG (SA00001-2, Proteintech) at room temperature. Proteins of interest were diluted in buffer with enhanced chemiluminescence solution. The bands were detected using a chemiluminescence imaging system (ChemiScope6100, Qinxing, Shanghai China). The density of bands was determined using ImageJ software and the results were expressed as the intensity signal in arbitrary units after normalization.

Statistical Analysis

Data from Experiment 1 were statistically analyzed using the PROC GLM procedures for one-way ANOVA by Statistical Analysis System v9.2 (SAS Inst. Inc., Cary, NC, United States). When significant differences were found ($p < 0.05$), Duncan's multiple comparisons test was performed and orthogonal polynomial contrasts were used to identify the form of the effect (linear or quadratic) over time (incubation days). Data from Experiment 2 on each sampling time were analyzed with a *t*-test using the PROC Ttest procedure in SAS v9.2, and differences were considered to be significant at $p < 0.05$.

RESULTS

Biochemical Index Related to Glucose Metabolism (Experiment 1)

The serum contents of glucose, pyruvate, and lactate increased ($p < 0.05$) in a linear and quadratic manner during embryogenesis

TABLE 1 | Forward and reverse primer sequences for PCR analysis.

Target genes	Forward primer (5'→3')	Reverse primer (5'→3')	Genebank accession no.	Product size (bp)
<i>PRKAA1</i>	GCGGCGGCGGATAACAGAAG	CATGCTTGCCAACTTGACTTTGC	XM_027447031.2	112
<i>PC</i>	CAACTACCTGCCAACCTGCTG	GGCTGTATTTGGTGCCTGATGGG	NM_205471.1	119
<i>PCK</i>	GAGCCATTGCCACCAGGAGTAAC	GCAGAACCGTGAGTTGGGATGAG	XM_021276483.2	99
<i>PFKFB2</i>	ACTTCTTCAGGCACGATAACAAGGAG	CGAGTTGTGTTGGTCGCATCAAAC	XM_038168182.1	136
<i>G6PC1</i>	CTGGCTCAACCTCGTCTTCAAGTG	GGCGTTGCTGTAGTAGTTGGTCTC	XM_027445511.2	85
<i>HKDC1</i>	AGGCAAGCCAGCATTGACAAGG	TCCCTCAGCATATCAACAACATCTTCC	XM_013092011.4	98
<i>PFKL</i>	GTGGGTGCCGTGAGAGAAGTTG	TGTAGAGGAACCTGGTGGTGTAGTG	XM_038183738.1	84
<i>PKM</i>	AGCCAACCATTGCGAGGAACAC	GTGGGTGCCGTGAGAGAAGTTG	XM_038184779.1	131
<i>FASN</i>	TCTCTGCCATCTCCCGAATTCC	TTAGCCACTGTGCCAACTCAAGC	XM_027471234.2	96
<i>SCD1</i>	AGTTCTCTCCGCTTCCAGC	TTCTCCATGACGGCATCCCC	XM_027460089.2	82
<i>PPARα</i>	ACCATCCTGATGATACCTTCTCTTCC	AAGTTGAGCATGTTCTGTGACAAGTTG	NM_001310383.1	86
<i>RXRα</i>	TGCGAGCCATTGTCTCTTCAAC	GATGCGTACACCTTCTCCCGTAAC	XM_027471073.2	88
<i>β-actin</i>	TACGCCAACACGGTGCTG	GATTTCATCATCTCTGCTTG	NM_00131042.1	215

PRKAA1, protein kinase AMP-activated catalytic subunit alpha 1; *PC*, pyruvate carboxylase; *PCK*, phosphoenolpyruvate carboxykinase; *PFKFB2*, 6-phosphofructo-2-kinase; *G6PC1*, glucose-6-phosphatase; *HKDC*, hexokinase; *PFKL*, phosphofructokinase; *PKM*, pyruvate kinase M1/2; *FASN*, fatty acid synthase; *SCD1*, stearoyl-CoA desaturase; *PPARα*, peroxisome proliferator-activated receptor alpha; *RXRα*, retinoid X receptor alpha.

(Figure 1A), reaching plateau periods during E28 - hatch, E31 - hatch, and E25-E31, respectively. The serum contents of ADP, acetyl CoA, and malonyl CoA increased ($p < 0.05$) linearly or quadratically (Figure 1A). Hepatic glycogen and pyruvate increased ($p < 0.05$) in a linear and quadratic manner, with the peak period of E28 - hatch or E28-E31, respectively (Figure 1B). As incubation day increased, hepatic NADH content decreased ($p < 0.05$) linearly and the ratio of NAD⁺ to NADH increased ($p < 0.05$) linearly, with maximal changes observed at the period of E28 - hatch (Figure 1B).

Hepatic Gene and Protein Expression Related to Glucose Metabolism (Experiment 1)

Hepatic mRNA expression related to energy regulation, gluconeogenesis, and glycolysis of protein kinase AMP-activated catalytic subunit alpha 1 (*PRKAA1*), pyruvate carboxylase (*PC*), phosphoenolpyruvate carboxykinase (*PCK*), 6-phosphofructo-2-kinase (*PFKFB2*), *G6PC1*, hexokinase (*HKDC*), phosphofructokinase (*PFKL*), and pyruvate kinase M1/2 (*PKM*) increased ($p < 0.05$) linearly or quadratically in response to the increased incubation day, and the plateau period was shown between E25 and E31 (Figure 2A). Hepatic protein expression of AMPKα1 and G6PC1 increased ($p < 0.05$) linearly or quadratically, and the higher level was presented between E22 and E28 (Figures 2B,C).

Effect of IOF LA on Biochemical Index and Fatty Acids (Experiment 2)

Compared to the control group, IOF LA group decreased ($p < 0.05$) serum contents of glucose, TG, and CHO on E28 and E31, and serum pyruvate content on E28 (Figure 3). IOF LA group increased ($p < 0.05$) serum contents of pyruvate, lactate, and acetyl CoA on E31, and hepatic pyruvate content on E28 and E31 (Figures 3A,B). For FA profiles, IOF LA group increased ($p < 0.05$) hepatic stearic acid (C18:0) and saturated FAs (SFAs) on E28, and myristic acid (C14:0) and

SFAs on E31, while IOF LA group decreased ($p < 0.05$) hepatic oleic acid (C18:1n-9), unsaturated FAs (UFAs), and the ratio of UFAs to SFAs on E28 by comparison to the control group (Table 2).

Effect of IOF LA on Hepatic Gene Relative Expression (Experiment 2)

IOF LA group decreased ($p < 0.05$) hepatic mRNA expression of *PRKAA1*, *PC*, *PCK*, *PFKFB2*, *PFKL*, *FASN*, *SCD1*, *PPARα*, and *RXRα* on E28, and increased ($p < 0.05$) mRNA expression of *PRKAA1*, *HKDC*, and *PFKL* on E31 (Figure 4).

DISCUSSION

Hepatic glucose metabolism is essential for energy homeostasis by balancing glucose storage and utilization during incubation. The blood glucose supply and muscle and hepatic glycogen storage were mainly mediated by the metabolic pathway of gluconeogenesis in embryogenesis (De Oliveira et al., 2008; Guo et al., 2013). In addition, both blood glucose and hepatic glycogen levels are considered as the criterion in assessing embryonic energetic status based on the data examined in carbohydrates metabolism (Christensen et al., 1999, 2000). Previous studies demonstrated that blood glucose concentration is positively associated with embryonic survival and hatchling weight (Christensen et al., 2000). Therefore, the avian embryo in the final stage of incubation has been considered as a suitable model for assessing the connection between glucose metabolism and energy status. In the present study, serum glucose and hepatic glycogen contents increased as incubation proceeded from E16 to hatch, and the plateau period was shown on the last week of the incubation (E28 - hatch). These parallel changes of serum glucose and hepatic glycogen were also confirmed in chick embryos (Latour et al., 1996; Christensen et al., 2001; Roy et al., 2013a). The rate-limiting enzymes of *PC*, *PCK*, *PFKFB2*, and *G6PC1* are responsible for the irreversible catalytic reactions in

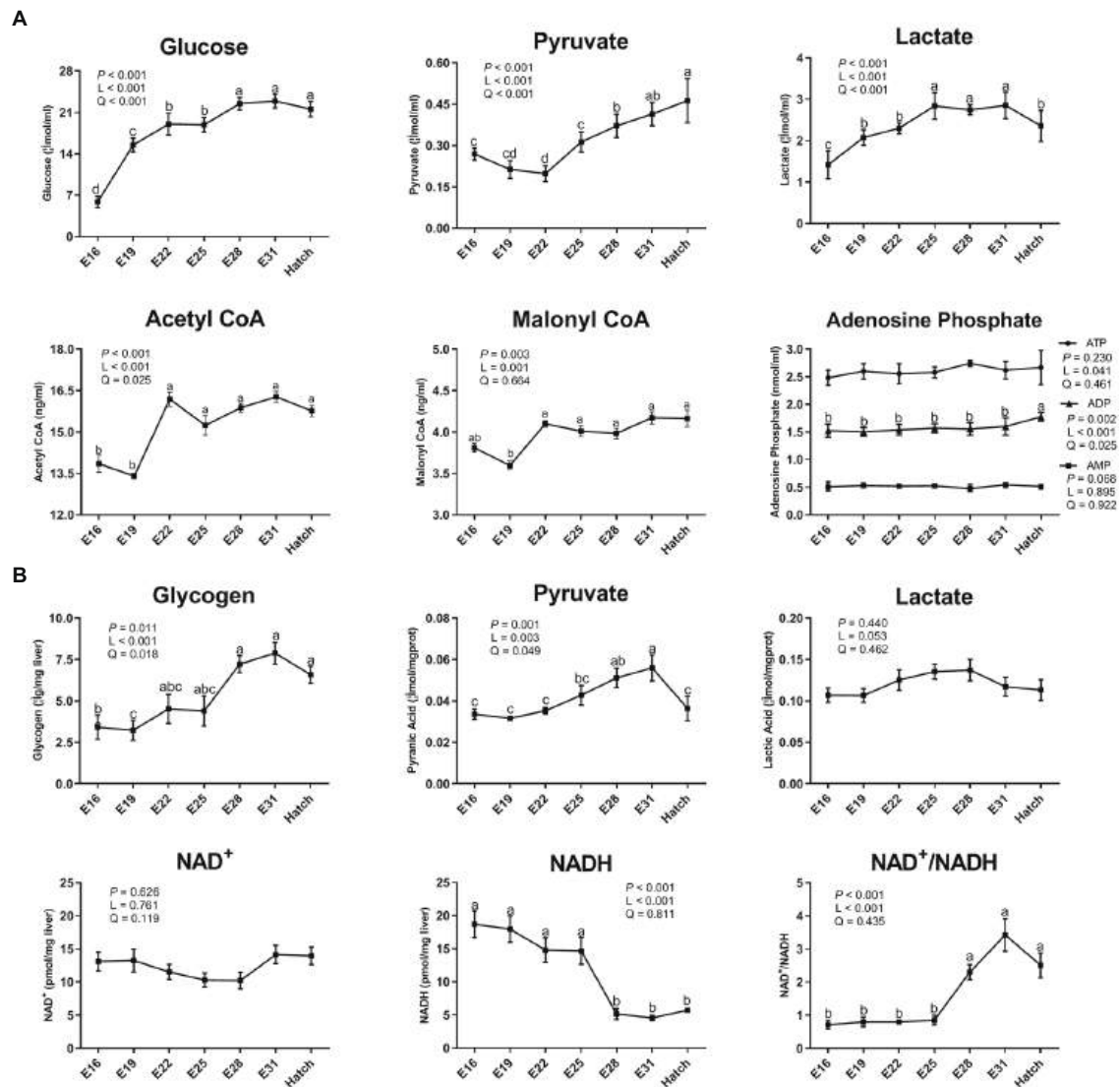


FIGURE 1 | Dynamic changes of serum and hepatic biochemical index in the avian model. **(A)** serum biochemical index; **(B)** hepatic biochemical index.

Abbreviations: CoA, coenzyme A; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; NAD, nicotinamide adenine dinucleotide; NAD⁺, the oxidized form of NAD; NADH, the reduced form of NAD. Data are expressed as mean \pm SEM ($n = 6$), and values on the same line with different lowercase letters indicated statistically significant differences (one-way ANOVA, $P < 0.05$). L, linear; Q, quadratic.

gluconeogenesis (Shen and Mistry, 1979; van Schaftingen and Gerin, 2002; Chesney, 2006). Hepatic gene mRNA expression of *PRKAA1*, *PC*, *PCK*, *PFKFB2*, and *G6PC1*, and the relative protein expression of *G6PC1* increased from E16 to hatch, and the maximal values were observed between E22 and E31. These results could partly explain the increasing serum glucose concentration and hepatic glycogen content during embryonic development. The avian embryo begins anaerobic catabolism of glucose before hatch, and glucose is metabolized into the highly versatile metabolite pyruvate (De Oliveira et al., 2008). And then, pyruvate can be anaerobically oxidized to lactate, accompanied by the conversion of NADH to NAD⁺ (Bar-Even et al., 2012; Luengo et al., 2021). In the current study, glycolysis was enhanced

as indicated by an increase in pyruvate and lactate contents in serum or liver and a decrease in hepatic NADH content during the last period of incubation (E28 - hatch). Glycolysis supplies the ATP molecules produced by a reversible reaction ($2\text{ADP} \leftrightarrow \text{ATP} + \text{AMP}$; Noor et al., 2010; Schormann et al., 2019). The increase in ADP content implied that ATP was hydrolyzed to generate energy for the maintenance of embryonic growth and development. It's suggested that glycolysis provides an additional energy source (except for FA oxidation) for the greater demands during the later period of incubation, especially during internal piping (break the air cell by the beak) and external piping (break the eggshell) periods (Moran, 2007; De Oliveira et al., 2008). Glycolysis is regulated by the key enzymes of HKDC, PFKL,

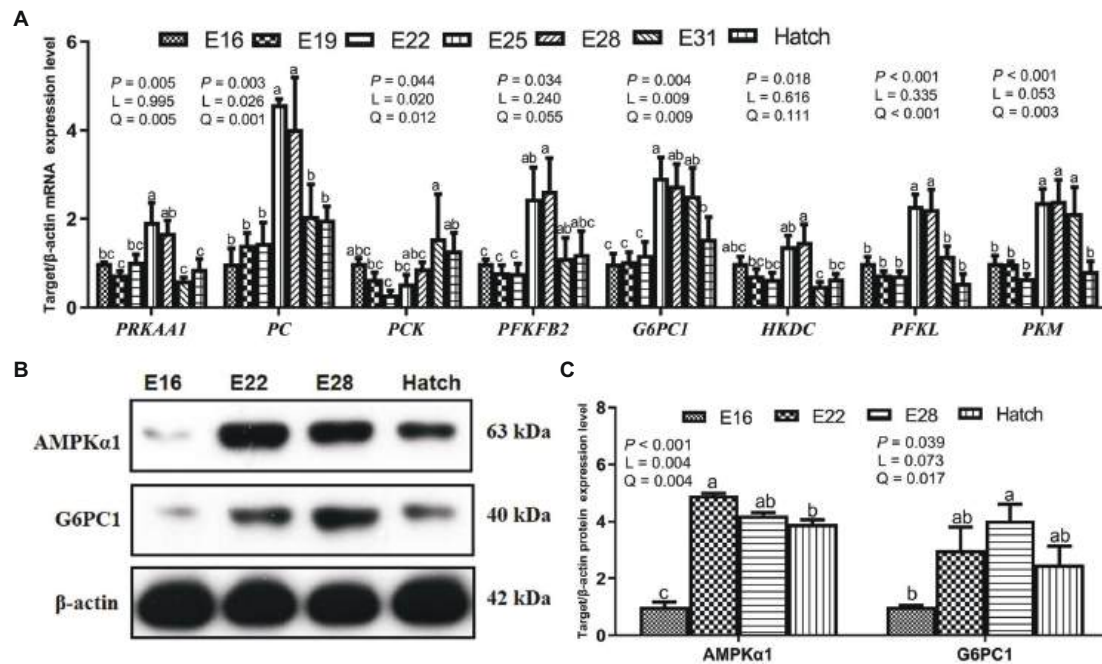


FIGURE 2 | Hepatic gene and protein expression related to glucose metabolism in the avian model. **(A)** Hepatic gene mRNA relative expression related to glucose metabolism in the avian model; **(B,C)** The expression of proteins related to glucose metabolism in the liver of avian embryos. *PRKAA1*, protein kinase AMP-activated catalytic subunit alpha 1; *PC*, pyruvate carboxylase; *PCK*, phosphoenolpyruvate carboxykinase; *PFKFB2*, 6-phosphofructo-2-kinase; *G6PC1*, glucose-6-phosphatase; *HKDC*, hexokinase; *PFKL*, phosphofructokinase; *PKM*, pyruvate kinase M1/2; *AMPKα1*, AMP-activated protein kinase alpha 1. Data are expressed as mean \pm SEM, and bars with different lowercase letters indicated statistically significant differences (one-way ANOVA, $p < 0.05$). L, linear; Q, quadratic.

and PKM (Bolaños et al., 2010). Hepatic gene mRNA expression of *HKDC*, *PFKL*, and *PKM* increased during embryonic development, and the plateau period was achieved between E25 and E31. A similar pattern was observed in chick and pigeon embryos reported previously (Roy et al., 2013b; Peng et al., 2018; Wan et al., 2018). Then, the increased protein expression level of AMPKα1 was observed in liver during E22 - E28. It's implied that the AMPK pathway is activated by falling energy status and promotes ATP production *via* increasing catabolism as well as switching off biosynthetic pathways (Hardie et al., 2012; Hardie, 2015). Briefly, these results suggested that the glucose homeostasis turnover between gluconeogenesis and glycolysis enhanced energy generation to meet the high energy requirements during embryonic development and as fuel storage for survival at the early post-hatch period (Li et al., 2008; Payne et al., 2019).

Previous studies have demonstrated that LA reduces glucose concentration in the diverse cell, animal, and human models (Conde-Aguilera et al., 2012; Matravada et al., 2016; Andersson-Hall et al., 2018; Holmäng et al., 2018). In the present study, the intervention of exogenous LA led to a decrease in serum contents of TG and CHO, and then reduced the concentration of glycerol, which was derived from TG hydrolysis and the main precursor of gluconeogenesis in late-term avian embryos (Sunny and Bequette, 2011; Neves et al., 2017; Xue et al., 2017). Moreover, IOF LA inhibited gluconeogenesis on E28 as displayed by a decrease in serum

glucose concentration and gene expression of *PC*, *PCK*, and *PFKFB2*. However, IOF LA increased stearic acid (C18:0) and decreased oleic acid (C18:1n-9) in liver, due to the weakened activity of desaturases responsible for the synthesis of UFAs, especially the $\Delta 9$ -desaturase activity (Kouba and Mourot, 1998; Bee, 2000; Novak et al., 2012; Lounis and Bergeron, 2017; Piccinin et al., 2019). Hepatic mRNA expression of *FASN*, *SCD1*, *PPARα*, and *RXRα* related to FA synthesis and oxidation was downregulated on E28. It's implied that FA oxidation could not satisfy all the energy demands for the embryos as the internal oxygen concentration became limited. In addition, IOF LA enhanced glycolysis on E31 as indicated by an increase in serum pyruvate and lactate contents, as well as the upregulation of hepatic gene expression of *HKDC* and *PFKL*. The synthesis of serum glucose and hepatic glycogen was suppressed, combined with the enhanced glycolysis, which led to a reduced energy fuel storage for late embryonic development. Hence, the embryonic mortality in IOF LA group (24.27%) was increased by 8% compared to the control group (16.67%). Similarly, *in vivo* studies showed that elevated maternal dietary LA (or conjugated LA) reduced fetal survival and increased embryonic mortality in rat or chicken models (Leone et al., 2010; Shrestha et al., 2019). It was concluded exogenous LA could inhibit hepatic gluconeogenesis and enhanced glycolysis, thus leading to the impairment of glucose homeostasis and energy status during embryonic development.

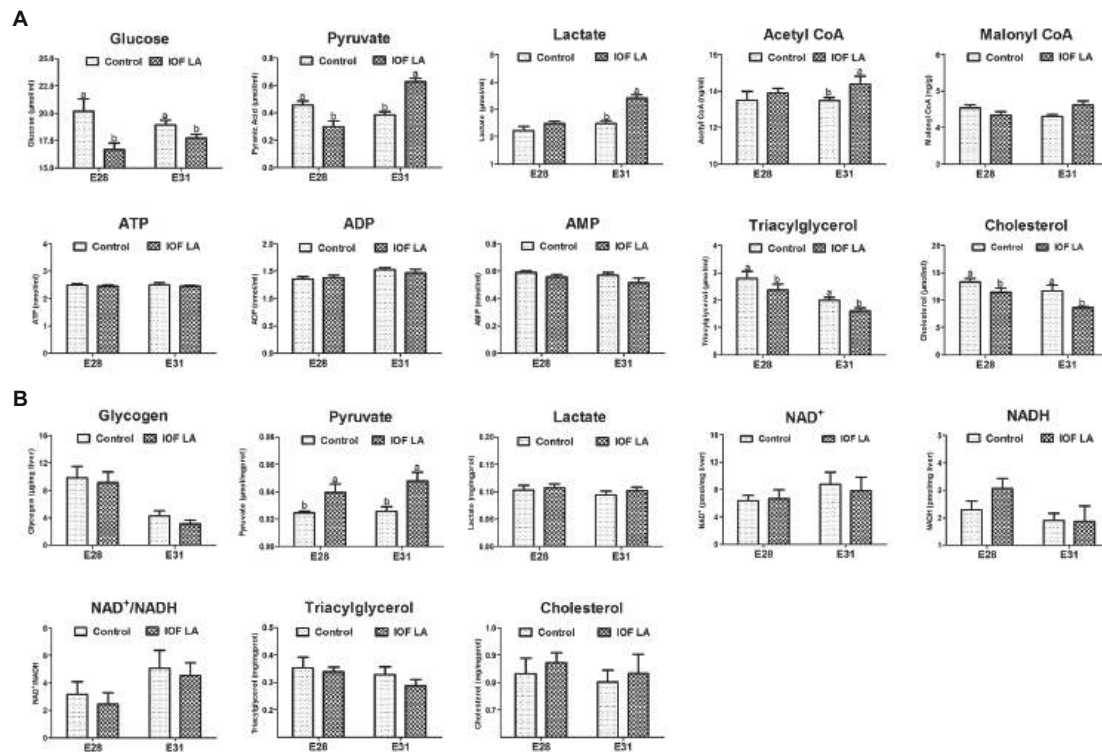


FIGURE 3 | Effect of *in ovo* feeding linoleic acid on the changes of biochemical index in the avian model. **(A)** serum biochemical index; **(B)** hepatic biochemical index. Control group was *in ovo* injected with PBS, and IOF LA was the treatment group *in ovo* feeding linoleic acid on E25. Abbreviations: CoA, coenzyme A; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; NAD, nicotinamide adenine dinucleotide; NAD⁺, the oxidated form of NAD; NADH, the reduced form of NAD. Data are expressed as mean ± SEM (*n* = 6), and bars on the same sampled day with different lowercase letters indicated statistically significant differences (*t*-test, *p* < 0.05).

TABLE 2 | Effect of *in ovo* feeding linoleic acid on the changes of hepatic fatty acid composition in the avian model (%).

Item	E28			E31		
	Control	IOF LA	SEM	Control	IOF LA	SEM
Myristic acid C14:0	5.77	5.41	0.341	5.08 ^b	6.96 ^a	0.379
Myristoleic acid C14:1	0.57	0.60	0.067	0.67	0.77	0.047
Palmitic acid C16:0	16.96	15.94	0.478	16.91	15.60	0.522
Palmitoleic acid C16:1	0.59	0.58	0.052	0.47	0.33	0.044
Stearic acid C18:0	12.65 ^b	16.42 ^a	0.715	16.28	17.15	0.476
Oleic acid C18:1n-9	30.18 ^a	27.33 ^b	0.831	26.86	25.84	0.883
Linoleic acid C18:2n-6	8.68	8.96	0.294	8.09	8.23	0.289
Eicosatrienoic acid C20:3n-6	0.34	0.41	0.021	0.41	0.40	0.039
Eicosatrienoic acid C20:3n-3	14.14	15.40	0.646	15.40	16.31	0.779
Nervonic acid C24:1n-9	0.89	0.67	0.105	0.95	0.86	0.041
Docosahexaenoic acid C22:6n-3	6.21	6.29	0.524	6.49	5.47	0.360
MUFAs	31.73	29.19	0.818	28.87	27.80	0.922
PUFAs	30.01	31.06	0.616	30.39	30.42	0.976
n-6 PUFAs	9.03	9.37	0.294	8.50	8.64	0.290
n-3 PUFAs	20.98	21.70	0.692	21.89	21.78	1.029
n-6/n-3	0.43	0.44	0.025	0.41	0.40	0.032
UFAs	61.74 ^a	60.25 ^b	0.441	59.25	58.21	0.399
SFAs	36.16 ^b	37.77 ^a	0.434	38.26 ^b	39.70 ^a	0.432
UFAs/SFAs	1.71 ^a	1.60 ^b	0.030	1.55	1.47	0.026

Control group was *in ovo* injected with PBS, and IOF LA was the treatment group *in ovo* feeding linoleic acid on E25. MUFAs are the sum of monounsaturated fatty acids that include C14:1, C16:1, C18:1n-9, and C24:1n-9. PUFAs are the sum of polyunsaturated fatty acids that include C18:2n-6, C20:3n-6, C20:3n-3, and C22:6n-3. N-6 PUFAs are the sum of n-6 polyunsaturated fatty acids that include C18:2n-6 and C20:3n-6. N-3 PUFAs are the sum of n-3 polyunsaturated fatty acids that include C20:3n-3 and C22:6n-3. UFAs are the sum of unsaturated fatty acids that include MUFAs and PUFAs. SFAs are the sum of saturated fatty acids that include C14:0, C16:0, and C18:0. Data are expressed as mean ± SEM (*n* = 6), and data at the same sampled day on the same line with different lowercase letters indicated statistically significant differences (*t*-test, *p* < 0.05).

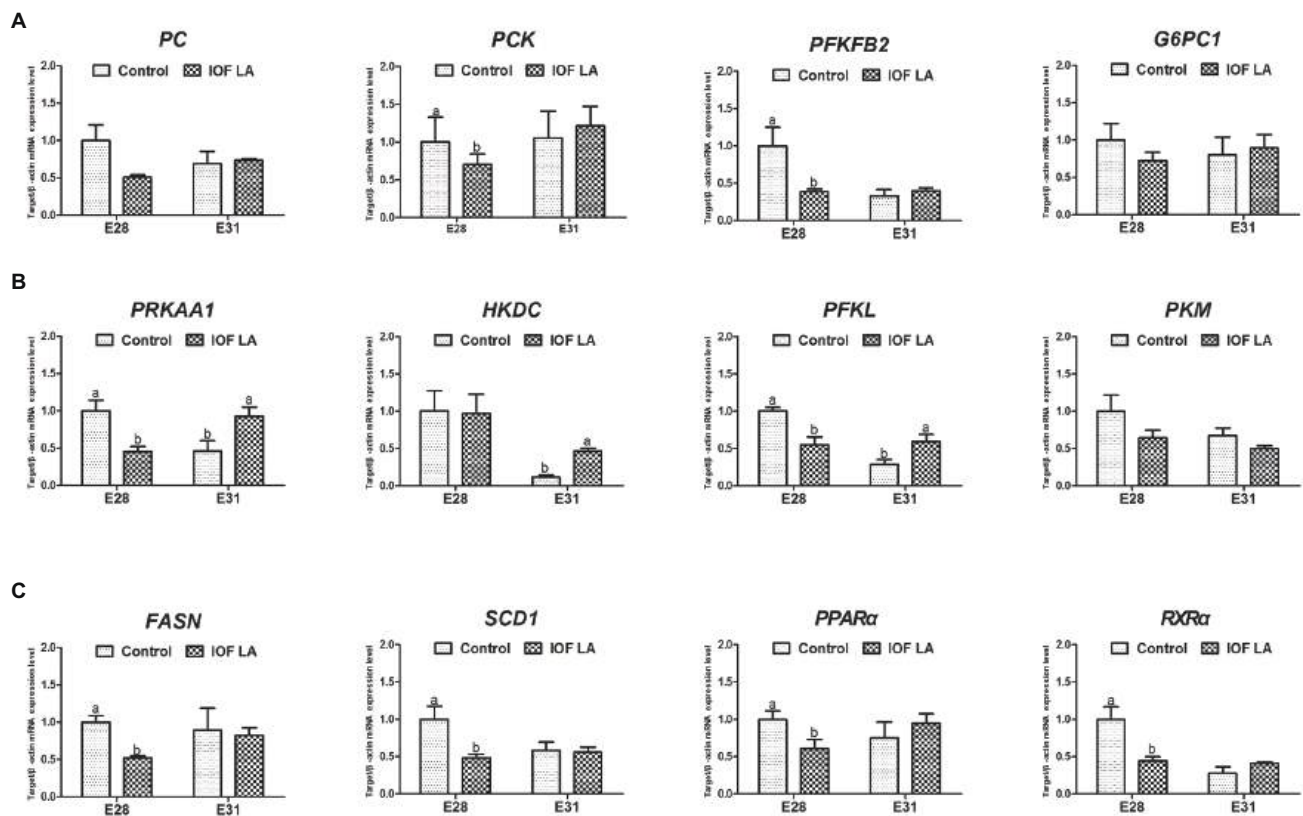


FIGURE 4 | Effect of *in ovo* feeding linoleic acid on the gene relative expression in the avian model. Control group was *in ovo* injected with PBS, and IOF LA was the treatment group *in ovo* feeding linoleic acid on E25. **(A)** gene expression related to gluconeogenesis: *PC*, pyruvate carboxylase; *PCK*, phosphoenolpyruvate carboxykinase; *PFKFB2*, 6-phosphofructo-2-kinase; *G6PC1*, glucose-6-phosphatase; **(B)** gene expression related to energy homeostasis and glycolysis: *PRKAA1*, protein kinase AMP-activated catalytic subunit alpha 1; *HKDC*, hexokinase; *PFKL*, phosphofructokinase; *PKM*, pyruvate kinase M1/2; **(C)** gene expression related to fatty acid synthesis and oxidation: *FASN*, fatty acid synthase; *SCD1*, stearoyl-CoA desaturase; *PPARα*, peroxisome proliferator-activated receptor alpha; *RXRα*, retinoid X receptor alpha. Data are expressed as mean ± SEM ($n=6$), and bars on the same sampled day with different lowercase letters indicated statistically significant differences (t -test, $p < 0.05$).

CONCLUSION

In summary, hepatic gluconeogenesis and glycolysis were enhanced to meet increasing energy demands during the late embryonic period, as evidenced by increasing contents of glucose, glycogen, pyruvate, and lactate in serum or liver, as well as hepatic target gene and protein expressions. IOF LA could inhibit gluconeogenesis and enhance glycolysis, leading to impaired glucose homeostasis and energy status in the developing embryos.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Care and Use Committee of South China Agricultural University.

AUTHOR CONTRIBUTIONS

XZ designed this study, carried out the experiments and measurements, and drafted the manuscript. QW, WZ, CL, and LH helped to analyze the experiment traits. WZ, XZ, and XH assisted with the incubation trial. HY and WW helped with the data analysis. LY and YZ participated in the study's design, coordination, and manuscript writing. All authors read and approved the final version of the manuscript.

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METABOLISM AND NUTRITION

Mineral requirements in ducks: an update

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ABSTRACT Mineral nutrition plays a critical role in growth and bone mineralization in meat ducks as well as reproductive performance in duck layers and duck breeders. In addition to improving production performance parameters, minerals are also essential to support several enzymatic systems to enhancing antioxidant ability and immune function. This review explores the biological function and metabolism of minerals in the body, as well as mineral feeding strategy of various species of ducks. Topics range from mineral requirement to the physiological role of macroelements such as calcium and phosphorus and microelements such as zinc and selenium, etc. As with the improvement of genetic evolution and upgrade of rearing system in duck production, mineral requirements and electrolyte balance are urgent to be re-evaluated using sensitive biomarkers for the modern duck breed characterized by the rapid growth rate and inadequate bone development and mineralization. For duck breeders, mineral nutrition is not only required for maximal egg production performance but

also for maintaining normal embryonic development and offspring's performance. Therefore, the proper amounts of bioavailable minerals need to be supplemented to maintain the mineral nutritional state of duck species during all phases of life. In addition, more positive effects of high doses microelements supplementations have been revealed for modern meat ducks subjected to various stresses in commercial production. The nutritional factors of mineral sources, supplemental enzymes, and antinutritional factors from unconventional ingredients should be emphasized to improve the effectiveness of mineral nutrition in duck feed formulation. Organic mineral sources and phytase enzymes have been adopted to reduce the antagonistic action between mineral and antinutritional factors. Therefore, special and accurate database of mineral requirements should be established for special genotypes of ducks under different rearing conditions, including rearing factors, environmental stresses and diets supplemented with organic sources, phytase and VD₃.

Key words: duck, mineral requirement, mineralization, organic source, sensitive biomarker

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INTRODUCTION

Duck meat consumption generally escalated during the past few decades because of its high nutrient content with an optimum essential amino acid, proper composition of fatty acid with a high polyunsaturated fatty acid, and a balanced ratio of omega-6 and omega-3 (Pingel and Germany, 2011). In 2019, Food and Agriculture Organization of the United Nations estimated that the annual duck slaughter has reached 6.42 billion birds in the world. Asia is the leading continent in duck production with a share of 82.2%, followed by Europe with

12.4% (Sumarmono, 2019). However, the improvement and update of feed nutrition and management system are lagging behind the demand of duck production (Baeza, 2016). So far, many studies have been conducted on macronutrients (metabolizable energy, crude protein, and amino acid), whereas the information on mineral nutrition of ducks was relatively in shortage. During 2005–2020, studies of mineral nutrition were mainly focused on macroelement of Ca and P nutrition (Table 1), with limited information on microelement nutrition of Cu, Fe, Mn, Zn, and Se in ducks (Table 2). The optimum Ca and P levels (0.65% Ca and 0.40% non-phytate phosphorus (NPP) for 0–2 wk; 0.60% Ca and 0.30% NPP for 2–7 wk) have been recommended for Pekin ducks at 0 to 7 wk of age by NRC (1994). However, these data sourced from previous studies over 5 decades (Dean et al., 1967; Lin and Shen, 1979) may not be applicable to modern duck breeds/varieties with the greater changes in growth potential and management. For example, owing to the

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Table 1. Summary of the recommended requirement of calcium and phosphorus in ducks during 2005-2020.

Minerals	Breed	Age	Dietary Ca and P levels	Evaluation indicator	Recommended level	References
Ca	Pekin duck	7-18 d	Ca: 0.74, 0.85, 0.95, 1.11%; NPP: 0.40%	Weight gain	0.95%	Rush et al., 2005
	Pekin duck	0-2 wk	Ca: 0.40, 0.60, 0.80, 1.00, 1.20%; NPP: 0.40%	Weight gain and feed/gain	0.796–0.806%	Xie et al., 2009a
	Pekin duck	3-6 wk	Ca: 0.36, 0.48, 0.66, 0.84%; NPP: 0.37%	Weight gain	0.72%	Xie et al., 2009b
	Pekin duck	0-3 wk	Ca: 0.55, 0.75, 0.95, 1.15%; TP: 0.60%	Weight gain and feed/gain	0.75%	Zhu et al., 2018
	Sheldrake	0-3 wk	Ca: 0.45, 0.60, 0.75, 0.90, 1.05, 1.20%; NPP: 0.40%	Weight gain and bone mineralization	0.60–0.87%	Zhu et al., 2019
	Cherry Valley duck	15-35 d	Ca: 0.5, 0.7, 0.9, 1.1%; NPP: 0.40%	Bone turnover and tibia quality	0.70%	Zhang et al., 2018
	Linwu duck	30-38 wk	Ca: 2.0, 2.5, 3.0, 3.5, 4.0%; NPP: 0.29%	Egg qualified rate and eggshell strength	3.50%	Huang et al., 2017
	Linwu duck	22-28 wk	Ca: 1.5, 2.0, 2.5, 3.0, 3.5%; NPP: 0.29%	Egg weight, tibial Ca content, and tibial ash content	2.79–2.98%	Huang et al., 2016
	Longyan duck	21-33 wk	Ca: 2.8, 3.2, 3.6, 4.0, 4.4%; NPP: 0.40%	Serum ALP activity and tibial fresh weight	3.20–3.60%	Xia et al., 2015
P	Pekin duck	1-14 d	NPP: 0.20, 0.30, 0.40, 0.50%; Ca: 0.80%	Weight gain and feed/gain	0.379–0.403%	Xie et al., 2009a
	Pekin duck	3-6 wk	NPP: 0.17, 0.25, 0.33, 0.41%; Ca: 0.66–0.72%	Weight gain and tibial P content	0.37%	Xie et al., 2009b
	Pekin duck	1-21 d	TP: 0.409, 0.476, 0.532, 0.563, 0.659, 0.710, 0.796, 0.863%; Ca: 0.56%	Weight gain	0.56%;	Rodehutsord et al., 2003
	Pekin duck	21-49 d	TP: 0.377, 0.415, 0.493, 0.539, 0.585, 0.681, 0.817, 0.949%; Ca: 0.61%	Weight gain	0.51%;	Rodehutsord et al., 2003
	Cherry Valley duck	1-21 d	NPP: 0.22, 0.34, 0.40, 0.46, 0.58%; Ca: 0.90%	Weight gain and feed intake	0.34%	Dai et al., 2018

considerable improvement of genetics between 1988 and 2014, modern Cherry Valley duck breeds had greater feed intake, growth rate, and feed efficiency than before (e.g., live weight at 44 d, 2.95 kg vs 3.50 kg; FCR at

3.25 kg live weight, 2.65 vs 1.85; market age, 49 d vs 42 d). In addition, the enhanced performance capacity of birds has necessitated an increasing mineral requirement to ensure the bone health. [Zhang et al. \(2019\)](#)

Table 2. Summary of the recommended requirement of microelement in ducks during 2005-2020.

Microelements	Breed	Age	Microelement content in basal diet	Dietary supplemental level	Evaluation indicator	Recommended level	References
Copper	Pekin duck	1-56 d	7.0 mg/kg	0, 4, 8, 12, 150 mg/kg	Growth performance	8 mg/kg	Attia et al., 2012
	Shanma duck	17-45 wk	4.63 mg/kg	0, 4, 8, 12, 16, 20, 24 mg/kg	Laying performance and egg quality	5 mg/kg	Fouad et al., 2016a
Zinc	Pekin duck	1-56 d	26 mg/kg	0, 30, 60, 120 mg/kg	Growth rate and Zn excretion.	30 mg/kg	Attia et al., 2013
	Longyan duck	21-41 wk	27.7 mg/kg	0, 10, 20, 40, 80, 160 mg/kg	Productive performance and Zn deposition	70-80 mg/kg	Zhang et al., 2020
	Longyan duck	23-43 wk	37 mg/kg	0, 15, 30, 45, 60, 75, 90 mg/kg	Productive performance and antioxidant capacity	30-45 mg/kg	Chen et al., 2017
Manganese	Shanma duck	17-36 wk	19.1 mg/kg	0, 15, 30, 45, 60, 75, 90 mg/kg	Laying performance and egg quality	90 mg/kg	Fouad et al., 2016b
Iron	Shanma duck	17-30 wk	52.2 mg/kg	0, 15, 30, 45, 60, 75 mg/kg	Egg weight	45-75 mg/kg	Xia. et al., 2016
	Linwu duck	50-60 wk	77,34 mg/kg	0, 20, 40, 60, 80 mg/kg	Laying performance and egg quality	71.88-84.50 mg/kg	Huang et al., 2015
Selenium	Cherry Valley duck	1-49 d	0.041 mg/kg	0, 0.2, 0.4, 0.6 mg/kg	Growth performance	0.40 mg/kg	Baltić et al., 2017
	Shanma duck	22-48 wk	0.042 mg/kg	0, 0.08, 0.16, 0.24, 0.32, 0.40 mg/kg	Egg production	0.18-0.24 mg/kg	Chen et al., 2015a

reported that intensive selection for growth rate in ducks resulted in tibial morphology changes, displaying rapid bone growth (e.g., bone length, approximately from 40 mm to 117 mm; bone width, approximately from 1.50 to 8.70 mm) and mineralization (e.g., tibia density, approximately from 0.26 to 0.67 g/cm³) in ducks from day 1 to day 35. Moreover, ducks rearing in the plated or caged systems were lacked of exercise than the litter floor system (Rodenburg et al., 2005). Therefore, mineral nutrition should be paid more attention to bone development and mineralization of ducks under the update rearing systems with limited space.

In NRC (1994), the recommended requirements of Mn (60 mg/kg), Zn (40 mg/kg), Fe (80 mg/kg), and Se (0.15 mg/kg) were sourced from the values in chicken. However, there are huge differences in digestive physiology (Jamroz et al., 2002), mineral digestibility (Adeola, 2006), and mineral deposition (Rodehutscord and Dieckmann, 2005) between chickens and ducks. Unlike chickens, ducks have a spindle-shaped widening of the esophagus and fusiform proventriculus and a quicker transit rate of chyme, resulting in a lower mineral availability (Fan, 2003). For special duck types, mineral requirement recommendations from INRA (1989) and NRC (1994) were mainly focused on large type meat ducks, such as Muscovy ducks and Pekin ducks. It is not clear whether these data were applied to small type meat ducks and duck layers due to the great differences in growth curve, digestive physiology and nutrient digestibility (Wasilewski et al., 2015). Therefore, as a result of changes in genetics, sex, and rearing factors of birds, the mineral requirements in ducks at different growth periods need be further reevaluated. In China, the standard of nutrient requirements of meat-type duck has been published by the national waterfowl industry system in 2012, covering the duck species of Pekin ducks, Muscovy ducks, and local meat-egg-type ducks at the starter, growth-finisher, and breeder periods (NY/T, 2012). However, some values of microelement requirements in the standard were recommended based on the data from duck production experiences, which need to be confirmed and reevaluated. Therefore, it is necessary to establish the database and feeding standard for the mineral requirement of special duck species at the different growth phases.

Calcium

Calcium, as the most abundant mineral in the body, plays an important role in growth development and bone mineralization in animals (Selle et al., 2009). The Ca requirement of Pekin duck but not duck breeder (laying period) was recommended by NRC (1994). Dietary Ca deficiency induced rickets in broilers and impaired eggshell quality in laying hens. Four series of experiments conducted by Dean et al. (1967) indicated that rickets occurred in ducks fed a corn-soybean meal diet with a Ca level of 0.17% while maximum weight gain and normal bone ash were achieved when the Ca level was increased to 0.56%. Lin and Shen (1979)

reported that the minimum Ca requirements were 0.48% and 0.58%, respectively, for maximum growth and optimum tibia ash content in mule ducks as determined by regression analysis. Leclercq et al. (1990) recommended that the optimum Ca requirements were 0.46% and 0.42% in Muscovy ducks at 3 to 8 wk and 8 to 12 wk of age, respectively. During 1989–2008, the studies of Ca and P nutrition on ducks have not shown much progress. However, the production potential of modern duck breed was improved by the genetic breeding improvement as well as the feed pattern of rearing ducks was changed from on ground to on netting bed or on cage during this time, which may have caused a change in the requirements of the modern-type meat ducks. Therefore, the applicability of values from NRC (1994) needs to be reevaluated in modern duck breeds/varieties.

Over the past decade, Xie et al. (2009a) investigated the interaction between dietary Ca and NPP levels on growth performance and bone ash in White Pekin ducks. The predicted requirements were 0.80% Ca and 0.38–0.40% NPP in Pekin ducklings from hatch to 2 wk of age (Xie et al., 2009a) as well as 0.72% Ca and 0.37% NPP in ducks from 3 to 6 wk of age (Xie et al., 2009b) based on body weight gain (BWG). These values were higher than those recommended by NRC (1994) (0.65% Ca and 0.40% NPP for 0–2 wk; 0.60% Ca and 0.30% NPP for 2–7 wk). In addition, both genetic selection and increasing nutrient density priority to improving growth performance resulted in the increased leg problems of meat ducks. Moreover, the welfare problems also were inadvertently involved in the genetic evolution with poor bone quality and mineralization in meat ducks. Zhang et al. (2018) found that above 0.7% Ca supplementation in a low-nutrient density diet decreased bone turnover and subsequently increased tibia quality by downregulating the expression of osteoclast differentiation genes. Therefore, the demands for supplemental Ca level could be greater than before to ensure bone development and quality of modern meat ducks. For local meat-egg-type ducks, Zhu et al. (2019) estimated that the 0.60 to 0.87% Ca was required to optimize BWG and bone mineralization in Sheldrake ducks from hatch to 21 d of age.

Huang et al. (2016, 2017) showed that local Linwu ducks required 2.79 to 2.98% Ca and 3.50% Ca to support laying performance and egg quality in the early- and peak-laying periods, respectively. The optimal laying performance and bone quality could be achieved in Shanma laying ducks fed diets containing 3.2 to 3.6% at the peak-laying period (Xia et al., 2015). Wang et al. (2014) examined the influence of Ca source (limestone vs oyster shell) and particle size (<0.1 mm vs 0.85 to 2 mm) on laying performance, egg quality, and bone properties in duck layers, indicating that a diet supplemented with limestone with a large particle size provided for superior eggshell and bone quality. Chen et al. (2015b) found that dietary Ca supplementation increased both *secreted phosphoprotein 1* and *carbonic anhydrase 2* mRNA expressions in the uterus and then

improve eggshell quality and microarchitecture partly by strengthening shell biomineralization, which might be influenced by Ca source and particle size. Therefore, the precise Ca requirement of ducks could be varied in the diets supplemented with different Ca sources.

Phosphorus

Phosphorus is essential for poultry to attain their optimum genetic potential in growth and skeletal development involving in the metabolic and structural processes. In practical duck feed formulation, an excess P level was added to the diets to ensure a safety factor and prevent P deficiency in ducks, leading to the waste of resources and serious pollution. The studies involved P requirements and availabilities are limited on ducks. The P requirement (0.40% NPP for 0–2 wk and 0.30% NPP for 2–7 wk) recommended by NRC (1994) was estimated based on values obtained from chicken, which were lacked of experimental data on ducks. In fact, the P availability was significantly different between duck and chicken species. As reported by Rodehutscord and Dieckmann (2005), the maximum in marginal efficiency of supplemented P as monobasic calcium phosphate source was 96% in White Pekin ducks and 74% in broiler chickens at 3 wk of age. In studies with graded P levels, Pekin ducks achieved a plateau BWG in the P concentration of 5.6 g/kg at 2 wk old, while the value was slightly lower (5.1 g/kg) at 5 wk old (Rodehutscord et al., 2003). However, these P recommendations in duck diets were much lower than the result of 0.72 to 0.79% (NPP 0.34–0.40%) reported by Dai et al. (2018). These inconsistent results implied that the applicability of P requirement of NRC (1994) for ducks needs to be further confirmed.

For the aspect of P availability from different inorganic P sources, Wendt et al. (2004) found that P availabilities were 100, 96, 92, 91, and 86% for monosodium phosphate, anhydrous dibasic calcium, calcium sodium magnesium phosphate, monodibasic calcium phosphate, and dihydrated dibasic calcium phosphate using low-P diet, respectively. Li et al. (2018) indicated that P availability of monocalcium phosphate was 109.85% higher than dicalcium phosphate based on BWG and tibia mineralization. These data suggested that the amounts of different P sources should be adjusted appropriately for feed formulations due to the different P availabilities.

Nutritional Factors on Ca and P Utilization

Many studies have indicated that Ca and P utilization in poultry is affected by nutritional factors, such as Ca:P ratio and the levels of phytase and VD_3 additions (Rodehutscord, 2006). For dietary Ca:P ratio, Rodehutscord and Dieckmann (2005) and Rush et al. (2005) recommended the appropriate Ca:TP ratio varied between 1.6 and 1.9 based on the level of dietary Ca requirement. Xie et al. (2009a,b) recommended that requirements of Ca and NPP ratio for maximum weight gain were 2.0 (Ca 0.806% vs NPP 0.403%) and

1.94 (Ca 0.72% vs NPP 0.37%) in Pekin ducks at starter and growth-finisher periods, respectively. Zhu et al. (2018) reported that as dietary Ca:TP ratio was increased ranging from 1.2 to 2.5, the growth rate and bone mineralization were decreased in ducks fed the low-P diet with 0.45% TP. Therefore, it is important to consider the Ca:P ratio as a determinant of Ca and P requirements in the duck diet.

Supplementing phytase is an effective way of improving the utilization of phytate P and reducing the amount for P supplementation and P excretion (Bedford and Schulze, 1998). Rodehutscord et al. (2006) found that the addition of phytase (0, 250, 500, 750, 1,000, 1,500, and 2,000 U) could linearly increase the utilizations of Ca and P, and a plateau in response was reached above 1500 U/kg in duckling diets in accordance with the results of 2 balance studies. Similarly, Orban et al. (1999) found that the addition of phytase can increase the phytate P utilization (0.06–0.08%) resulting in the greater growth performance and bone quality in meat ducks at 3 to 6 wk old. Adeola (2010, 2018) indicated that supplementing the low-P duck diet with phytase resulted in both linear and quadratic increases in ileal digestibility and retention of P in both the starter and growth phases. Yang et al. (2009) found that adding 500 U/kg phytase reduced supplemental NPP level from 0.45 to 0.25% in duck layers diets without affecting the laying performance and bone mineral deposits. The mean P equivalency values of phytase supplemented with graded levels of inorganic P or phytase for 500, 1,000, and 1,500 phytase U/kg of diet were 0.453, 0.847, and 1.242 g/kg of duck diet, respectively (Adeola, 2010, 2018). Moreover, studies on broilers have found that high dose of phytase addition could degrade phytic acid to the greatest extent and obtain better production performance (Manobhavan et al., 2016). Therefore, it is necessary to determine the content of phytate P in feed ingredients and P equivalency values between phytase and inorganic P.

Supplementation of VD_3 in diets can increase Ca deposition, bone density, and immune function, which plays a great role in maintaining the normal growth and development of poultry (Świątkiewicz et al., 2017). With the increase of VD_3 levels (0, 250, 500, 1,000, 2,000, 3,000 IU/kg), feed intake and BWG were increased linearly and the maximum value was reached at 2,000 IU/kg in Pekin ducklings fed low Ca:NPP ratio diets (0.4% Ca vs 0.2% NPP) at 1 to 14 d of age, whereas the addition of VD_3 had no effect on growth performance in ducklings fed the normal Ca:NPP ratio diets (0.8% Ca vs 0.4% NPP) (Wang et al., 2010). Rush et al. (2005) observed that there was no response to increasing concentrations of VD_3 from 826 to 8,260 IU/kg on performance characteristics or bone ash contents in drakes during 0 to 13 d of age. The above inconsistent results implied that the positive effect of VD_3 on Ca and P metabolism is closely related to Ca and P contents in diets as well as nutritional status of Ca and P in ducks. Currently, the use of 25-hydroxycholecalciferol (25- OH-D_3) as biologically active metabolite of VD_3 is

more popular in poultry diets (Soares et al., 1995). The relative biological value of 25-OH-D₃ in comparison to VD₃, calculated using slope ratio based on tibia compressive strength and daily weight gain, were 1.44 and 1.37 times in Pekin ducks at 1 to 21 d and 22 to 42 d of age, respectively (Shi, 2013). Ren et al. (2016, 2017) confirmed that the inclusion of the mixture of 25-OH-D₃ and canthaxanthin in a diet increased antioxidant ability and serum P level in newly hatched ducklings. The enhanced P absorption and skeletal P deposition might be due to that VD₃ supplementation could increase the *NaP-IIb* and *PiT-2* mRNA expressions and decrease *PiT-1* mRNA expression in the small intestine of broilers (Shao et al., 2019). Although recent studies have paid more attention on the Ca and P requirements of different duck breeds at different growth stages, it is unclear that Ca and P requirements for the growth performance were enough to meet the need of skeletal development of ducks. Moreover, the databases of Ca and P supplementation should be established for feed formulation with addition of different Ca and P sources as well as supplemental 25-OH-D₃ and phytase.

Electrolyte Balance

Dietary electrolyte balance influences the nutrient metabolism by affecting the acid–base balance and pH in the microenvironment. When the acid–base balance is destroyed in the organism, the catalytic efficiency of enzymes is reduced and then results in metabolic abnormalities (Mushtaq et al., 2013). Most commonly, the electrolyte balance is described by a simple formula Na + K-Cl and expressed as mEq/kg meal. Adeola (2006) recommended 0.18 and 0.16% for Na requirement of meat ducks during 0 to 2 and 2 to 7 wk old, respectively. Dean (1972) suggested that ducklings require approximately 0.14% Na and 0.12% Cl for maximum weight gain, which was closed to the recommendations of Pekin ducks in NRC (1994) (0.15% Na and 0.12% Cl) and NY (2012) (0.15% Na and 0.12% Cl). INRA (1989) recommended Na and Cl requirements to be 0.16 and 0.14% for Muscovy ducks, respectively, which are close to those for Pekin ducks. On transfer to saline drinking water (284 mmol/L Na⁺, 6.0 mmol/L K⁺), there was a gradual loss of body weight accompanied by a reduction in the food and water intake (Fletcher and Holmes, 1968). Numerous studies have shown that the amount 250 mEq/kg is considered optimal for normal physiological function in broilers, but limited information in meat ducks (Mushtaq et al., 2013). Liu and Wang (2005) recommended 250–350 mEq/kg for duck layer diets (CP 16%) at growth-finisher period. Birds covered with a feather without sweat glands are susceptible to heat stress and respiratory alkalosis occurs at high temperatures consequent to the excessive loss of carbon dioxide induced by panting (Farghly et al., 2017; Rizk et al., 2019). Treatment with aqueous electrolyte solutions eliminates the adverse effects on broilers and layers (Ahmad and Sarwar, 2006). Subjected to high-temperature environment in summer, the electrolyte

balance of 250–300 mEq/kg was recommended for caged duck layers at the early laying stage (Wang et al., 2011). Therefore, heat-stressed condition results in poor growth rate and poor eggshell quality of highly productive layers (Nawab et al., 2018).

In addition, NRC (1994) recommended magnesium was 500 mg/kg diet for Pekin ducks. Ding and Shen (1992) reported that dietary excess Mg level (690, 1,070, 1,690, 2,150, 2,380 mg/kg) did not affect laying performance in Tsaiya ducks, whereas there was a negative correlation between eggshell thickness and eggshell Mg content, revealing that the increase in eggshell magnesium content probably associated with the impairment of eggshell quality. However, no significant correlation was observed in Leghorn hens. The inconsistent results suggested that there might be some differences on the mineral deposition and microstructure in eggshell between duck and chicken species.

Copper

Copper is a necessary mineral in poultry nutrition as being a cofactor for many enzymes, for example, cytochrome oxidase, lysyl oxidase, tyrosinase, phydroxyphenyl pyruvate hydrolase, and CuZnSOD (Leeson, 2009). However, Cu requirement in meat duck was not given by NRC (1994). INRA (1989) recommended 5, 4, and 3 mg Cu/kg diet for Muscovy ducks at starter, growth, and finisher phases, respectively, whereas Adeola (2006) recommended 8 mg Cu/kg diet for Pekin ducks at 0 to 7 wk of age. Fouad et al. (2016a) reported that a basal diet containing 5 mg Cu/kg was sufficient for laying performance and egg quality of Shanma laying ducks from 17 to 45 wk of age. In addition, dietary high Cu level of 150 mg/kg supplementation decreased plasma lipids, triglycerides, and cholesterol contents and increased plasma AST and ALT activities (Fouad et al., 2016a). Similarly, dietary 60 mg Cu/kg also decreased the cholesterol contents in plasma and egg yolk, which will help prevent cardiovascular disease for human health (Attia et al., 2011). However, it cannot be recommended due to its toxic effect on organ morphology and EU regulation of 35 mg/kg as the maximum permitted level.

Owing to the limited supplemental Cu level, organic Cu sources have recently received much more attention due to their higher electrical and melting points and bioavailability as well as low electrochemical migration and feed cost in poultry. For instance, Attia et al. (2012) have shown that organic lysine-Cu was more potent for decreasing plasma triglycerides than the inorganic source. Cu nanoparticles with larger surface area and greater capability to cross the small intestine could avoid antagonism with other nutrients and improve growth performance effectively, which was confirmed in piglets, fish, and broilers (Scott et al., 2018). Zhang (2004) found that Cu nanoparticles supplementation improved feed intake and immunity and increased the secretion of GnRH and GH to promote the growth and carcass quality of meat ducks.

Zinc

Zinc is a nutritionally essential mineral needed for catalytic, structural, and regulatory functions in animals. [Wight and Dewar \(1976\)](#) have reported that the growing ducks fed Zn-deficient maize starch-spray-dried egg albumen displayed the retarded growth and severe lesions of pedal epidermis. Severe Zn deficiency in diets results in a lower hatchability rate, abnormal embryonic development, and poor performing offspring ([Kienholz et al., 1961](#)). A 60 mg Zn/kg diet is required to maintain the optimum productive performance of Pekin ducks at 0 to 7 wk of age by [NRC \(1994\)](#). [INRA \(1989\)](#) recommended that the Zn requirements of Muscovy ducks were 40, 30, and 20 mg/kg at starter, growth, and finisher periods, respectively. [Wen et al. \(2018\)](#) found that Zn content in the traditional corn-soybean meal diet was inadequate to support optimum growth of Pekin ducks. [Attia et al. \(2013\)](#) showed that supplementation with 30 mg Zn/kg in corn-soybean meal basal diet (containing 26 mg Zn/kg) was optimal for growth performance of Pekin ducks from hatching to 56 d of age. In addition, Zn as an essential cofactor for thymulin can enhance the immune system and infectious disease resistance in poultry ([Park et al., 2004](#)). Zn deficiency could inhibit the growth and development of immune organs, leading to the decline of immune function, which was attributed to the apoptosis of immune cells via Fas/Fas-L pathway. For duck layers, addition of 30 to 45 and 70 to 80 mg Zn/kg to the corn-soybean basal diets could maintain the productive performance and improve immune function at the growing and breeder phases, respectively ([Chen et al., 2017](#); [Zhang et al., 2020](#)). These varies suggested that Zn requirements still need to be reevaluated precisely in special duck species at different growth stages.

Recent study has demonstrated that dietary high level of 120 mg Zn/kg exhibited growth-promoting effect and improved the intestinal morphology and barrier integrity on Pekin ducks from 1 to 35 d of age ([Wen et al., 2018](#)). In caged systems, dietary high level of 140 mg Zn/kg level increased the ultrastructural palisade layer thickness contributing to greater eggshell thickness of duck breeders than 40 mg Zn/kg ([Huang et al., 2020](#)). Owing to the higher bioavailability organic Zn, the greater improvement achieved by organic Zn supplementation has been reported in broilers and hens ([Huang et al., 2019](#)). [Guo et al. \(1999\)](#) reported that organic Zn of Met-Zn exhibited greater laying performance of local Jingjiang ducks at laying period. However, [Attia et al. \(2013\)](#) showed that a level of 30 mg Zn/kg from inorganic source displayed better effects of growth rate and Zn excretion than organic source. Because studies in broilers have demonstrated that the bioavailability of Zn might be depended on the chelation strengths, and organic Zn with moderate or strong chelation strength exhibits higher bioavailability compared to inorganic Zn as determined by tissue *MT* mRNA expression ([Huang et al., 2009](#)). One hypothesis is that organic Zn sources with moderate or strong chelation

strength could resist interference from dietary competitive ligands in the digestive tract and directly reach the intestinal brush border, displaying greater Zn bioavailability. Therefore, it is necessary to determine whether the improvements in productive performance were related to the higher Zn bioavailability of organic Zn with optimal chelation strength.

Manganese

Biochemically, Mn functions as an integral component of the enzymes pyruvate carboxylase, arginase, and superoxide dismutase. [NRC \(1994\)](#) and [Adeola \(2006\)](#) recommended a diet containing a minimum of 60 mg Mn/kg for growing Pekin ducks at 0 to 7 wk of age. Ducks fed a basal diet containing 11.62 mg Mn/kg for 2 wk presented the slipped tendon or perosis symptoms of Mn deficiency, characterized by swelling and flattening of the hock joint, along with subsequent slipping of the Achilles tendon from the condyles, whereas a 40 mg Mn/kg of diet was adequate for normal growth and prevention of perosis of ducks ([Van Reen and Pearson, 1955](#)). However, [Zhu et al. \(1999\)](#) indicated that dietary Mn levels had no effect on growth performance and increased tissue Mn contents. The differences between the studies may depend on the differences in the Mn content in basal diets, experimental periods and the genetic differences, ages, and physiological states of the birds. [Fouad et al. \(2016b\)](#) reported that a corn-soybean meal diet containing 19.2 mg Mn/kg was sufficient for laying performance and egg quality and adding 90 mg Mn/kg basal diet is required to increase Mn-containing superoxide dismutase (**MnSOD**) activity and yolk Mn content in Shanma laying ducks, suggesting that a higher Mn requirement was estimated for ducks by using some sensitive biomarkers (e.g., heart MnSOD activity as well as *MnSOD* mRNA and protein expressions). In addition, Mn is essential for embryonic development, normal growth of bones, and reproduction. For laying hens and breeding hens, some researchers have demonstrated that Mn deficiency marginally showed little or no effect on egg production and eggshell quality, the offspring performance was negatively influenced accompanying with the slipped tendon or perosis symptoms ([Olgun, 2017](#)). It is suggested that dietary Mn requirement for egg production might not be sufficient to maintain the optimal embryonic development and offspring performance in duck breeders. Therefore, the optimal Mn requirement in duck breeders needs to be reevaluated for egg production as well as embryonic development and offspring performance in the future.

Iron

Iron has a very specific function in animals as a component of the protein heme in the red blood cell's protein hemoglobin and in the muscle cell's protein myoglobin ([Theil, 2004](#)). So far, iron requirement of meat ducks was not recommended by [NRC \(1994\)](#). [Adeola \(2006\)](#) recommended a diet required 80 mg

Fe/kg for growing Pekin ducks at 0 to 7 wk of age. [INRA \(1989\)](#) recommended that the Fe requirements of Muscovy ducks were 40, 30, and 20 mg/kg at starter, growth, and finisher periods respectively. However, these values were lacked of experimental evidence in meat ducks. Therefore, studies about Fe nutrition in meat duck need to be further strengthened. In laying ducks, [Xia et al. \(2016\)](#) showed that 52.2, 97.2, and 127.2 mg Fe/kg are required to maintain performance and enhance hemoglobin and hematocrit levels of local Shanma ducks, respectively. [Huang et al. \(2015\)](#) shown that dietary supplemented with 71.9 to 84.5 mg Fe/kg were estimated to obtain the better egg production performance and egg quality of local Linwu laying ducks at 50 to 60 wk old. These data about Fe requirement were evaluated based on traditional conventional indicators for maximal performance, which may not be able to effectively reflect the sensitive response of iron nutritional status in ducks. Therefore, in broilers, some sensitive and specific indicators, such as enzymes (e.g., succinate dehydrogenase) and genes expression (e.g., cytochrome C oxidase) related to iron metabolism, have been selected to reevaluated Fe requirement, which tended to be higher than those from maximal performance ([Ma et al., 2016](#)).

Some antinutritional factors in feedstuffs have antagonistic effects on iron absorption, such as phytic acid ([Hunter, 1981](#)), pectin ([Miyada et al., 2011](#)), and tannin ([Delimont et al., 2017](#)). For example, iron can bind to cellulose or tannins to form insoluble complexes to inhibit the Fe absorption in animals. Recently, more unconventional ingredients with high contents of antinutritional factors tended to be used in feed formulation of meat ducks than broilers. Therefore, supplemental Fe levels in duck diet should not be referred to the data from studies in broilers. Organic sources with higher bioavailability could prevent antagonistic action between Fe and antinutritional factors as well as reduce fecal Fe excretion and environmental pollution.

Selenium

Selenium as an integral part of selenoproteins participated in the regulation of various physiological processes in the body. Se deficiency damaged fibroblast membranes and decreased collagen synthesis, resulting in myodegeneration in ducks ([Brown et al., 1982](#)). Se requirements were 0.2 and 0.1 mg/kg in Pekin ducks and Muscovy ducks, respectively. [Chen et al. \(2015a\)](#) indicated that Se requirement based on daily egg production were 0.18 and 0.24 mg/kg for duck layers at early-laying and peak-laying periods, respectively. Generally speaking, there are 2 major Se sources for poultry, namely inorganic Se (selenite) and organic Se in the form of selenomethionine (Se-yeast). Organic Se sources shows greater bioavailability (75.7%) than Se bound in the inorganic form (49.9%) ([Mahan and Peters, 2004](#)), augmenting antioxidant defense against free radicals and natural immunity of the organism ([Surai, 2002](#)).³⁴⁸

Compared with inorganic Se, diet required 0.40 mg Se/kg as Se-yeast source to improve the growth rate of Cherry Valley ducks from 1 to 49 d of age ([Baltić et al., 2017](#)). [Zhang \(2013\)](#) reported that adding 0.19 mg Se/kg in a basal diet containing 0.042 mg Se/kg was sufficient to maintain growth rate and improve the antioxidant ability and immune function of ducklings at the starter period. Considering the enhanced antioxidant ability due to Se supplementation, [He et al. \(2013\)](#) have demonstrated that Se-yeast + VE could reduce negative effects of AFB1 on growth and hepatic function. [Li \(2008\)](#) found that dietary higher level of 0.4 mg Se/kg could alleviate the cold-stressed effect on growth performance of ducklings. Therefore, the beneficial effect and mechanism of organic Se sources and levels should be examined in further studies in ducks subjected to the stress and disease conditions.

SUMMARY

Over the past 60 yr, most mineral nutrition studies have been limited to the effects of Ca, P, Zn, and Se on growth performance of meat ducks and laying performance of duck layers. Thus, more studies should be conducted to determine the requirements of Cu, Fe, and Mn and electrolyte balance of meat ducks and duck breeders. Especially, mineral nutrition in female duck breeder is not only required for maximal egg production performance but also for maintaining normal embryonic development and offspring's performance. As with the improvement of genetic evolution and upgrade of rearing systems in duck production, mineral requirements should be urgent to be reevaluated for modern duck breed characterized by the rapid growth rate and inadequate bone development and mineralization. It is also suggested that sensitive and specific enzymes and target genes related to mineral metabolism should be used to determine mineral requirements of ducks. In addition, considering the high susceptibility of modern poultry to various stresses in commercial production, more attention should be paid for the positive effects of high doses addition for meat ducks subjected to the stressed challenges. Recently, some unconventional ingredients with antinutritional factors were preferred to be used in feed formulation of ducks, some nutritional strategies with supplemental enzymes (e.g., phytase and NSP enzymes) or organic sources could be adopted to reduce the antagonistic action between mineral and antinutritional factors. It is necessary to clarify how to adjust the mineral addition in diets supplementations of phytase and VD₃. Therefore, special and accurate database of mineral requirements should be established for different genotypes of ducks (meat-type vs egg-type) under different rearing conditions, including rearing factors (caged system and litter floor), environmental stresses (heat stress vs cold stress) and diets supplemented with different mineral sources (inorganic source vs organic source), phytase and VD₃.

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DISCLOSURES

The authors declare that there is no conflict of interest related to the preparation and publication of this article.

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Responses of Combined Non-starch Polysaccharide Enzymes and Protease on Growth Performance, Meat Quality, and Nutrient Digestibility of Yellow-Feathered Broilers Fed With Diets With Different Crude Protein Levels

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The aim of this study was to investigate the responses of non-starch polysaccharide (NSP) enzymes and protease combination on growth performance, meat quality, and nutrients digestibility of yellow-feathered broilers fed with corn-soybean meal basal diets with normal and subnormal crude protein (CP) levels. The experimental design was completely randomized with a 2 × 2 factorial arrangement of treatments, including six replicates of 20 birds per pen. Two basal diets were formulated with normal CP level as positive control (PC) and subnormal CP level without extra essential amino acid (AA) supplementation as negative control (NC). The basal diets were supplemented without or with NSP enzymes and protease. Broilers fed with the NC diet had lower ($P < 0.05$) final body weight (BW), average daily weight gain (ADG) on days 1–21, 22–56 and 1–56 and higher ($P < 0.05$) feed-to-gain ratio (F/G) on day 22–56 than those fed with PC diet. The broilers fed with the NC diet had higher ($P < 0.05$) L* and b* values in thigh muscle, crypt depth in the duodenum, and dry matter (DM) digestibility as well as lower ($P < 0.05$) villus height, musculature thicknesses, and villus height: crypt depth in the duodenum than those fed with the PC diet. Dietary NSP enzymes and protease combination increased ($P < 0.05$) the ADG and F/G of the broilers on days 1–56, and pH values in breast and thigh muscles as well as the digestibility of DM, gross energy (GE), CP and most AAs of the broilers on day 56. Compared with the PC diet, the combination of NSP enzymes and protease exhibited greater ($P < 0.05$) improvements in the digestibility of DM, CP, and some AAs (Asp, Ile, and Leu) in the broilers fed with the NC diet. In conclusion, reducing CP diet without essential AA supplementation impaired the growth performance and meat color of the thigh muscles of the broilers.

The combination of NSP enzymes and protease effectively improved the growth performance, meat quality, and nutritional values of the broilers. In terms of the digestibility of DM, CP, and some AAs, the magnitude of response to the addition of NSP enzymes and protease was greater in the low nutritional-quality diet with a subnormal CP level.

Keywords: non-starch polysaccharide enzymes, protease, nutrients utilization, yellow-feathered broilers, crude protein

INTRODUCTION

With the increasing demand and price volatility of ingredients, a concerted effort to improve the nutritive value of poultry feeds with exogenous enzyme supplementation has been ongoing in the poultry industry (1, 2). The existing knowledge is that exogenous enzymes could play a great role in enhancing the potential feeding value of poultry diets (3–5). In addition to the use of individual exogenous enzymes, combinations of exogenous enzyme preparations (i.e., carbohydrase, phytase, and protease) have also been proposed to be more effective in enhancing growth performance and nutrient availability in poultry (6–8). A number of studies have demonstrated that a combination of exogenous non-starch polysaccharide (NSP) enzymes and protease supplementation was used expectantly to improve growth performance and nutrient utilization, and reduce nutrient excretion in broilers (9) and hens (7, 10). The efficiency of exogenous enzyme combinations varied depending on dietary nutritional quality and substrate specificity (8, 11). Responses to exogenous enzyme additions were expected to be greater in diets the lower ingredient quality and nutritional density (12–14). The multi-enzyme addition of xylanase, amylase, and protease exhibited greater effects on increasing energy digestibility of corn-soybean meal diets with lower energy contents compared with diets with higher energy levels in broiler chickens (15). However, several studies have indicated that there was no beneficial responses of exogenous enzyme supplementation on performance and nutrient digestibility of broiler chickens fed WITH diets varying in nutrient density (16, 17). The lack of effect of dietary exogenous enzymes could be that a greater reduction in the nutritional levels of diets could have possibly elicited the beneficial effects of enzyme supplementations (16, 18). So far, research data on possible interactions between dietary crude protein (CP) levels and exogenous enzyme supplementation in broiler diets were not only limited but also inconsistent and as a result, more information is still required. Therefore, this study was conducted to investigate whether there were different responses of combined NSP enzymes and protease supplementation on growth performance, carcass traits, meat quality and nutrient utilization in broiler chickens fed diets with normal and subnormal CP levels.

METHODS AND MATERIALS

Experimental Design and Diets

The study procedures were reviewed and approved by the Institutional Animal Care and Use Committee of South China

Agricultural University to ensure compliance with welfare and humane practices (SCAU-10564)). The experimental design was completely randomized with a 2×2 factorial arrangement of treatments. Two basal diets were formulated with normal CP level as positive control (PC) and subnormal CP level without extra essential amino acid (AA) supplementation as negative control (NC). The basal diets were supplemented without (control) or with exogenous NSP enzymes and protease combination (enzyme). The NSP enzymes (Kemzyme[®] FHS, enzyme activities: xylanase 80,000 U/g, and β -glucanase 6,000 U/g) and protease (Kemzyme[®], heat- and acid-resistant protected protease, activity: 8,000 U/g) were provided by Kemin (China) Technologies Co., Ltd. (Zhuhai, Guangdong, China). The recommended level of 200 mg/kg enzyme mixtures was added to the diets at the expense of corn (14), providing 16,800 U of xylanase, 1,180 U of β -glucanase, and 1,530 U of protease per kg diet based on the analysis.

A total of 480-day-old male yellow-feathered broilers were kept in floor pens in a building with a controlled environment. The birds were weighed individually and divided into four dietary treatments with 6 replicate pens of 20 birds per pen. The room temperature was initially set at 34°C and was accordingly reduced by 2°C per week to maintain 20°C. A lighting program of 18-h light and 6-h darkness was maintained throughout the trial except for the first day when the birds had 24 h of light. The PC diet was formulated to meet the nutrient requirements of the National Agricultural Industry Standard (NY/T 3645-2020) for yellow-feathered broilers and contained CP levels of 22 and 19.5% for broilers at the starter and grower-finisher phases, respectively (Table 1). The NC diets were formulated with subnormal CP levels of 20 and 18% without extra essential AA supplementation compared to the PC diet, while other nutrients met the requirements for broilers in the two phases. Both the PC and NC diets were without or with supplemental exogenous enzymes according to the experimental design. Each diet contained 5 g/kg titanium dioxide (TiO₂) as an indigestible marker for calculation of nutrient digestibility coefficients (19). The diets were pelleted using a conditioning temperature of 65°C. All the diets were fed in a pelleted form throughout the experimental period. The experimental diets and water were provided *ad libitum*.

At 21 and 56 days of age, after 12-h feed withdrawal, the birds were weighed, and feed consumption was recorded by each replicate pen. Average daily gain (ADG), average daily feed intake (ADFI), and feed:gain ratio (F:G) were calculated following any necessary corrections for mortality. Mortality was very low and averaged 95% throughout the experimental period. On day 56, based on the average body weight (BW) of birds in

TABLE 1 | Composition and nutrient levels of the positive and negative control diets.

Item	Starter period (days 1–21)		Grower-finisher period (days 22–56)	
	PC	NC	PC	NC
Ingredient, %				
Corn	55.28	62.18	56.37	61.54
Soybean meal	29.3	23.38	26.57	22.2
Corn gluten meal	8.2	8.2	6.0	6.0
Lard oil	2.08	1.1	7.0	6.2
Limestone	1.06	1.06	1.2	1.2
Dicalcium phosphate	2.0	2.0	1.1	1.1
L-lysine-HCl	0.41	0.41	0.2	0.2
DL-Methionine	0.11	0.11	0.06	0.06
Choline chloride	0.1	0.1	0.08	0.08
Sodium bicarbonate	0.26	0.26	0.24	0.24
Sodium chloride	0.20	0.20	0.18	0.18
Vitamin-mineral premix ¹	1.0	1.0	1.0	1.0
Total	100	100	100	100
Calculated value, %				
Metabolizable energy, Kcal/kg	2,950	2,950	3,253	3,251
Crude protein ²	22.03	20.02	19.51	18.03
Calcium	0.95	0.94	0.8	0.79
Total phosphate	0.71	0.69	0.53	0.52
Lysine	1.15	1.02	0.95	0.86
Methionine	0.50	0.47	0.40	0.38
Methionine + cysteine	0.90	0.86	0.70	0.67
Threonine	0.85	0.80	0.67	0.62
Tryptophan	0.20	0.17	0.16	0.14

¹Premix provided the following per kilogram of diets for the starter period: VA 12,000 IU, VD₃ 600 IU, VE 45 IU, VK 2.5 mg, VB₁ 2.4 mg, VB₂ 5 mg, VB₆ 3.5 mg, VB₁₂ 0.1 mg, niacin 42 mg, D-calcium pantothenate 10 mg, folic acid 1 mg, biotin 15 mg, Fe 80 mg, Cu 8 mg, Mn 80 mg, Zn 85 mg, I 7 mg, Se 15 mg; Premix provided the following per kilogram of diets for the grower-finisher period: VA 9,000 IU, VD₃ 500 IU, VE 35 IU, VK 2.2 mg, VB₁ 2.4 mg, VB₂ 5 mg, VB₆ 6 mg, VB₁₂ 0.07 mg, niacin 35 mg, D-calcium pantothenate 18 mg, folic acid 7 mg, biotin 1 mg, Fe 80 mg, Cu 7 mg, Mn 60 mg, Zn 80 mg, I 0.60 mg, and Se 15 mg.

²Analyzed values based on triplicate determinations.

PC, positive control; NC, negative control.

each replicate pen, two birds per each replicate were selected and euthanized by CO₂ inhalation, and then killed by bleeding. The left breast and thigh meats were removed and weighed to determine the percentages of breast and thigh meats relative to live BW at processing. The breast and thigh muscles were sliced and weighed. The concentration of hydrogen ion was estimated using a microprocessor pH meter (model pH 211; Hanna Instruments, Woonsocket, RI), which was set into incisions on the cranial left side of the muscles. Two measurements were recorded, and the mean pH value of the muscles of each carcass was calculated. Hunter L* (lightness), a* (redness), and b* (yellowness), of the meat were measured using a Minolta CR410 chromameter (Konica Minolta Sensing, Osaka, Japan) in two different fields of the internal face of the cranial position of the post-mortem. Segments of about 1.5 cm from the middle of the duodenum and jejunum were excised and flushed with ice-cold saline and immediately placed in 4% paraformaldehyde for morphometric analysis. The indices of villus height, crypt depth, and muscular thickness were measured by computer-aided light microscope image analysis. Excreta samples were collected per cage over three consecutive days (from days 54 to 56) for

determination of nutrient digestibility. Multiple subsamples from three consecutive days were pooled, homogenized, and stored in airtight containers at 4°C until further analysis. Diets and excreta samples were analyzed for DM, GE, CP, and AAs.

Sample Analyses

Measurement of DM was performed according to the Association of Official Analytical Chemists standard procedures (AOAC 930.15; AOAC, 2007) at 135°C for 2 h. GE values were measured using an isoperibol oxygen bomb calorimeter (Kalorimeter C7000 processo, IKA, Staufen, Germany). Determination of CP content was performed with the Kjeldahl method (method 984.13; AOAC, 2007) on a Kjeltac TM 8400 apparatus (FOSS Inc., Eden Prairie, MN, United States). One xylanase unit was defined as the amount of enzyme that releases 48 µmol of reducing sugar as xylose from wheat arabino xylan per minute at pH 4.2 and 50°C. One protease unit was defined as the amount of enzyme that releases 1 µg of phenolic compound, expressed as tyrosine equivalents, from a casein substrate per minute at pH 7.5 and 40°C. TiO₂ concentrations were determined in triplicate for diets and digesta samples, respectively, with the colorimetric method.

TABLE 2 | Effects of NSP enzymes and protease on growth performance of yellow-feathered broilers fed with diets with different CP levels during days 1–56.

Item	PC ¹		NC ¹		SEM	Dietary CP ²		SEM	Enzyme treatment ^{2,3}		SEM	P-value		
	Control	Enzyme	Control	Enzyme		PC	NC		Control	Enzyme		CP	Enzyme	CP × enzyme
Days 1–21														
BW, g/bird	414.5	431.1	407.5	424.3	2.85	422.8 ^a	415.9 ^b	2.01	411 ^b	427.7 ^a	2.01	0.03	<0.0001	0.97
ADG, g/bird/day	19.8	19.9	18.7	19.6	0.14	19.9 ^a	19.1 ^b	0.10	19.2 ^b	19.7 ^a	0.10	0.01	<0.0001	0.86
ADFI, g/bird/day	43.6	41.65	42.3	41.4	1.09	42.63	41.84	0.77	42.9	41.5	0.77	0.48	0.21	0.63
F/G	2.20	2.09	2.26	2.12	0.06	2.19	2.19	0.04	2.28 ^a	2.11 ^b	0.04	0.95	0.007	0.68
Days 22–56														
BW, g/bird	2,405	2,489	2,361	2,401	14.0	2,447 ^a	2,381 ^b	9.92	2,383 ^b	2,445 ^a	9.90	0.0001	0.0003	0.14
ADG, g/bird/day	56.9	58.8	55.8	56.5	0.41	57.8 ^a	56.1 ^b	0.29	56.3 ^b	57.6 ^a	0.29	0.0005	0.005	0.14
ADFI, g/bird/day	130.5	129.2	132.4	128.5	1.93	129.9	130.5	1.36	131.4	128.8	1.36	0.76	0.19	0.53
F/G	2.29	2.20	2.37	2.28	0.035	2.25 ^b	2.32 ^a	0.025	2.33 ^a	2.23 ^b	0.03	0.04	0.01	0.96
Days 1–56														
ADG, g/bird/day	43.1	44.7	42.3	43.1	0.25	43.9 ^a	42.7 ^b	0.18	42.7 ^b	43.9 ^a	0.17	0.001	0.002	0.14
ADFI, g/bird/day	97.9	96.4	98.6	95.8	1.51	97.15	97.2	1.07	98.3 ^a	96.1 ^b	1.07	0.96	0.17	0.71
F/G	2.27	2.16	2.33	2.23	0.035	2.22	2.28	0.025	2.30 ^a	2.20 ^b	0.03	0.08	0.007	0.91

^{a,b}Within a column, means without a common superscript differ ($P < 0.05$).

^{1,2,3}Data represent the means of 6 and 12 replicates ($n = 6$ and 12).

PC, positive control with normal CP level; NC, negative control with subnormal CP level; ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; F/G, feed:gain ratio; for enzyme treatment, the basal diets were supplemented without or with a combination of NSP enzymes and protease supplementation that was supplied with 16,000 U of xylanase, 1,200 U of β -glucanase, and 1,600 U of protease per kg diet.

The nutrient digestibility of DM, GE, CP, and AAs of the diets were calculated according to the following formulas:

$$\text{Nutrient digestibility (\%)} = \{1 - [(TiO_2 \text{ feed} / TiO_2 \text{ excreta}) \times (\text{Nutrient}_{\text{excreta}} / \text{Nutrient}_{\text{feed}})]\} \times 100.$$

Statistical Analyses

All the data were analyzed by two-way ANOVA using the general linear model procedure of SAS 9.2 (SAS Institute, 2010). The model included the main effects of dietary CP levels, dietary enzyme treatments, and their interactions. Either one replicate cage or one broiler served as an experimental unit, and the values presented in the Tables are means with pooled standard error of mean (SEM). Differences among the means were tested with the LSD method, and statistical significance was set at $P < 0.05$.

RESULTS

Growth Performance and Carcass Traits

The results of growth performance and carcass traits are shown in **Tables 2, 3**. The NC group had lower ($P < 0.05$) final BW and ADG of broilers on days 1–21, 22–56, and 1–56 and higher ($P < 0.05$) F/G on days 22–56 than the PC group. Compared with the control group, the combination of dietary NSP enzymes and protease increased ($P < 0.05$) the final BW and ADG of broilers on days 1–21, 22–56, and 1–56 but decreased ($P < 0.05$) the F/G ($P < 0.05$) on days 22–56. Dietary CP levels and enzyme supplementation had no effect ($P > 0.05$) on ADFI on days 1–21, 22–56, and 1–56 and on the percentage of dressing, eviscerated yield, breast muscle, thigh muscle, and abdominal fat on day 56. There were no significant interactions ($P > 0.05$) between dietary CP levels and enzyme supplementation in all the above-mentioned indices.

Meat Quality

The results of meat quality are shown in **Table 4**. The broilers fed with the NC diet had higher ($P < 0.05$) L* and b* values in the thigh muscle of the broilers on day 56 than those fed the PC diet. Dietary NSP enzyme and protease supplementation increased ($P < 0.05$) pH values and had no effect ($P > 0.05$) on L* and b* values in both breast and thigh muscles of the broilers on day 56. There were no significant interactions ($P > 0.05$) between dietary CP levels and enzyme supplementation in the measured indices related to meat quality.

Intestinal Histomorphology

The results of intestinal histomorphology are shown in **Table 5**. Broilers fed with the NC diet had lower ($P < 0.05$) villus height, musculature thicknesses, villus height: crypt depth, and higher crypt depth ($P < 0.05$) in the duodenum of broilers on day 56 than those fed with the PC diet. The broilers fed with the NC diet had lower ($P < 0.05$) musculature thickness in the jejunum on day 56 than those fed with the PC diet. Dietary CP level had no effect ($P > 0.05$) on villus height, crypt depth, and villus height: crypt depth in the jejunum. Dietary enzyme

TABLE 3 | Effects of NSP enzymes and protease on carcass traits of yellow-feathered broilers fed with diets with different CP levels on day 56.

Item, %	PC ¹		NC ¹		SEM	Dietary CP ²		SEM	Enzyme treatment ^{2,3}		SEM	P-value		
	Control	Enzyme	Control	Enzyme		PC	NC		Control	Enzyme		CP	Enzyme	CP x enzyme
Dressing	76.7	75.69	75.64	76.54	0.97	76.20	76.09	0.69	76.17	76.12	0.69	0.91	0.95	0.34
Eviscerated yield	62.02	62.08	62.11	62.24	1.05	62.05	62.175	0.74	62.07	62.16	0.74	0.72	0.78	0.62
Breast muscle	14.87	13.90	13.27	13.52	0.94	14.39	13.395	0.72	14.07	13.71	0.72	0.33	0.55	0.41
Thigh muscle	19.1	19.36	18.97	17.49	1.02	19.23	18.23	0.69	19.04	18.43	0.67	0.30	0.71	0.53
Abdominal fat	2.98	2.54	2.21	2.32	0.41	2.76	2.265	0.29	2.60	2.43	0.24	0.24	0.68	0.51

^{1,2,3}Data represent the means of 6 and 12 replicates ($n = 6$ and 12).

PC, positive control with normal CP level; NC, negative control with subnormal CP level; for enzyme treatment, the basal diets were supplemented without or with a combination of NSP enzymes and protease supplementation that was supplied with 16,000 U of xylanase, 1,200 U of β -glucanase, and 1,600 U of protease per kg diet.

TABLE 4 | Effects of NSP enzymes and protease on meat quality of yellow-feathered broilers fed with diets with different CP levels on day 56.

Item	PC ¹		NC ¹		SEM	Dietary CP ²		SEM	Enzyme treatment ^{2,3}		SEM	P-value		
	Control	Enzyme	Control	Enzyme		PC	NC		Control	Enzyme		CP	Enzyme	CP × enzyme
Breast muscle														
L*	55.52	56.1	55.5	52.6	1.83	55.8	54.1	0.74	55.5	54.4	1.29	0.36	0.52	0.35
a*	8.37	8.81	8.24	7.61	0.81	8.59	7.93	0.72	8.31	8.21	0.57	0.42	0.9	0.52
b*	2.69	2.64	2.42	2.72	0.76	2.66	2.57	0.69	2.56	2.68	0.53	0.90	0.87	0.81
pH	6.75	7.20	6.62	7.12	0.08	6.975	6.87	0.29	6.69 ^b	7.16 ^a	0.06	0.24	<0.0001	0.80
Thigh muscle														
L*	54.9	56.0	59.6	58.1	1.44	55.49 ^b	58.83 ^a	0.89	57.3	57.1	0.89	0.01	0.87	0.33
a*	9.16	9.82	11.77	10.29	0.71	9.49	11.03	0.47	10.47	10.06	0.47	0.15	0.95	0.97
b*	1.97	2.11	2.93	2.04	0.67	2.04 ^b	2.49 ^a	0.50	2.45	2.08	0.50	0.04	0.57	0.15
pH	6.87	6.97	6.72	7.07	0.08	6.92	6.90	0.06	6.80 ^b	7.02 ^a	0.06	0.75	0.01	0.18

^{a,b}Within a column, means without a common superscript differ ($P < 0.05$).

^{1,2,3}Data represent the means of 6 and 12 replicates ($n = 6$ and 12).

PC, positive control with normal CP level; NC, negative control with subnormal CP level; L*, lightness; a*, redness; b*, yellowness; for enzyme treatment, the basal diets were supplemented without or with a combination of NSP enzymes and protease supplementation that was supplied with 16,000 U of xylanase, 1,200 U of β -glucanase, and 1,600 U of protease per kg diet.

TABLE 5 | Effects of NSP enzymes and protease on intestinal histomorphology of yellow-feathered broilers fed with diets with different CP levels on day 56.

Item, μm	PC ¹		NC ¹		SEM	Dietary CP ²		SEM	Enzyme treatment ^{2,3}		SEM	P-value		
	Control	Enzyme	Control	Enzyme		PC	NC		Control	Enzyme		CP	Enzyme	CP \times enzyme
Duodenum														
Villus height	887	879	723	814	50	883 ^a	769 ^b	35	805	847	35	0.03	0.41	0.33
Crypt depth	146	154	174	172	9.9	150 ^b	173 ^a	7	160	163	7	0.03	0.75	0.62
Musculature thicknesses	174	190	152	151	14.3	182 ^a	152 ^b	10	163	171	10	0.04	0.63	0.56
Villus height: Crypt depth	6.10	5.93	4.22	4.74	0.42	6.02 ^a	4.48 ^b	0.29	5.16	5.34	0.29	0.001	0.69	0.42
Jejunum														
Villus height	952	911	907	969	45	932	938	33	930	940	33	0.95	0.87	0.29
Crypt depth	155	153	167	162	13.2	154	165	10	161	158	10	0.41	0.79	0.92
Musculature thicknesses	198	196	178	155	0.67	197 ^a	167 ^b	14	188	176	14	0.04	0.42	0.48
Villus height: Crypt depth	6.37	6.10	5.47	6.07	0.43	6.24	5.77	0.31	5.92	6.09	0.31	0.27	0.74	0.33

^{a,b}Within a column, means without a common superscript differ ($P < 0.05$).

^{1,2,3}Data represent the means of 6 and 12 replicates ($n = 6$ and 12).

PC, positive control with normal CP level; NC, negative control with subnormal CP level; for enzyme treatment, the basal diets were supplemented without or with a combination of NSP enzymes and protease supplementation that was supplied with 16,000 U of xylanase, 1,200 U of β -glucanase, and 1,600 U of protease per kg diet.

supplementation and interactions between dietary CP levels and enzymes supplementation ($P > 0.05$) did not influence the measured indices of intestinal histomorphology.

Nutrient Utilization

The results of nutrient utilization are shown in **Table 6**. Dietary CP levels affected ($P < 0.05$) DM digestibility but had no effect ($P > 0.05$) on the digestibility of GE and CP. Dietary enzyme supplementation influenced ($P < 0.05$) the digestibility of DM, GE, CP, and most AAs. There were significant interactions ($P < 0.05$) between dietary CP levels and enzyme in digestibility of DM, CP, and some AAs (Asp, Ile, and Leu). The broilers fed with the NC diet had higher ($P < 0.05$) DM digestibility than those fed with the PC diet. The combination of dietary NSP enzyme and protease increased ($P < 0.05$) the digestibility of DM, GE, CP, and most AAs. Dietary enzyme supplementation exhibited greater improvements in the digestibility of DM, CP, and some AAs (Asp, Ile, and Leu) of broilers in NC group than in the PC group. No significant interactions ($P > 0.05$) between dietary CP levels and enzyme supplementation were observed in other indices mentioned above.

DISCUSSION

The efficacy of exogenous enzymes in poultry has been previously reported, and there was considerable evidence to support their beneficial effects on poultry productivity (2, 20). In the current study, the relative responses of feed exogenous enzymes were assessed in yellow-feathered broilers fed with diets with different dietary CP levels, namely, normal and suboptimal CP levels. Several studies have suggested that reducing CP levels in diets with adequate supplementation of indispensable amino acids could not completely compromise the growth performance of broilers (21–23). The NC diet was expected to decrease the final BW and ADG on days 1–21, 22–56, and 1–56 compared to the PC diet. When the broilers consumed a feed intake similar to the two different CP diets, birds fed with the NC diet had lower total CP and essential AA intake than those fed with the PC diet, and the poor performance could be attributed to possible deficiency in essential AAs. In addition, the broilers fed with the NC diet had greater F/G than those fed with the PC diet on days 22–56, but no differences in F/G were observed between PC and NC diets on days 1–21 and 1–56. The different aged responses to the nutritional levels of diets were agreed with previous study (24). One explanation was that endogenous enzymatic activities and the microbial community in the digestive tract might not be completely developed in the early stage, which could be due to lack of significant growth response of broilers fed with diets with different nutritional densities (18, 25). In the current study, a combination of NSP enzymes and protease supplementation significantly improved the growth performance of broilers fed with both the PC and NC diets regardless of CP levels on days 1–21, 22–56, and 1–56. The positive results were in agreement with those reported in chick broilers (7) and ducks (26). However, they were inconsistent with studies that reported that mixture enzyme additions had no impact or negative impact on performance variables (17, 27). The varied effects of enzyme treatments on

growth performance could depend on enzymes sources and activities, dietary compositions, and specific substrates and age of birds. The nutritional quality of a diet was probably an important factor that influenced the efficiency of an enzyme product. Responses to exogenous enzyme product additions were expected to be greater in diets with lower nutritional quality (12–14). In the present study, no interaction between dietary CP level and supplemental enzyme mixture level was observed in growth performance. One explanation could be that the extent of CP reduction in the NC diet could have possibly elicited the greater responses of enzyme addition to promote growth.

The carcass traits results in the current study indicated that lowering the CP levels in diets had no effect on the relative percentage of breast and thigh muscles and abdominal fat of yellow-feathered broilers, which was similar to the previous finding (28). However, other studies have shown a significant increase in abdominal fat deposition when broilers or ducks were fed with low-CP diets (29, 30). Our study demonstrated that the light and yellow colors of thigh muscles were influenced by dietary CP levels. The greater amount of meat light color reflected a greater degree of protein denaturation and a lower heme pigment concentration in broilers fed with NC diets. Twenty-hours after slaughtering, supplemental dietary enzymes increased the pH values of breast and thigh muscles in broilers on day 56, which was in association with decreased drip loss of the meat. In contrast, other authors have reported that commercial enzyme supplementation was less effective in improving meat quality parameters of broiler chickens (31, 32). The effects of enzyme treatments were inconsistent and varied because of pre-slaughter responses to stress, storage time and temperature, and glycogen reserves at slaughter. Therefore, further studies are needed to determine the effects of enzyme supplementation on the meat quality of broiler chickens.

It is well-documented that exogenous enzyme products could play great role in enhancing the feeding value of poultry diets with different nutritional qualities (3, 5). In the present study, broilers given the NC diet had lower DM digestibility than birds given the PC diet, but there was no effect on GE and CP digestibility. The decreased DM digestibility could be attributed to the lower villus height and villus height:crypt depth ratio of the duodenum in broilers fed with the NC diet. However, dietary nutrient density had no effect on the digestibility of nitrogen, calcium, phosphorus, and most amino acids in the broilers (12). The combination of NSP enzymes and protease supplementation was effective in increasing the digestibility of DM, GE, CP, and most AA of the corn-soybean basal diet used in our study, attributing to chick growth more efficiently (33). The beneficial effects noted in the current study were in agreement with previous studies (33). The NSP content of feeds could impair nutrient digestibility, both directly because of physical hindrance and indirectly because of physiological changes in the gut such as increased digesta viscosity (26). Xylanase and glucanase could degrade cell wall structures and then release encapsulated nutrients most likely contributing to overall improvements in nutrient utilization by reducing the digesta viscosity and increasing the accessibility to digestive enzymes. Then, protease contributed to the hydrolysis of large protein

TABLE 6 | Effects of NSP enzymes and protease on digestibility of DM, GE, CP, and amino acids of yellow-feathered broilers fed with diets with different CP levels.

Item, %	PC ¹		NC ¹		SEM	Dietary CP ²		SEM	Enzyme treatment ^{2,3}		SEM	P-value		
	Control	Enzyme	Control	Enzyme		PC	NC		Control	Enzyme		CP	Enzyme	CP x enzyme
DM	73.3 ^{bc}	74.7 ^a	71.0 ^c	74.1 ^a	0.32	74.0	72.5	0.69	72.2	74.4	0.69	0.0001	<0.0001	0.003
GE	76.4	79.8	76.2	80.0	0.24	78.1	78.1	0.74	76.3 ^b	79.9 ^a	0.74	0.87	<0.0001	0.36
CP	50.8 ^b	53.0 ^a	49.7 ^c	53.5 ^a	0.39	51.9	51.6	0.72	50.3	53.3	0.72	0.42	<0.0001	0.05
Asp	53.3 ^{bc}	56.2 ^a	52.1 ^c	57.7 ^a	1.16	54.8	54.9	0.82	52.7	56.9	0.82	0.35	<0.05	0.04
Thr	57.1	62.2	57.2	64.0	1.48	59.6	60.6	0.60	57.1 ^b	63.1 ^a	0.60	0.47	0.0002	0.41
Ser	58.2	56.5	57.1	59.9	0.92	57.4	58.5	0.78	57.7 ^b	58.2 ^a	0.77	0.78	0.0006	0.59
Glu	56.9	60.0	57.4	60.3	1.78	58.4	58.9	1.06	57.2 ^b	60.1 ^a	1.06	0.66	0.004	0.46
Ala	48.2	53.5	49.1	52.9	1.45	50.8	51.0	0.95	48.6 ^b	53.2 ^a	0.95	0.58	<0.0001	0.41
Val	57.6	59.2	58.3	60.4	2.29	58.4	59.4	1.58	58.0 ^b	59.8 ^a	1.58	0.92	<0.0001	0.52
Ile	52.5 ^c	56.3 ^b	53.7 ^c	59.4 ^a	1.30	54.4	56.6	0.63	53.1	57.9	0.63	0.74	<0.0001	0.01
Leu	59.9 ^b	62.0 ^a	56.5 ^c	62.4 ^a	1.80	60.9	59.5	1.28	58.2	62.2	1.28	0.002	0.03	0.001
Tyr	64.8	67.6	62.7	66.3	1.59	66.2 ^a	64.5 ^b	0.84	63.7 ^b	66.9 ^a	0.84	0.00	0.00	0.82
Phe	59.4	59.2	63.1	64.6	1.81	59.3	63.9	0.99	61.3	61.9	0.99	0.72	0.56	0.76
Lys	56.4	60.0	55.5	58.7	2.37	58.2	57.1	1.38	56.0 ^b	59.3 ^a	1.38	0.19	0.00	0.70
His	59.8	57.4	59.0	59.6	2.91	58.6	59.3	1.77	59.4	58.5	1.77	0.49	0.62	0.46
Arg	57.1	62.3	59.4	64.6	1.64	59.7	62.0	1.07	58.3 ^b	63.4 ^a	1.08	0.38	0.00	0.85
Pro	61.2	64.6	60.9	63.7	1.28	62.9	62.3	0.81	61.0 ^b	64.2 ^a	0.81	0.41	<0.0001	0.52

^{a,b,c} Within a column, means without a common superscript differ ($P < 0.05$).

^{1,2,3} Data represent the means of 6 and 12 replicates ($n = 6$ and 12).

PC, positive control with normal CP level; NC, negative control with subnormal CP level; CP, crude protein; GE, gross energy; for enzyme treatment, the basal diets were supplemented without or with a combination of NSP enzymes and protease supplementation that was supplied with 16,000 U of xylanase, 1,200 U of β -glucanase, and 1,600 U of protease per kg diet.

molecules into more absorbable peptides and AAs, enhancing the overall digestion and absorption of CP and AAs (34). Additionally, the beneficial effects on CP and AA digestibility could be mediated by reduction in secretion of amino acid-rich endogenous proteins. Moreover, exogenous NSP enzymes and protease could work together to provide a proper condition for the action of exogenous amylase on starch, which can result in increased DM and energy digestibility (13). Interestingly, there was no effect of enzyme supplementation on feed intake and intestinal histomorphology of the duodenum and jejunum, suggesting that the observed performance responses were due to changes in the digestibility of energy and CP rather than nutrient intake. These findings were in accordance with those who also reported no effect of addition of a mixture of enzymes on the digestibility of energy and CP in the starter phase of broiler chickens (18, 27). The inconsistent and varied effects of enzyme products depended on dietary nutritional composition and levels, enzyme product profile, and age of the birds. Responses to exogenous enzyme addition were expected to be greater in a diet with low-nutrient density than one that provided higher nutrient density to the animals at or above requirements (12–14). Previous study have showed that the combined addition of pectinase, protease, and amylase significantly improved AMEn when added to a corn soybean meal basal diet with lower energy and protein levels (16, 17). In the current study, an interaction between dietary CP level and enzymes was present for the digestibility of DM, CP, and some AAs (Asp, Ile, and Leu). The increased digestibility of DM, CP, and some AAs (Asp, Ile, and Leu) by supplemental enzymes was more apparent in birds fed with the NC diet with reduced CP concentration. It is noteworthy that DM and Leu digestibility of birds fed with the enzyme-supplemented NC diet was restored to the similar level in birds fed with the PC diet. The different responses of enzymes to dietary CP levels offered more flexibility in the use of enzymes for corn-soybean-based meal diets to reduce feed cost in poultry production.

In conclusion, the dietary subnormal CP level without essential AA supplementation impaired the growth performance and meat color of the thigh muscle of yellow-feathered broilers on day 56. The combination of NSP enzymes and protease

supplementation effectively improved growth performance, pH value of muscle meat, and the nutritional value of a corn-soybean-based meal diet of yellow-feathered broilers. The magnitude of the responses to exogenous enzyme addition was greater in diets with subnormal nutritional CP level in terms of the digestibility of DM, CP, and some AAs.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by South China Agricultural University (SCAU-10564). Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

CW and TY conceived and designed research. JY, WZ, and QW was responsible for data analysis and interpretation. KZ and XM interpreted the results. LW and KN was responsible for manuscript revision. YZ and XL wrote the paper. All authors contributed to the article and approved the submitted version.

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Predicting the metabolizable energy and metabolizability of gross energy of conventional feedstuffs for Muscovy duck using in vitro digestion method

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Abstract

In total, two experiments were conducted to evaluate the effectiveness of an in vitro digestion method for predicting the metabolizable energy (ME) and metabolizability of gross energy (ME/GE) values using in vitro digestible energy (IVDE) and the digestibility of gross energy (IVDE/GE) content, respectively, of conventional feedstuffs for Muscovy ducks. In experiment 1, the apparent metabolizable energy (AME), true metabolizable energy (TME), AME/GE, and TME/GE of eight-grain feedstuff samples (two corn samples, three sorghum samples, and three barley samples) and eight protein feedstuff samples (two soybean meal samples, three cottonseed meal samples, and three rapeseed meal samples) were determined by the tube-feeding method with six different ducks for each sample. In experiment 2, a computer-controlled simulated digestion system (CCSDS) containing simulated digestive fluid was used to determine the enzymatic hydrolysis energy value of feedstuffs, which was defined as IVDE in our study. The simulated gastric fluid containing pepsin and simulated small intestinal fluid containing amylase, trypsin, and chymotrypsin for the in vitro gastric and intestinal digestion, respectively. The IVDE and in vitro digestibility of GE (IVDE/GE) of 16 feedstuff samples were determined using the CCSDS with five replicates per sample. The results showed that the IVDE and IVDE/GE were positively correlated with ME and ME/GE of feedstuffs, respectively. The coefficient of determination of eight regression models in predicting ME (grain feedstuffs: $AME = 1.050 \times IVDE - 0.9293$, $TME = 1.032 \times IVDE + 0.6478$; protein feedstuffs: $AME = 1.331 \times IVDE - 6.685$, $TME = 1.269 \times IVDE - 3.490$) and ME/GE (grain feedstuffs: $AME/GE = 1.069 \times IVDE/GE - 6.516$, $TME/GE = 1.068 \times IVDE/GE + 0.7764$; protein feedstuffs: $AME/GE = 1.093 \times IVDE/GE - 19.21$, $TME/GE = 1.196 \times IVDE/GE - 13.25$) of feedstuffs for Muscovy ducks ranged from 0.8610 to 0.9921. The accuracy of the regression model was acceptable as the difference between measured and predicted ME and ME/GE values was less than 0.45 MJ/kg (100 kcal/kg) and 2.62% for 14 of the 16 feed samples, respectively. In conclusion, the in vitro digestion method can be used to predict the ME and ME/GE of conventional feedstuffs for Muscovy ducks with acceptable accuracy.

Lay Summary

Metabolizable energy (ME) is one of the major factors in formulating diets for ducks and most studies on the ME values of ingredients have been conducted on Peking ducks, with limited research on Muscovy ducks. Compared with the time-consuming in vivo digestion method, in vitro simulating digestion as a rapid and reliable method has been performed to predict ME and metabolizability of gross energy. Therefore, the precision of the in vitro digestion method was evaluated for Muscovy duck feed in our study.

Key words: in vitro digestion method, metabolizable energy, metabolizability, Muscovy duck.

Abbreviations: AME, apparent metabolizable energy; CCSDS, computer-controlled simulated digestion system; CSM, cottonseed meal; CV, coefficient of variation; DM, dry matter; GE, gross energy; IVDE, in vitro digestible energy; IVDE/GE, in vitro digestibility of gross energy; ME, Metabolizable energy; ME/GE, metabolizability of gross energy; R², coefficient of determination; RSM, rapeseed meal; SBM, soybean meal; TME, true metabolizable energy

Introduction

In 2020, China Animal Agriculture Association estimated that the annual slaughter of meat ducks was 4.68 billion, of which Muscovy ducks account for about 267 million (Hou and Liu, 2021). Metabolizable energy (ME) is one of the major factors in formulating diets for ducks. However, most studies on the ME values of ingredients have been conducted on Peking ducks (Hong et al., 2002; Adedokun and Adeola, 2005; Zhao et al., 2008) and limited research was available

on Muscovy ducks (Hoai et al., 2011; Coluccy et al., 2015). The capacity to utilize the nutrients varied due to the difference in digestive physiology between breeds and ages of ducks (Elkin, 1987). For example, the different digestive enzyme activities between Muscovy ducks and Peking ducks obtained in our lab, such as amylase (3,380 vs. 4,417 U/mL), trypsin (601 vs. 1,196 U/mL), and chymotrypsin (274 vs. 429 U/mL) (Wang et al., 2021) might lead to the difference in the digestibility of nutrients in the diet. Therefore, the recommended

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energy requirements and ME values of ingredients based on Peking ducks were not suitable to formulate diets in Muscovy ducks for optimal production performance and economic efficiency. The traditional true metabolizable energy (TME) assay proposed by [Sibbald \(1976\)](#) was used to determine the ME of feedstuffs in the past few decades ([Dudley-Cash, 2009](#)). The TME assay requires an adaptation period for 5 d and a fasting period for 36 h before tube feeding, and a 36-h excrement collection period after tube feeding with at least three participants, displaying the disadvantages of time-consuming and laborious ([Yegani et al., 2013](#)). Therefore, an in vitro simulating digestion as a rapid and reliable method has been performed to predict energy digestibility in pigs ([Noblet and Jaguelin-Peyraud, 2007](#); [Regmi et al., 2008, 2009](#)) and chickens ([Losada et al., 2009, 2010](#)). In vitro method using a computer-controlled simulated digestion system (CCSDS) has been developed to mimic the stomach-intestinal digestion of ducks, which are included in the simulated the temperature, pH as well as concentrations of digestive enzymes and ions. The CCSDS system requires total 25 h and only one person to complete the measurement of the energy value of feeds for ducks following the automatic operation according to the program. However, most of the existing in vitro digestion literatures were focused on Peking ducks, few studies were performed on Muscovy ducks. Based on the in vitro digestion model in our lab, the feasibility of an in vitro CCSDS method was conducted to predict ME and metabolizability of gross energy (ME/GE) values of feedstuffs for Muscovy duck in the present study.

Materials and methods

The animal care and use protocol was approved by the Institutional Animal Care and Use Committee of South China Agricultural University (SCAU-10564), and the study was performed following the Regulations for the Administration of Affairs Concerning Experimental Animals.

Ingredients and experimental design

A total of 16 samples of six different plant feedstuffs obtained from different provinces in China were applied in our study. Including three energy sources of grain (corn, sorghum, and barley) and three plant protein sources (soybean meal [SBM], cottonseed meal [CSM], and rapeseed meal [RSM]). Two local varieties of corn were collected from Shandong and Henan provinces, respectively. Grain sorghum (sorghum A), sweet sorghum (sorghum B), and red sorghum (sorghum C) were collected from Shandong, Zhejiang, and Anhui provinces, respectively. Three different colored naked barley (black, yellow, and white) were collected from Liaoning, Shandong, and Hebei provinces, respectively. Two SBM samples were collected from conventional or generic soybean lines in Shandong and Hubei Provinces, respectively. CSM samples with high (CSM A), medium (CSM B), and low (CSM C) gossypol content was collected from Hebei, Shandong, and Xinjiang, respectively. RSM A (one zero normal RSM) and RSM C (normal RSM) samples were collected from Hebei and Heilongjiang province, respectively, and the RSM B sample (double zero RSM) containing the low levels of glucosinolates and erucic acid were collected from Shandong province. All the samples were ground through a 0.5-mm screen to ensure a fine particle size, and each sample was analyzed in triplicate for GE, crude protein, ether extract, starch, ash, neutral deter-

gent fiber, and acid detergent fiber. In experiment 1, apparent metabolizable energy (AME), TME, AME/GE, and TME/GE of feedstuff samples in the present study were determined with six different Muscovy ducks per sample by the tube-feeding method ([McNab and Blair, 1988](#)). Each feedstuff was treated as an individual experiment. The grain feedstuffs such as corn, sorghum, and barley were fed as the only dietary ingredient, while the plant protein feedstuffs such as SBM, CSM, and RSM were tested as part of a complete diet (60% corn starch + 40% test ingredient). In experiment 2, in vitro digestible energy (IVDE) and metabolizability of GE (IVDE/GE) were determined with five replicates of each sample according to the procedure described by [Zhao et al. \(2014a\)](#).

Tube-feeding assay in vivo

In experiment 1, 96 male Muscovy ducks of 20-wk-old were weighed individually (4.20 ± 0.24 kg) and assigned to determine the AME and TME of 16 feedstuffs and the remaining 12 ducks were assigned to estimate of endogenous energy losses. In brief, the threaded plastic retainer rings with a 4.3 cm diameter hole in the center were sutured on the skin around the vent through 12 2-mm holes around the periphery before the tube-feeding experiment ([Adeola et al., 1997](#)). And preweighed ducks were housed in individual cages (0.50 m length \times 0.42 m width \times 0.55 m height) and the cages were maintained in an environmentally controlled room (25 °C) under a 24-h light cycle for a 7-d adaptation period. After that, at 36 h after feed withdrawal, a 60 g test diet was delivered to the ducks' crop by a tube-feeding apparatus consisting of a 60-mL catheter-tip syringe and a 35-cm long NalgeneTM tubing with an inside diameter of 8 mm ([Ragland et al., 1997](#)). Finally, the excreta collection apparatus could screw onto the modified retainer ring attached to the duck with the threads of the ring after tube-feeding ([Adeola et al., 1997](#)) and the total excreta samples were collected during the next 36 h after being tube-feeding and the ducks for determining endogenous energy losses continued to be deprived of feed for 36 h to correct AME to TME. The total excreta dried at 55 °C for 48 h, next the excreta samples were ground through a 0.5-mm screen prior and then stored at -20 °C for the determination of GE.

In vitro digestion method

In experiment 2, a CCSDS containing simulated digestive fluid was used to determine the enzymatic hydrolysis energy value of feedstuffs, which was defined as IVDE in our study. The method has been reported to be used to correct the IVDE and IVDE/GE by regression models to predict ME and ME/GE of feedstuffs for rooster ([Zhao et al., 2014a](#)) and Peking ducks ([Zhao et al., 2014b](#); [Zhang et al., 2019](#)) and DE and DE/GE of feedstuffs for pig ([Regmi et al., 2008, 2009](#); [Chen et al., 2014](#)), respectively. The simulated gastric fluid containing pepsin and simulated small intestinal fluid containing amylase, trypsin, and chymotrypsin were used for the in vitro gizzard and small intestine digestion, respectively. Briefly, 2 g of the grain samples or 1 g of the plant protein samples and 20 mL simulated gastric fluid made of 1,550 U of pepsin/mL were mixed and incubated for 4 h at 42 °C and accompanied by the gastric buffer solution (16.9 mmol/L Na⁺, 26.5 mmol/L Cl⁻, and pH 2.0) ([Sturkie, 1976](#)). When the digestion process of the gastric phase was finished, the small intestinal buffer solution was circularly pumped into digestion chambers for 0.5 h, and then 2 mL simulated small intestinal fluid was

injected into each digestion chamber and the buffer solution continued to circulate for 15 h. The small intestinal buffer solution (104.39 mmol/L Na⁺, 51.25 mmol/L Cl⁻, and pH 7.79 for grain feedstuffs; 93.53 mmol/L Na⁺, 46.25 mmol/L Cl⁻, and pH 7.80 for protein feedstuffs) and simulated small intestinal fluid (amylase 307.26 U/mL, trypsin 54.68 U/mL, and chymotrypsin 24.90 U/mL for grain feedstuffs; amylase 381.88 U/mL, trypsin 72.84 U/mL, and chymotrypsin 11.98 U/mL for protein feedstuffs) were prepared with the optional digestive parameters for grain feedstuffs and plant protein feedstuffs aforementioned, respectively (Wang et al., 2021). In addition, 2 mL intestinal fluid was diluted by 20 mL neutral pepsin solution added in the gastric phase, therefore, the activities of enzymes in the simulated small intestinal fluid were equal to 11 times of those in the small intestinal fluid of Muscovy duck. After the simulated digestion process in vitro, the undigested residues from each replicate were predried to constant weight and then extracted fat by ethanol, finally, the defatted residues were dried at 105 °C to constant weight for the measurement of GE.

Sample collection and analysis

The dry matter of feedstuff samples and excreta samples in experiment 1 were determined according to the Association of Official Analytical Chemists standard procedures (method 934.01; AOAC, 1990) with 105 °C for 5 h. The GE values of feedstuff samples and excreta samples in experiment 1 and the defatted residues in Experiment 2 were measured using an isoperibol oxygen bomb calorimeter (Kalorimeter C7000 processo, IKA, Staufen, Germany). The crude protein, ash, and ether extract content of feedstuff samples in experiment 1 were analyzed according to the classical procedures of the Association of Official Analytical Chemists (method 954.01,

method 942.05, and method 920.39), respectively. The neutral detergent fiber and acid detergent fiber contents of feedstuff samples in experiment 1 were determined as described by Van Soest et al. (1991). The starch content of feedstuffs was determined using the commercial kit obtained from the Nanjing Jiancheng Bioengineering Institute (A148-1-1, Nanjing, China).

Calculation and statistical analysis

AME and TME for grain feedstuffs were calculated as follows:

$$\text{AME, MJ/kg} = (\text{EI} - \text{EO}) / \text{FI};$$

$$\text{TME, MJ/kg} = (\text{EI} - \text{EO} + \text{EEL}) / \text{FI};$$

AME and TME for protein feedstuffs were calculated as follows:

$$\text{AME, MJ/kg} = (\text{AME}_t \text{AME}_b \times b) / (1 - b);$$

$$\text{TME, MJ/kg} = (\text{TME}_t \text{TME}_b \times b) / (1 - b);$$

EI: gross energy of tube-feeding diets, MJ/kg; EO: gross energy of the excreta, MJ/kg; FI: feed intake, g; EEL: endogenous energy loss, MJ/kg; AME_t: AME of the complete diet (basal diet plus the ingredient); AME_b: AME of the basal diet; TME_t: TME of the complete diet (basal diet plus the ingredient); TME_b: TME of the basal diet; b, the proportion of the basal diet in the complete diet.

IVDE were calculated as follows:

Table 1. Analyzed composition of feedstuff samples in the present study (DM basis)

Items ¹	Gross energy, MJ/kg	Crude protein, %	Ether extract, %	Starch, %	Ash, %	Neutral detergent fiber, %	Acid detergent fiber, %
Grain feedstuffs							
Corn A	18.68	11.25	3.99	76.95	1.44	13.06	2.75
Corn B	18.73	10.31	4.15	77.81	1.92	9.56	2.47
Sorghum A	18.62	9.82	3.94	65.60	1.35	13.01	2.88
Sorghum B	18.45	10.02	3.82	67.72	1.26	21.46	3.44
Sorghum C	18.06	10.79	3.63	66.19	1.5	24.99	3.82
Barley A	17.51	9.81	1.77	36.70	2.67	40.73	5.79
Barley B	18.12	10.97	1.91	38.95	3.13	40.37	5.92
Barley C	18.16	11.74	1.88	39.67	2.43	40.78	5.76
Protein feedstuffs							
SBM A	17.82	47.18	1.94	3.85	6.61	24.49	11.92
SBM B	19.15	48.78	2.16	3.68	8.33	16.73	6.73
CSM A	18.78	32.94	0.64	2.21	7.11	43.49	18.6
CSM B	19.28	36.32	0.73	2.34	8.4	37.8	16.72
CSM C	19.2	37.17	0.68	2.03	7.17	34.77	12.98
RSM A	19.7	39.64	1.76	6.74	7.26	35.68	20.85
RSM B	19.81	41.01	1.65	6.21	7.16	34.05	22.24
RSM C	21.97	38.27	1.81	6.96	8.5	28.6	17.01

¹ The average data based on triplicate determinations. SBM, soybean meal; CSM, cottonseed meal; RSM, rapeseed meal.

Table 2. ME and ME/GE measured by *in vitro* and *in vivo* digestion methods (DM basis)

Items	<i>In vivo</i> method			<i>In vitro</i> method			AME- IVDE	TME- IVDE	AME/GE- IVDE/GE	TME/GE- IVDE/GE
	AME, MJ/kg ¹	CV	AME/ GE, % ¹	CV	TME, MJ/kg ¹	CV	IVDE, MJ/kg ²	CV	IVDE/ GE, % ¹	CV
Grain feedstuffs										
Corn A	14.63	1.85	78.32	1.85	15.93	1.70	14.91	0.29	79.82	0.29
Corn B	14.11	2.21	75.33	3.99	15.42	2.02	14.26	0.87	76.13	0.89
Sorghum A	13.02	3.67	69.92	2.05	14.44	3.35	13.45	1.50	72.23	1.06
Sorghum B	15.04	1.54	81.52	1.16	16.21	1.43	15.39	0.59	83.41	0.59
Sorghum C	14.77	3.07	81.78	3.22	16.23	2.80	14.52	1.88	80.40	0.80
Barley A	13.02	2.80	74.36	2.80	14.44	2.52	13.45	1.43	76.81	1.43
Barley B	13.54	3.44	74.72	3.44	14.97	3.11	13.75	0.66	75.88	0.66
Barley C	12.67	3.11	69.77	2.31	13.80	2.79	12.88	1.06	70.93	1.06
Protein feedstuffs										
SBM A	11.63	3.27	65.26	9.82	13.80	2.65	13.73	2.16	77.05	2.16
SBM B	12.28	4.01	64.13	8.65	14.96	3.78	14.42	1.50	75.30	3.41
CSM A	7.71	9.26	41.05	10.97	10.34	6.81	10.75	1.14	57.24	1.14
CSM B	9.67	7.68	50.16	13.53	12.46	6.07	12.50	1.46	64.83	1.46
CSM C	7.74	2.35	40.31	2.47	10.39	1.87	11.06	1.40	57.60	1.79
RSM A	9.59	5.57	48.68	6.37	12.25	4.36	12.35	0.93	62.69	0.93
RSM B	9.60	5.32	48.46	7.38	12.28	4.17	12.47	0.68	62.95	0.68
RSM C	11.24	4.66	51.16	6.87	12.33	3.81	12.57	1.42	57.21	0.93

¹ AME, apparent metabolizable energy; TME, true metabolizable energy. The values represent the mean of six replicates per sample (DM basis).² AME/GE, ratio of AME to GE, mean of six determinations per sample (DM basis).³ TME/GE, ratio of TME to GE, mean of six determinations per sample (DM basis).⁴ IVDE, in vitro digestible energy, mean of five determinations per sample (DM basis).⁵ IVDE/GE, in vitro digestibility of GE, mean of five determinations per sample (DM basis).

CV, coefficient of variation; SBM, soybean meal; RSM, rapeseed meal; CSM, cottonseed meal.

Table 3. Pearson correlation coefficients (*r*) between ME and IVDE and between ME/GE and IVDE/GE in grain and protein feedstuffs

Grain feedstuffs	<i>r</i>		Protein feedstuffs	<i>r</i>	
	IVDE, MJ/kg ²	IVDE/GE, % ⁵		IVDE, MJ/kg ²	IVDE/GE, % ⁵
AME, MJ/kg ¹	0.97**		AME ¹	0.95**	
TME, MJ/kg ¹	0.95**		TME ¹	0.99**	
AME/GE, % ³		0.97**	AME/GE ³		0.93**
TME/GE, % ⁴		0.95**	TME/GE ⁴		0.99**

* $P < 0.05$, ** $P < 0.01$.

¹ AME, apparent metabolizable energy; TME, true metabolizable energy. The values represent the mean of six replicates per sample (DM basis).

² IVDE, in vitro digestible energy, mean of five determinations per sample (DM basis).

³ AME/GE, ratio of AME to GE, mean of six determinations per sample (DM basis).

⁴ TME/GE, ratio of TME to GE, mean of six determinations per sample (DM basis).

⁵ IVDE/GE, in vitro digestibility of GE, mean of five determinations per sample (DM basis).

Table 4. Models for predicting ME from IVDE or ingredient composition and predicting the ME/GE from IVDE/GE

Items	Model	R ²	P value
Grain feedstuffs			
AME ¹ , MJ/kg	$1.050 \times \text{IVDE}^4 - 0.9293$	0.9445	< 0.001
AME ¹ , MJ/kg	$0.8229 \times \text{GE}^5 - 1.202$	0.1385	0.3640
AME ¹ , MJ/kg	$-0.06783 \times \text{CP}^6 + 14.57$	0.0028	0.9008
TME ¹ , MJ/kg	$1.032 \times \text{IVDE}^4 + 0.6478$	0.9097	< 0.001
TME ¹ , MJ/kg	$0.7323 \times \text{GE}^5 + 1.786$	0.1093	0.4239
TME ¹ , MJ/kg	$-0.1322 \times \text{CP}^6 + 16.58$	0.0107	0.8079
AME/GE ³ , %	$1.069 \times \text{IVDE/GE}^7 - 6.516$	0.9357	< 0.001
TME/GE ³ , %	$1.068 \times \text{IVDE/GE}^7 + 0.7764$	0.8960	< 0.001
Protein feedstuffs			
AME ¹ , MJ/kg	$1.331 \times \text{IVDE}^4 - 6.685$	0.9117	< 0.001
AME ¹ , MJ/kg	$1.329 \times \text{GE}^5 - 14.37$	0.1040	0.4359
AME ¹ , MJ/kg	$0.9952 \times \text{CP}^6 - 0.6050$	0.1744	0.3033
TME ¹ , MJ/kg	$1.269 \times \text{IVDE}^4 - 3.490$	0.9921	< 0.001
TME ¹ , MJ/kg	$1.357 \times \text{GE}^5 - 12.48$	0.1300	0.3802
TME ¹ , MJ/kg	$0.3799 \times \text{CP}^6 + 8.328$	0.0304	0.6795
AME/GE ³ , %	$1.093 \times \text{IVDE/GE}^7 - 19.21$	0.8610	0.002
TME/GE ³ , %	$1.196 \times \text{IVDE/GE}^7 - 13.25$	0.9881	0.002

¹ AME, apparent metabolizable energy; TME, true metabolizable energy. The values represent the mean of six replicates per sample (DM basis).

² AME/GE, ratio of AME to GE, mean of 6 determinations per sample (DM basis).

³ TME/GE, ratio of TME to GE, mean of 6 determinations per sample (DM basis).

⁴ IVDE, in vitro digestible energy, mean of 5 determinations per sample (DM basis).

⁵ GE, gross energy, mean of 5 determinations per sample (DM basis).

⁶ CP, crude protein, mean of 5 determinations per sample (DM basis).

⁷ IVDE/GE, in vitro digestibility of GE, mean of 5 determinations per sample (DM basis).

R², coefficient of determination.

$$\text{IVDE, MJ/kg} = [(\text{sample DM weight} \times \text{sample GE}) / - (\text{defatted residue DM weight} \times \text{defatted residue GE})] / \text{sample DM weight};$$

Correlation analysis and the linear regression models between ME and IVDE and between ME/GE and IVDE/GE were estimated using the GraphPad Prism version 8.3.0 (GraphPad Software, USA). The difference between the determined and predicted values of ME or ME/GE using

t-test procedure (SAS Inst. Inc., Cary, NC), in accordance with methods for testing TME additivity (Sibbald, 1977; Hong et al., 2001). * $P < 0.05$, ** $P < 0.01$.

Results and discussion

Several studies have shown that in vitro simulated digestion method have been applied to predict the ME/GE of feedstuffs for chickens (Weurding et al., 2001; Zhao et al., 2014b) and Peking ducks (Zhao et al., 2014b; Zhang et al., 2019), and to predict the DE/GE for pigs (Regmi et al., 2008, 2009; Park et al., 2012; Pan et al., 2018). Zhao et al. (2014a) reported that the in vitro simulated digestion method could be used to accurately predict the AME or TME of feed for poultry with reproducibility and additivity. However, most of the existing literature on in vitro digestion was on pigs (Chen et al., 2014), chickens (Zhao et al., 2014a), and Peking ducks (Zhao et al., 2014b; Zhang et al., 2019), limited studies were performed on Muscovy ducks. Therefore, based on in vitro digestion parameters of Muscovy ducks in our lab (Wang et al., 2021), this study aimed to investigate the feasibility of predicting ME and ME/GE of feedstuffs for Muscovy ducks using the in vitro simulated digestion method. GE, crude protein, ash, starch, ether extract, neutral detergent fiber, and acid detergent fiber of 16 feedstuffs were presented in Table 1. In our study, the excreta were collected from a plastic retainer ring sutured on the skin around the vent according to the procedure described by Adeola et al. (1997), which was due to ducks that consume water in much greater quantities than chickens (Siregar and Farrell, 1980) and the result is highly liquid excreta. Traditional excreta collection methods utilizing collection pans are subject to errors from contamination with feathers and losses due to splatter when the forcefully ejected excreta make contact with the collection pan. Therefore, the consequences of utilizing pan collection are sample loss and contamination of sample with orts, dander, and feathers, resulting in less accurate estimates of bioavailable nutrients. Moreover, the excreta collection device glued around the cloaca and retained by a custom adjustable harness suggested by Lancaster et al. (2019) may also be acceptable in the future.

The coefficient of variation (CV) was often used as a statistical measure of precision, displaying better reproducibility and smaller differences between repeated measurements (Zhao et al., 2014b). In the present study, the mean CV of IVDE, AME, TME, IVDE/GE, AME/GE, and TME/GE for the 16 feed samples were 1.04, 2.71, 2.47, 0.85, 2.60, and 2.43 respectively

Table 5. The determined and predicted AME and TME of 16 feedstuffs (DM basis)

Items ¹	IVDE, MJ/kg	AME, MJ/kg			SEM	P value	TME, MJ/kg			SEM	P value
		Determined ²	Predicted ³	Difference ⁴			Determined ²	Predicted ³	Difference ⁴		
Grain feedstuffs											
Corn A	14.91	14.63	14.73	-0.10	0.17	0.718	15.93	16.03	-0.10	0.15	0.534
Corn B	14.26	14.11	14.04	0.07	0.18	0.790	15.42	15.36	0.06	0.20	0.823
Sorghum A	13.45	13.02	13.19	-0.17	0.20	0.860	14.44	14.53	-0.09	0.14	0.763
Sorghum B	15.39	15.04	15.23	-0.19	0.10	0.060	16.21	16.53	-0.32	0.17	0.248
Sorghum C	14.52	14.77	14.32	0.45	0.18	0.136	16.23	15.63	0.60	0.48	0.334
Barley A	13.45	13.02	13.19	-0.17	0.14	0.419	14.44	14.53	-0.09	0.14	0.342
Barley B	13.75	13.54	13.51	0.03	0.15	0.912	14.97	14.84	0.13	0.16	0.618
Barley C	12.88	12.67	12.59	0.08	0.13	0.835	13.80	13.94	-0.14	0.14	0.669
Protein feedstuffs											
SBM A	13.73	11.63	11.59	0.04	0.19	0.900	13.80	13.93	-0.13	0.28	0.789
SBM B	14.42	12.28	12.51	-0.23	0.33	0.644	14.96	14.81	0.15	0.23	0.812
CSM A	10.75	7.71	7.62	0.09	0.15	0.730	10.34	10.15	0.19	0.18	0.795
CSM B	12.50	9.67	9.95	-0.28	0.37	0.524	12.46	12.37	0.09	0.57	0.976
CSM C	11.06	7.74	8.04	-0.30	0.18	0.378	10.39	10.55	-0.16	0.44	0.874
RSM A	12.35	9.59	9.75	-0.16	0.45	0.141	12.25	12.18	0.07	0.21	0.892
RSM B	12.47	9.60	9.91	-0.31	0.19	0.285	12.28	12.33	-0.05	0.22	0.912
RSM C	12.57	11.24	10.05	1.19	0.55	0.524	12.33	12.46	-0.13	0.30	0.672

¹ IVDE, in vitro digestible energy, mean of 5 determinations per sample (DM basis).² The values were determined with 6 adult ducks for each sample.³ The values were calculated according to the equations of ME based on the IVDE.⁴ Difference is calculated as determined – predicted.**Table 6.** The determined and predicted ME/GE of 16 feedstuffs (DM basis)

Items	IVDE/ GE ¹ , %	AME/GE ² , %			SEM	P value	TME/GE ³ , %			SEM	P value
		Determined ⁴	Predicted ⁵	Difference ⁶			Determined ⁴	Predicted ⁵	Difference ⁶		
Grain feedstuffs											
Corn A	79.82	78.32	78.81	-0.49	2.78	0.905	85.28	86.02	-0.74	3.55	0.545
Corn B	76.13	75.33	74.87	0.46	2.61	0.995	82.33	82.09	0.24	3.60	0.839
Sorghum A	72.23	69.92	70.70	-0.78	2.74	0.798	77.55	77.92	-0.37	3.54	0.778
Sorghum B	83.41	81.52	82.65	-1.14	2.58	0.076	87.86	89.86	-2.00	3.51	0.253
Sorghum C	80.40	81.78	79.43	2.35	2.94	0.171	89.87	86.64	3.22	3.58	0.341
Barley A	76.81	74.36	75.60	-1.24	2.76	0.528	82.47	82.81	-0.35	3.54	0.349
Barley B	75.88	74.72	74.60	0.12	2.89	0.794	82.62	81.82	0.80	3.56	0.630
Barley C	70.93	69.77	69.30	0.47	2.81	0.996	75.99	76.52	-0.53	3.54	0.682
Protein feedstuffs											
SBM A	77.05	65.26	65.00	0.26	3.88	0.882	77.44	78.90	-1.46	2.48	0.805
SBM B	75.30	64.13	63.09	1.03	3.78	0.811	78.12	76.81	1.31	2.43	0.828
CSM A	57.24	41.05	43.36	-2.30	3.32	0.920	55.06	55.21	-0.15	2.38	0.811
CSM B	64.83	50.16	51.65	-1.50	4.14	0.660	64.63	64.29	0.34	2.77	0.996
CSM C	57.60	40.31	43.75	-3.44	3.36	0.476	54.11	55.64	-1.53	2.64	0.891
RSM A	62.69	48.68	49.31	-0.63	3.16	0.178	62.18	61.73	0.46	2.41	0.910
RSM B	62.95	48.46	49.59	-1.13	3.18	0.359	61.99	62.04	-0.05	2.42	0.930
RSM C	57.21	51.16	43.33	7.84	3.3	0.660	56.12	55.18	0.94	2.50	0.685

¹ IVDE/GE, in vitro digestibility of GE, mean of 5 determinations per sample (DM basis).² AME/GE, ratio of AME to GE.³ TME/GE, ratio of TME to GE.⁴ The values were determined with 6 adult ducks for each sample.⁵ The values were calculated according to the equations of ME/GE based on the IVDE/GE.⁶ Difference is calculated as determined – predicted.

(Table 2), which indicated that the degree of variation of ME and ME/GE were greater than that of IVDE and IVDE/GE. Similar results were found in corn grain (Pan et al., 2018), sorghum grain, barley (Regmi et al., 2008), and wheat (Regmi et al., 2009) for pigs and triticale for broiler chickens (Yegani et al., 2013). For grain feedstuffs, the difference between IVDE and AME (0.15–0.43 MJ/kg) and between IVDE/GE and AME/GE (0.80%–2.46%) was lower than that between IVDE and TME (0.92–1.71 MJ/kg) and between IVDE/GE and TME/GE (4.44%–9.47%), respectively. It might suggest that the in vitro digestion method could be used to predict the AME and AME/GE with higher accuracy than TME and TME/GE of feedstuffs for Muscovy ducks. However, Zhao et al. (2014b) reported that IVDE could be used to predict the TME of corn for Peking ducks with acceptable accuracy. These inconsistent results might be due to the different digestive enzyme activities used by in vitro method between Muscovy ducks and Peking ducks, such as amylase (3,380 vs. 4,417 U/mL), trypsin (601 vs. 1,196 U/mL), and chymotrypsin (274 vs. 429 U/mL) (Wang et al., 2021). On the contrary, the difference between IVDE and AME (1.33 to 3.32 MJ/kg) and between IVDE/GE and AME/GE (6.05%–17.29%) was higher than that between IVDE and TME (0.07–0.67 MJ/kg) and between IVDE/GE and TME/GE (0.21%–3.49%) for protein feedstuffs, respectively (Table 2). It was in line with the previous studies that IVDE could be used to predict the TME of SBM and CSM with relatively high accuracy for Peking ducks (Zhao, 2008; Zheng, 2009). In addition, the TME (14.96 MJ/kg) and IVDE (14.42 MJ/kg) values for soybean meal in our study seemed to be higher than TME [3,394 kcal/kg (14.26 MJ/kg)] and IVDE [3,301 kcal/kg (13.87 MJ/kg)] reported for chickens, respectively (Zhao et al., 2014a), which might be due to the greater ability to digest organic matter, crude fiber, crude protein and nitrogen-free extract in ducks than chickens (Muztar et al., 1977; Mohamed et al., 1984).

Pearson correlation analysis showed that a highly positive correlation was found between IVDE concentration and AME ($r = 0.9719$ and 0.9548) and TME ($r = 0.9538$ and 0.9960) as well as between IVDE/GE and AME/GE ($r = 0.9673$ and 0.9279) and TME/GE ($r = 0.9466$ and 0.9941) for grain and protein feedstuffs, respectively (Table 3), indicating that IVDE and IVDE/GE was a good predictor of ME and the in vivo metabolizability of GE. It was consistent with the previous results that IVDE could be used to predict ME of different feedstuffs (wheat, sorghum, SBM, CSM, RSM, sunflower meal, fish meal, DDGS, cassava, coconut meal, palm meal, peanut meal, sesame meal, and rice gluten meal) for Peking ducks (Zhao et al., 2008, 2014b; Zhang et al., 2019) and roosters (Zhao et al., 2014a). The coefficient of determination (R^2) was used to characterize the accuracy of the prediction models (Kaps and Lamberson, 2004). In our study, regression analysis showed significant linear effects ($P < 0.01$) of IVDE on AME ($R^2 = 0.9445$ and 0.9117) and TME ($R^2 = 0.9097$ and 0.9921) as well as IVDE/GE on AME/GE ($R^2 = 0.9357$ and 0.8610) and TME/GE ($R^2 = 0.8960$ and 0.9881) for grain and protein feedstuffs, respectively (Table 4). The R^2 of eight regression models were ranging from 0.8610 to 0.9921, which were greater than R^2 (0.81) for an AME prediction model of eight samples from IVDE in broiler chicks (Yegani et al., 2013). Next, the difference of ME and ME/GE between determined and predicted values was conducted to further evaluate the predictive accuracy of regression models.

Previous studies have proposed that the difference of less than 100 kcal/kg and 2.62%, respectively, between determined and predicted values of ME and ME/GE was considered to be an acceptable accuracy (Bourdillon et al., 1990; Valdes and Lee-son, 1992). In our study, 14 out of 16 feed samples showed differences between determined and predicted values less than 0.45 MJ/kg (100 kcal/kg) and 2.6% for ME (AME and TME) and ME/GE (AME/GE and TME/GE), respectively (Tables 5 and 6), and there was no difference between the determined and predicted values of ME or ME/GE ($P > 0.05$), indicating the regression models were accuracy acceptable to predict the ME and ME/GE of feedstuffs for Muscovy ducks. In addition, the GE and crude protein might not be used to predict the AME and TME of feedstuffs due to the low R^2 of the regression models. In conclusion, the in vitro digestion method can be used to predict the ME and ME/GE of conventional feedstuffs for Muscovy ducks with acceptable accuracy.

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Conflict of Interest Statement

There is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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In vitro evaluation of efficacy of nonstarch polysaccharides enzymes on wheat by simulating the avian digestive tract

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Abstract

In this study, the efficacy of different nonstarch polysaccharide (NSP) enzyme sources on wheat ingredients and wheat basal diets in vitro were evaluated by simulating the avian digestive tract. In Exp. 1, pH level was increased from 2.0 to 8.0 by simulating the avian digestive tract. The relative enzyme activities of xylanase A, B, and C and β -glucanase X at pH 3.0–3.5 were higher ($P < 0.05$) than those at pH 2.0 or 7.0–8.0. The optimal pH levels of 3.5 and 7.0 were screened by simulating the proventriculus and small intestine, respectively to evaluate the efficacy of NSP enzyme on wheat sources. In Exp. 2, wheat 1 contained the highest content of NSP fractions and the lowest digestibility in vitro dry matter (IVDMD) and energy (IVED) in wheat samples. Therefore, wheat 1 was selected for hydrolysis research under different NSP enzyme sources and levels (1,500, 4,500, 13,500, 40,500, 121,500 U xylanase/kg and 250, 500, 1,000, 2,000, 4,000 U β -glucanase/kg) in vitro. The hydrolysis of wheat on the basis of the released reducing sugar content was determined by xylanase sources $A > B > C$ ($P < 0.05$) and β -glucanase sources of $X > Y$ ($P < 0.05$). On the basis of the hydrolysis, the optimum dose of xylanase A and β -glucanase X were 40,500 U/kg and 2,000 U/kg, respectively. Subsequently, the completely randomized designs involving 2 NSP enzymes treatments \times 2 endogenous digestive enzymes treatments (Exp. 3), as well as 2 wheat basal diets \times 2 NSP enzymes treatments (Exp. 4) were used to evaluate the efficacy of NSP enzymes on dietary nutrient digestibility. The addition of NSP enzymes (40,500 U xylanase A/kg and 2,000 U β -glucanase X/kg) increased the IVDMD and IVED of wheat 1 without endogenous enzymes ($P < 0.05$), while the IVDMD and IVED of wheat 1 with endogenous enzyme were only slightly increased ($P > 0.05$). The addition of NSP enzymes could increase the IVDMD and IVED of corn–wheat–soybean meal diet ($P < 0.05$), but had no effect on those of wheat–cottonseed meal rapeseed meal diet ($P > 0.05$). In conclusion, xylanase and β -glucanase additions could effectively eliminate the adverse effects on wheat and wheat basal diets at the optimal pH levels of 3.5 and 7.0 by simulating the proventriculus and small intestine parts, respectively. The efficacy of NSP enzymes was influenced by the enzyme sources, dietary type, and the interaction of endogenous enzymes.

Lay Summary

The inclusion level of wheat in poultry feeds is limited by nonstarch polysaccharides (NSP). Feeding NSP will increase the intestinal viscosity and residence time of the digesta, reduce nutrient digestion, and absorption of nutrients by birds, thereby damaging the intestinal function and growth performance. The degradation of NSP in feed by supplementing NSP enzymes has a positive effect on nutrient availability and growth performance. Therefore, there is a need for a quick and reliable method to assess the efficacy of NSP enzymes from different types, sources, and processing techniques. Compared with the expensive and time-consuming in vivo method for animal feeding experiments, in vitro digestion has been proved to be a rapid method for predicting the efficacy of exogenous enzymes in various parts of the avian digestive tract. Therefore, in this study, the efficacy of different NSP enzyme sources on wheat ingredients and wheat basal diets were evaluated in vitro by simulating the avian digestive tract.

Key words: energy digestibility, enzymatic efficacy, in vitro, nonstarch polysaccharides, wheat

Abbreviations: ADF, acid detergent fiber; CF, crude fiber; CP, crude protein; CSM, cottonseed meal; DM, dry matter; GE, gross energy; IVDMD, in vitro dry matter digestibility; IVED, in vitro energy digestibility; NDF, neutral detergent fiber; NSP, nonstarch polysaccharide; RSM, rapeseed meal; SBM, soybean meal

Introduction

Due to the shortage of corn supply and the rising price of corn, wheat, as an important cereal, has been widely used in poultry diets. The inclusion level of wheat in poultry feeds is limited by nonstarch polysaccharides (NSP), which will damage the growth performance of birds leading to increased intestinal viscosity and reduced nutrient digestion (Annison,

1993; Adeola and Bedford, 2004). Some studies have shown that NSP enzymes supplementation could break down NSP fractions in complex cell wall matrices into smaller fragments, thereby resisting possible anti-nutritional effects, and displaying a positive effect on nutrient utilization and performance (Smeets et al., 2018; Olukosi and Bedford, 2019). However, whether adding NSP enzymes is superior to other enzymes in improving enzymatic degradation remains controversial

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due to the varied efficacy of commercially available exogenous enzymes (Malathi and Devegowda, 2001). The efficacy of feed NSP enzymes depends on the enzymes sources, their substrate specificity and the stability subjected to pH levels and endogenous enzymes in the digestive tract (Aftab and Bedford, 2018). For example, changes in the range of optimal pH or maximum active pH have been noted in commercial sources of feed-grade xylanase and β -glucanase (Ao et al., 2008). Therefore, a fast and reliable method is needed to evaluate the efficacy of NSP enzymes of different types, sources, and processing techniques. Compared with the expensive and time-consuming in vivo method for animal feeding experiments, in vitro digestion has been proved to be a rapid method for predicting the efficacy of exogenous enzymes in various parts of the simulated avian digestive tract (Yegani et al., 2013; Zhang et al., 2019). Previous studies have demonstrated that the method of determining dry matter and energy digestibility in vitro displayed favorable repeatability in evaluating the nutritional values of feedstuffs with pigs and poultry as experimental animals (Zhang and Adeola, 2017; Zhang et al., 2019). In a two-stage in vitro digestion assay, feed sample containing exogenous enzymes were exposed to different pH values and endogenous digestive enzymes in gastrointestinal digestion by simulating the gastric and intestinal phases (Ao et al., 2008; Zhao et al., 2014). Hence, it will be more accurate to evaluate the efficacy of feed NSP enzymes under the in vitro conditions simulating the enzymes existing in the intestine, which can also directly provide information about the effects of NSP enzyme supplementations on the nutrient digestion and growth performance of animals in vivo. Based on the above views, this study aimed to evaluate the changes in the activities of different feed-grade NSP enzyme sources (xylanase and β -glucanase) subjected to pH levels by simulating the avian digestive tract in vitro. Then, the efficacy of NSP enzyme on dry matter and energy digestibility of wheat and wheat basal diet was evaluated by simulating the avian digestive tract in vitro using endogenous enzymes.

Materials and Methods

Sample preparation

A total of five commercially available NSP enzymes were collected in 2020, including three xylanase sources and two β -glucanase sources. Three commercial xylanase sources were xylanase-A (produced by *Trichoderma longibrachiatum*, 213,120 U/g), xylanase-B (produced by *Pichia pastoris*, 304,140 U/g), and xylanase-C (produced by *Bacillus subtilis*, 139,103 U/g), respectively. Two commercial β -glucanase sources were β -glucanase-X (produced by *Trichoderma reesei*, 52,980 U/g) and β -glucanase-Y (produced by *B. subtilis*, 55,089 U/g), respectively. In September and October 2019, the four wheat ingredients in China were sourced from Henan (wheat 1, 12.61% crude protein [CP], 1.26% crude fiber [CF]), Shandong (wheat 2, 12.78% CP, 2.09% CF), Hebei (wheat 3, 13.79% CP, 1.89% CF), and Heilongjiang (wheat 4, 16.45% CP, 1.66% CF) at harvest time, respectively. The wheat samples were milled into particles smaller than 0.5 mm using a mill (FW100, Taisite Instrument Co., Ltd., Tianjin, China). The particles were used to analyze NSP content and evaluate the efficacy of NSP enzymes in vitro. The activities of xylanase and β -glucanase were measured and validated according to the same laboratory procedures as the definitions because recommendations of xylanase and β -glu-

canase activities were different among suppliers. The xylanase activity was determined by reducing sugar method using the 3, 5-dinitrosalicylic acid (DNS) reagent with xylan (Sigma-Aldrich, MO, USA) as substrate. The β -glucanase activity was determined using the reducing-sugar method with β -glucan (S11183, Shanghai Yuanye Biological Co., Ltd., Shanghai, China) as substrate according to König et al. (2002).

pH and buffers (Exp. 1)

In order to obtain the optimum pH levels for each NSP enzyme source, eight pH levels were used in Exp. 1, including pH levels of 2.0, 2.5, 3.0, and 3.5 for simulating the proventriculus and pH levels of 6.5, 7.0, 7.5, and 8.0 for simulating the small intestine, respectively. Each pH treatment included five replicates of conical flasks. The pH gradient was obtained by dissolving 2 mL enzyme and 1 g substrate in different buffers. Sodium chloride and potassium chloride buffer solution (0.1 M, pH 7.0) was adjusted to the pH of 2.0, 2.5, 3.0, and 3.5 by adding 2 M HCl. A phosphate buffer solution (0.1 M, pH 6.5) was adjusted to the pH of 6.5, 7.0, 7.5, and 8.0 by adding 1 M NaOH. In addition, 2 mL of penicillin (Sigma C-0378, Sheboygan Falls, WI, USA) solution (0.5 g 100 mL/L ethanol) was added to inhibit microbial activity. One milliliter of the prepared solution with the equal activity of NSP enzyme solution or blank buffer solution was pipetted into 250 mL conical flasks. Each flask across all treatments was placed in a water-bath at 41.0 ± 0.5 °C for 4 h under gentle agitation. The enzyme activity at pH 3.5 was set to 100%. The relative enzyme activity under other pH values was expressed as the percentage of the enzyme activity at the pH level of 3.5.

In vitro hydrolysis of wheat by NSP enzymes addition (Exp. 2)

First, each wheat sample was used to determine the NSP compositions and constituent monosaccharides. Then, in vitro digestibility of dry matter (IVDM) and energy (IVED) was determined in four wheat samples in triplicate using the procedure described in previous study (Zhao et al., 2014). NSP and monosaccharides were determined by gas chromatography (Englyst et al., 1982; Dida, 2016; Amicucci et al., 2019). The wheat samples containing the highest total NSP content and the lowest IVED were screened, so as to compare the effect of adding NSP enzyme sources and levels on hydrolysis in vitro. Sources and levels of xylanase and β -glucanase (1,500, 4,500, 13,500, 40,500, 121,500 U xylanase/kg and 250, 500, 1,000, 2,000, 4,000 U β -glucanase/kg) were used to evaluate the degree of wheat hydrolysis. Briefly, about 2 g (± 0.01) wheat samples were weighed into 250 mL conical flasks. As described in Exp. 1, the reaction mixture for simulating the pH environment of proventriculus was prepared by diluting the buffer solution and HCL solution. The mixture was maintained at 41.0 ± 0.5 °C for 4 h under gentle agitation. Subsequently, phosphate buffer and 1 M NaOH for the reaction mixture were added for simulating pH environment of the small intestine. Hydrolysis was continued for 15 h using the same water-bath conditions. At the end of the hydrolysis process, the slurry was centrifuged at 300 g for 10 min at room temperature and rinsed with 20 mL distilled water for 3 times. All supernatants before and after washing were collected to determine the total reducing sugar content. Each treatment was analyzed in four replicates. The reducing sugar contents released from NSP enzymes were calculated by the subtraction of the average value from the blank control.

In vitro nutrient digestibility of wheat basal diets with NSP enzyme (Exps. 3 and 4)

First, in order to study the interaction effects between endogenous digestive enzymes and exogenous NSP enzymes, a completely randomized design involved with and without endogenous enzymes additions \times with and without NSP enzymes additions (Exp. 3). The endogenous digestive enzymes included 401.56 U amylase/mL (Sigma A3306, Sigma-Aldrich Co., St. Louis, MO, USA), 108.75 U trypsin/mL (Amersco 0785, Amersco Inc., Solon, OH, USA), and 39.01 U chymotrypsin/mL (Amersco 0164, Amersco Inc., Solon, OH, USA) as reported by Zhao et al. (2014). The supplemental levels of NSP enzymes (combination of xylanase A and β -glucanase X) were added per kilogram diet based on the results from Exp. 2. Then, a completely randomized design involving two wheat basal diet \times two NSP enzymes treatments was used to evaluate the nutrient digestibility by addition of NSP enzymes in vitro digestion (Exp. 4). The two basal diets were composed of corn–wheat–soybean meal (SBM) diet and wheat–cottonseed meal–(CSM)–rapeseed meal (RSM) diet, respectively. To meet the nutrients requirements of poultry, both wheat basal diets were fortified with a mineral and vitamin premix to simulate the normal commercial poultry diets. The composition and nutrient levels of two wheat basal diets were presented in Table 1. Two NSP enzymes treatments were the wheat basal diets with NSP enzymes in the combination of xylanase A and β -glucanase X and without NSP enzymes addition. The IVDM and IVED were determined on wheat with five replicates of each treatment for Exps. 3 and 4. A computer-controlled simulated digestion system was designed to automatically process the in vitro gastrointestinal digestion procedure described by Zhao et al. (2014). Briefly, 2 g of either wheat or wheat basal diets samples were incubated with gastric buffer solution containing 1,550 U pepsin/mL (Sigma 10070, Sigma-Aldrich Co., St. Louis, MO, USA) for 4 h in dialysis tubing (Membra-cel MD 44-14, 14000 Dalton, Viskase Companies Inc., Darien, IL, USA) at 41 °C with an occasional vortex to simulate the peptic or gastric phase. After gastric digestion, small intestinal digestion was initiated with the small intestinal buffer solution circularly pumped into digestion chambers for 0.5 h, 2 mL of simulated small intestinal fluid containing 401.56 U amylase/mL, 108.75 U trypsin/mL, and 39.01 U chymotrypsin/mL was injected into each digestion chamber, and the buffer solution continued to circulate for 15 h at 42 °C. The buffer solutions were prepared with 800,000 U/L penicillin to prevent microbial degradation in accordance with the guidelines of the computer-controlled simulated digestion system. After the simulated digestion was finished, the undigested residues from each replicate were defatted and dried at 105 °C for 5 h to the constant weight for the determination of the IVDMD and IVED.

Sample analyses

The measurement of dry matter (DM) was performed according to the Association of Official Analytical Chemists standard procedures (AOAC 2001–2012) at 135 °C for 2 h. The gross energy (GE) values were measured using an isoperibol oxygen bomb calorimeter (Kalomimeter C7000 processo, IKA, Staufen, Germany). The determination of CP content was performed with the Kjeldahl method, AOAC International method 984.13 (AOAC, 2007) on a Kjeltec™ 8400 apparatus (FOSS Inc., Eden Prairie, MN, USA). The ash content was analyzed using a muffle furnace at 600 °C for 2 h, AOAC

Table 1: Composition and nutrient levels of two wheat basal diets (as-fed basis, %)

Ingredient	Corn–wheat–SBM diet	Wheat–CSM–RSM diet
Corn	20.00	—
Wheat	52.56	75.00
Soybean meal	17.90	—
Cottonseed meal	—	7.90
Rapeseed meal	—	8.00
Mixed oil	1.20	1.73
Wheat middling	5.02	4.02
Limestone	1.00	0.94
Dicalcium phosphate	1.15	1.05
NaCl	0.38	0.38
L-Lysine sulphate	0.14	0.36
DL-Methionine	0.15	0.12
Choline chloride	0.10	0.10
Vitamin and mineral premix ¹	0.40	0.40
Total	100.00	100.00
Nutrient levels ²	0.00	0.00
Metabolizable energy, MJ/kg	12.14	12.14
Crude protein	17.50	17.50
Calcium	0.85	0.85
Total phosphorus	0.60	0.60
Lysine	0.85	0.85
Methionine	0.40	0.40
Methionine + cysteine	0.82	0.83
Crude fiber	2.94	2.95
Insoluble NSP	9.66	9.76
Soluble NSP	2.71	2.30
Total NSP	14.31	13.69

¹Provided per kilogram of diet: vitamin A, 8,000 IU; vitamin D₃, 4,000 IU; vitamin E, 20 IU; thiamin, 1.5 mg; riboflavin, 8 mg; pyridoxine, 3.0 mg; vitamin B₁₂, 0.02 mg; calcium pantothenate, 10 mg; folate, 0.15 mg; niacin, 50 mg; Biotin, 0.20 mg Choline (Choline chloride), 750 mg; Cu (CuSO₄·5H₂O), 10 mg; Fe (FeSO₄·7H₂O), 60 mg; Zn (ZnSO₄·7H₂O), 90 mg; Mn (MnSO₄·H₂O), 80 mg; Se (NaSeO₃), 0.3 mg; I (KI), 0.2 mg.

²Calculated values.

International method 942.05 (AOAC, 2007). The contents of neutral detergent fiber (NDF; without sodium sulfite and alpha amylase, expressed inclusive of the residual ash) and acid detergent fiber (ADF; expressed inclusive of the residual ash) were measured as described by Van Soest et al. (1991). The constituent monosaccharides were determined as alditol acetates with gas liquid chromatography as described by Englyst et al. (1994). The constituent monosaccharide values were converted to the equivalent polysaccharide values using the conversion factor of 0.88 for pentoses (arabinose and xylose) and 0.90 for hexoses (mannose, galactose, and glucose). The contents of total NSP (TNSP), soluble NSP (SNSP), and insoluble NSP fractions (INSPP) were calculated as the sum of the constituent monosaccharides in the respective

fractions. The xylanase activity in the enzyme samples was determined using the DNS-reducing sugar method with xylan as substrate. Xylanase activity assay was measured according to Poutanen (1992). Xylan solution was prepared as substrate by dissolving 1.0% (w/v) xylan in sodium citrate buffer (0.05 M, pH 6.0). The reaction contained 0.1 mL of suitably diluted enzyme solution and 0.9 mL of xylan solution for 10 min at 50 °C. The liberated sugar amount was determined by DNS method using xylose as the standard. One unit of xylanase activity was defined as the amount of enzyme that catalyzes the release of 1 μ mol of xylose equivalent within 1 min. The activity of β -glucanase was determined according to the method as described in König et al. (2002). The assay system consisted of 1.5 mL of 0.7% β -glucan samples. The reaction was carried out at 55°C for 30 min, and then stopped by adding 3 mL of DNS reagent and boiling for 10 min. The absorbance of the reaction mixture was determined at 540 nm using a spectrophotometer (Model 680, Bio-Rad). One Unit (U) is the amount of enzyme which releases 1 micromole of reducing sugars (glucose equivalent) from β -glucan per minute at 50 °C and pH 6.8.

Calculation and statistical analysis

The IVDMD and IVED were calculated using the following formulas:

IVDMD = (sample DM weight – defatted residue DM weight)/sample DM weight;

IVED = [(sample DM weight \times sample GE) – [defatted residue DM weight \times defatted residue GE]]/(sample DM weight \times sample GE);

The Proc Univariate procedure of SAS 9.2 (SAS, 2008) was used to confirm a normal distribution of the data, equal variances, and to identify outliers. An observation was considered an outlier if the value was more than three standard deviations away from the mean. However, no outliers were found in the present study. The data for the relative enzyme activity (Exp. 1) and the release amount of reducing sugar (Exp. 2) were subjected to one-way ANOVA with equal variances among treatments by using the General Linear Model procedure of SAS 9.2 with different pH levels (Exp. 1) and either NSP enzyme sources or levels (Exp. 2) as the main effects, respectively. The data for IVDMD and IVED in Exps. 3 and 4 were subjected to two-way ANOVA by using the General Linear Model procedure of SAS 9.2, including the main effects of endogenous enzymes, NSP enzymes, and their interaction (Exp. 3), as well as the main effects of NSP enzymes, wheat basal diets, and their interaction (Exp. 4). Each replicate served as the experimental unit for all statistical analyses. The treatment comparisons for significant differences were tested by the LSD method. Significant differences were set at $P \leq 0.05$.

Results

The relative enzyme activity at various pH levels (Exp. 1)

The relative activities of xylanase and β -glucanase sources at various pH levels were presented in Figure 1. The activities of xylanase A, B, and C were the highest at pH values of 3.5, 3.5, and 2.5, respectively (Figure 1A-1C). By simulating the digestion of proventriculus or small intestine, the pH level was increased from 2.0 to 8.0, and the activities of xylanase A, B, and C were significantly decreased when the pH value was

increased from 2.0 or 6.5 to 8.0 ($P < 0.05$). The relative activity of xylanase B decreased when the pH level decreased from 3.5 to 2.5 ($P < 0.05$), while the relative activities of xylanase A and C did not decrease significantly at the varied pH levels ($P > 0.05$). By simulating small intestine, the increase of pH from 6.5 to 8.0 had no effect on the relative activities of xylanase A, B, and C ($P > 0.05$). The activities of β -glucanase X and Y were the highest at pH 3.0 and 2.0, respectively (Figure 1D-1E). When the pH level increased from 3.5 to 8.0, the relative activity of β -glucanase Y decreased gradually ($P < 0.05$), while no effects were observed when the pH level increased from 2.0 to 3.5 ($P > 0.05$). Compared with the increase of pH value from 6.5 to 7.0, the relative activities of β -glucanase X and Y were decreased when the pH level increased from 7.5 to 8.0 ($P < 0.05$). In contrast, the pH value increased from 6.5 to 7.0 and was not affected by different pH values from 6.5 to 7.0 ($P > 0.05$). Therefore, the optimal pH levels of 3.5 and 7.0 for NSP enzyme in vitro digestion were screened by simulating proventriculus and small intestine, respectively.

The nutrient and NSP composition and nutrient digestibility of wheat (Exp. 2)

The CP, CF, NDF, and ADF values of the four wheat samples ranged from 12.61% to 16.45%, 1.26% to 2.09%, 10.57% to 13.18%, and 2.97% to 3.62%, respectively (Table 2). The NSP, SNSP, and INSP values of wheat samples ranged from 9.916% to 10.445%, 1.489% to 1.613%, and 8.416% to 8.832%, respectively (Table 3). Wheat 1 has the highest monosaccharide contents. The IVDMD and IVED values of wheat samples ranged from 73.12% to 81.41% and 75.77% to 83.01%, respectively (Table 2). Among the four wheat samples, wheat 1 containing the greatest TNSP, SNSP, and INSP contents displayed the lowest IVDMD and IVED values and was selected to evaluate the efficacy of the NSP enzymes.

In vitro hydrolysis of wheat by NSP enzyme sources and levels (Exp. 2)

Compared with the addition of xylanases B and C, wheat 1 supplemented with xylanase A released the highest reducing sugar content ($P < 0.05$; Figure 2A). The reducing sugar content of wheat 1 released by β -glucanase X was higher than that of β -glucanase Y ($P < 0.05$; Figure 2B). When xylanase A was increased from 1,500 to 121,500 U/kg or β -glucanase X was increased from 250 to 4,000 U/kg, the content of wheat 1 released reducing sugar increased quadratically ($P < 0.05$; Figure 2C and 2D). When the supplemental levels of A and β -glucanase X were 40,500 U/kg and 2,000 U/kg, respectively, the net reducing sugar release of wheat 1 was the highest.

In vitro nutrient digestibility of wheat basal diets with NSP enzymes (Exps. 3 and 4)

Endogenous digestive enzymes, exogenous NSP enzymes, and their interactions had significant effects on IVDMD and IVED of wheat ($P < 0.05$; Table 4). The addition of endogenous digestive enzymes increased the IVDMD and IVED of wheat with and without NSP enzymes. The addition of NSP enzymes (40,500 U xylanase A/kg and 2,000 U β -glucanase X/kg) increased the IVDMD and IVED of wheat without endogenous enzymes ($P < 0.05$) and did not significantly improve IVDMD and IVED on wheat supplemented with endogenous enzyme ($P > 0.05$). IVDMD of wheat basal diet was significantly affected by NSP enzymes ($P < 0.05$), while IVED was not ($P > 0.05$; Table 5). The IVDMD of the Wheat-CSM–

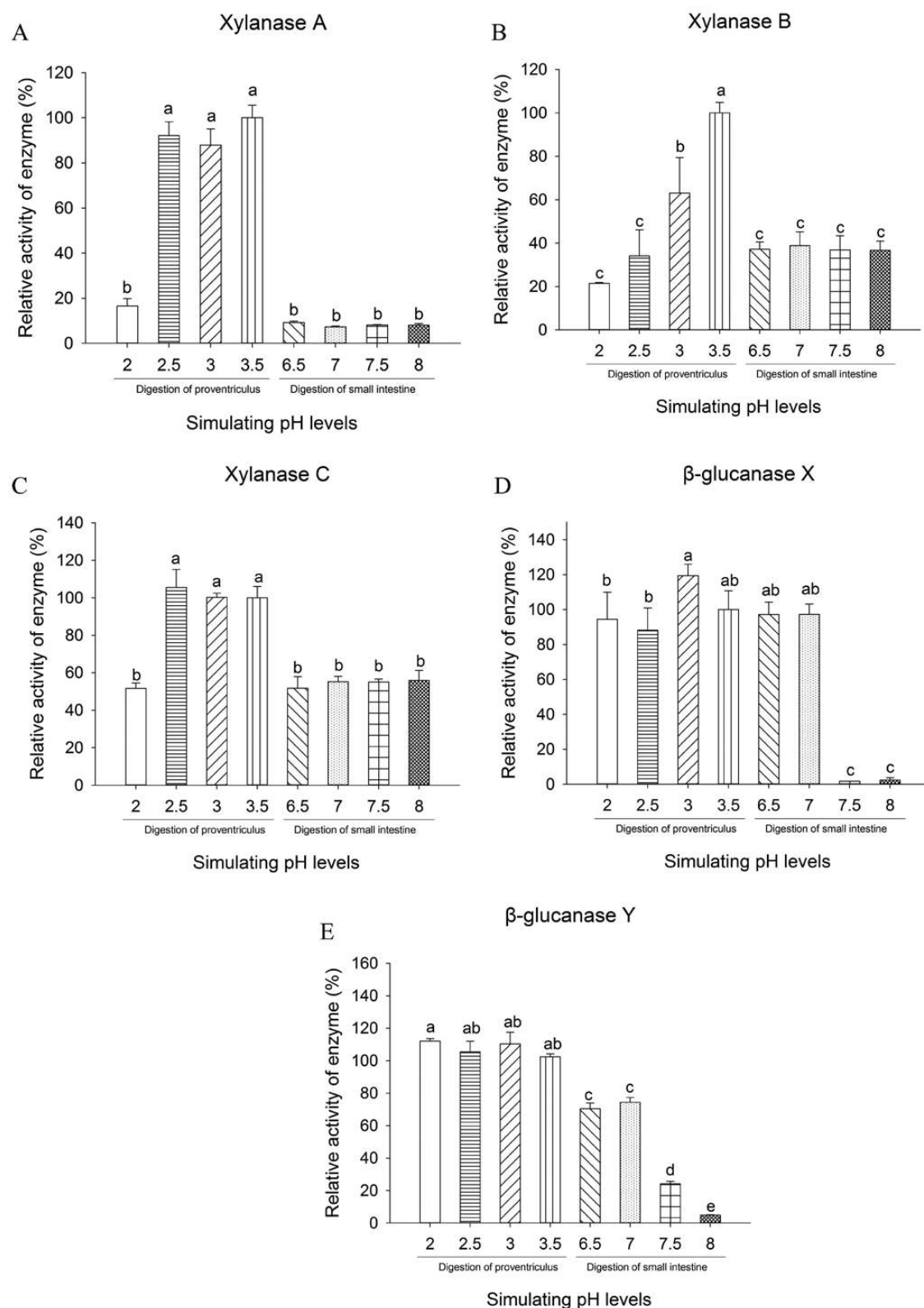


Figure 1. The relative activity of nonstarch polysaccharides enzymes at different simulating pH levels. All values were expressed as means \pm standard error. The values represent the mean of four replicates per sample. ^{a-e}Means within a bar lacking a common superscript letter are different ($P < 0.05$). The pH levels of 2.0, 2.5, 3.0, and 3.5 and pH levels of 6.5, 7.0, 7.5, and 8.0 were used for simulating pH levels of the proventriculus and small intestine, respectively. The relative activity was expressed as a percentage of activity obtained at the pH at 3.5. The activities of xylanase (A–C) and β -glucanase (D–E) were determined by reducing sugar method with xylan and β -glucan as substrate, respectively.

RSM diet was greater than that of the Corn–wheat–SBM diet ($P < 0.05$) between the two basal wheat diets, while no differences ($P > 0.05$) were observed in IVED. Additionally, the interactions between NSP enzymes and dietary type also had significant effects on the IVED values of wheat basal diets ($P < 0.05$; Table 5). The addition of NSP enzymes increased IVED of corn–wheat–SBM diet ($P < 0.05$), but had no significant effect on IVED of the wheat–CSM–RSM diet ($P > 0.05$).

Discussion

Current knowledge of the role of NSP-degrading enzymes on nutrients digestibility and bird growth performance has been well documented at a wide range of NSP enzyme sources and levels of NSP enzyme (Malathi and Devegowda, 2001; Smeets et al., 2014). However, there is no evidence that the addition of NSP enzymes has a decisive effect on the growth performance of wheat-fed broilers (Adeola and Cowieson, 2011). NSP enzymes might encompass differences in substrate affinity, pH, and temperature, this may affect the degree of enzymatic degradation (Smeets et al., 2014). The pH of the digestive tract is one of the key factors limiting the optimal efficacy of these enzymes (Ao et al., 2008). In this study, the relative activities of xylanase A and B and β -glucanase X declined dramatically as pH level decreased from 3.5 to 2.0 by simulating the digestion of proventriculus. These data were consistent with the results obtained in pigs (Thacker and Baas, 1996) and poultry

(Ao et al., 2008). Compared with xylanase B and β -glucanase X sources, as the pH level increased from 2.5 to 3.5, the relative enzyme activities of xylanase A and C and β -glucanase Y remained unchanged, indicating that the varied sensitivity of different enzymes sources in response to pH changes. On the whole, the optimal pH levels of different xylanase and β -glucanase were approximately pH of 3.0–3.5 by simulating the proventriculus. Compared with the digestion phase of the proventriculus, the relative enzyme activity decreased dramatically when the pH ranged from 6.5 to 8.0 by simulating the digestion of the small intestine, which has been confirmed by the previous results (Bailey and Poutanen, 1989; Thacker and Baas, 1996). There was no difference in the relative activities of xylanase A, B, and C when the pH levels increased from 6.5 to 8.0, which disagreed with the previous results in pigs (Baas and Thacker, 1996). The maximum activities of xylanase and β -glucanase were observed at around pH 7.0 by simulating the digestion of small intestine. Moran (1982) reported that the average pH values of the proventriculus and small intestine of the chicken GI tract were 2.5 (0.4–5.4) and 7.2 (5.7–8.2), respectively. In the current study, it was found that the activities of xylanase and β -glucanase sources declined in the pH range of most segments of the avian gastrointestinal tract, suggesting that the various pH levels of different segments of the avian digestive tract may be a limiting factor of exogenous NSP enzymes.

Table 2. Nutrients composition and in vitro digestibility of dry matter and energy of four wheat samples from different locations (dry matter basis, %)¹

Item ²	GE, MJ/kg	CP	Ash	CF	NDF	ADF	IVDMD	IVED
Wheat 1	17.7	12.61	1.84	1.26	10.57	2.97	73.12	75.77
Wheat 2	17.8	12.78	1.78	2.09	13.18	3.62	79.43	81.65
Wheat 3	17.8	13.79	1.62	1.89	12.97	3.40	79.62	82.09
Wheat 4	17.8	16.45	1.83	1.66	12.83	3.29	81.41	83.01
Average	17.8	13.91	1.77	1.73	12.39	3.32	78.40	80.63
CV, %	0.3	12.8	5.8	20.7	9.9	8.2	4.6	4.1

¹The average values represent the measured data in triplicate per sample.

²Wheat samples 1–4 were sourced from the Henan, Shandong, Hebei, and Heilongjiang provinces of China in harvest year of 2019, respectively.

Table 3. The nonstarch polysaccharides composition in four wheat samples from different locations (dry matter basis, %)¹

Item ^{2,3}		Rhamnose	Arabinose	Galactose	Xylose	Glucose	Mannose	Ribose	Fucose	TNSP
Wheat 1	SNSP	0.032	0.677	0.029	0.558	0.056	0.238	0.011	0.013	1.613
	INSP	0.135	3.447	0.055	4.208	0.643	0.131	0.131	0.082	8.832
	TNSP	0.167	4.125	0.084	4.765	0.699	0.369	0.142	0.095	10.445
Wheat 2	SNSP	0.024	0.634	0.020	0.535	0.041	0.222	0.002	0.012	1.489
	INSP	0.122	3.338	0.025	4.058	0.604	0.116	0.105	0.069	8.436
	TNSP	0.147	3.972	0.045	4.593	0.645	0.338	0.106	0.081	9.925
Wheat 3	SNSP	0.023	0.640	0.021	0.535	0.042	0.221	0.009	0.008	1.500
	INSP	0.125	3.318	0.031	4.039	0.608	0.118	0.107	0.070	8.416
	TNSP	0.148	3.958	0.053	4.574	0.649	0.340	0.116	0.078	9.916
Wheat 4	SNSP	0.023	0.647	0.024	0.529	0.043	0.228	0.007	0.016	1.518
	INSP	0.117	3.336	0.036	4.063	0.617	0.121	0.106	0.062	8.457
	TNSP	0.140	3.983	0.060	4.592	0.660	0.350	0.113	0.078	9.975

¹The average values represent the measured data in triplicate per sample.

²Wheat samples 1–4 were sourced from the Henan, Shandong, Hebei, and Heilongjiang provinces of China, respectively.

³The TNSP, SNSP, and INSP contents were calculated as the sum of the constituent monosaccharides in the respective fractions.

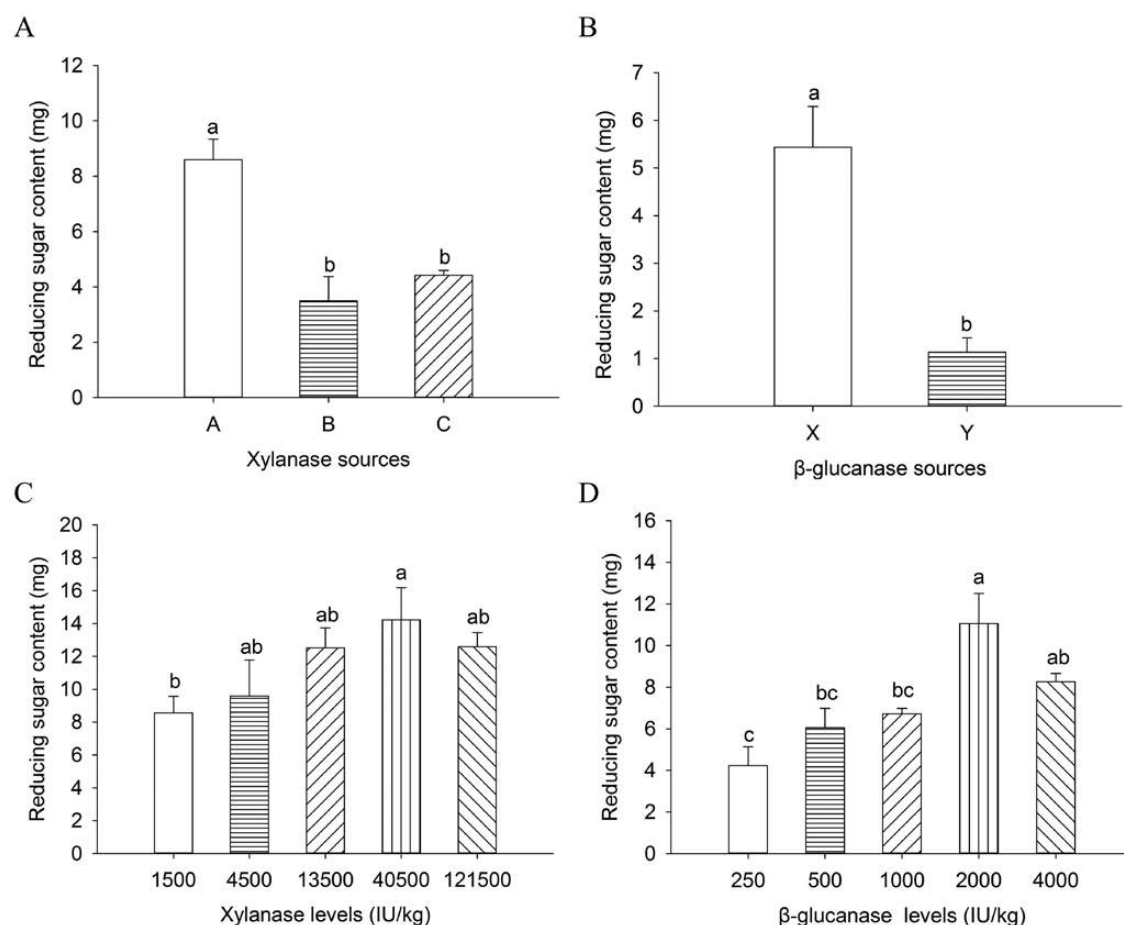


Figure 2. Effects of NSP enzyme sources (A and B) and levels (C and D) on the reducing sugar content released from wheat by in vitro hydrolysis. All values were expressed as means \pm standard error. The values represent the mean of four replicates per sample. ^{a-c}Means within a bar lacking a common superscript letter are different ($P < 0.05$). Wheat 1 with the greatest TNSP, SNSP, and INSP contents among the four wheat samples was selected for in vitro hydrolysis of NSP enzymes. The degree of hydrolysis in wheat were used to evaluated by adding the equal NSP enzymes activities from different commercial xylanase (A) and β -glucanase sources (B). Due to the highest reducing sugar content from xylanase A and β -glucanase X after the whole phase digestion, the dose effect of xylanase A (C) and β -glucanase X (D) on the reducing sugar content were determined to obtain the optimum levels for in vitro digestion.

The analyzed values of nutrient composition of wheat samples in this study was within the range of values reported in previous studies (Choct et al., 1999; Knudsen, 2014). TNSP, the sum of SNSP and INSP in the sample, ranged from 9.916% to 10.445% (DM basis). The amount of the NSP fraction on wheat was similar to that reported by Kim et al. (2003) and Knudsen (2014). The constituent sugar profile confirmed that arabinoxylans (estimated according to the total amount of arabinose and xylose) accounted for approximately 50% of the TNSP content of wheat, which agreed with the observation in previous studies (Meng et al., 2005). The changes in CP, CF, NDF, ADF, and NSP values may be related to the differences among the grain varieties, growth conditions, and harvesting seasons of cereal grains (Choct et al., 1999; Knudsen, 2014). Energy digestibility is an important factor in determining the nutritional value of feed ingredients, reflecting the feed conversion efficacy of animals (Cerrate et al., 2019). In the current study, the IVDE values of four wheat samples ranged from 75.77% to 83.01%, with an average value of 80.63%, which was lower than the apparent total digestive tract energy digestibility (88.95%) of poultry in vivo (King et al., 1997). The difference between in vivo and in vitro energy digestibility may be explained by the inability

to fully mimic the in vitro gastrointestinal (GIT) environment without the use of a microbial inoculant. Meunier et al. (2008) similarly reported that, using a dynamic in vitro model that did not simulate hindgut fermentation, the IVDE value of wheat was lower than DE in vivo, and the gap between in vitro and in vivo increased with the increase of NSP level (Meunier et al., 2008). Therefore, the accuracy of prediction on in vitro energy prediction could be improved by simulating hindgut fermentation as the third step in our further study to simulate the digestive process of poultry.

One of the anti-nutritional factors limiting the bioavailable energy content of wheat ingredients is the high NSP contents (Annison, 1993; Ball et al., 2013). Changes in NSP fractions of wheat and barley measured in broilers measured been proved to correlate closely with changes in energy digestibility (Annison, 1991). SNSPs, such as mixed-linked β -glucans, have been reported to increase intestinal transit time, delay gastric emptying and glucose absorption, increase pancreatic secretion, and slow absorption. In this study, wheat 1, containing the highest contents of TNSP, INSP, and SNSP, and substituting soluble or INSP fractions, had the lowest IVDMD and IVED values in the samples, which confirmed the inverse association between in vitro DM digestibility

Table 4. Interaction effects of endogenous digestive enzymes and NSP enzymes on in vitro dry matter and energy digestibility of wheat (%)¹

Endogenous enzymes treatment	NSP enzymes treatment	IVDMD ²	IVED ²
–	–	37.03 ^c	39.20 ^c
	+	47.53 ^b	49.67 ^b
+	–	80.65 ^a	81.09 ^a
	+	83.72 ^a	84.29 ^a
	SEM	2.27	2.69
Endogenous enzymes	–	37.26 ^b	38.52 ^b
	+	82.18 ^a	82.69 ^a
	SEM	1.71	1.67
NSP enzymes	–	53.81 ^b	54.23 ^b
	+	65.62 ^a	66.98 ^a
	SEM	1.71	1.67
P-value	Endogenous enzymes	<0.001	<0.001
	NSP enzymes	<0.001	<0.001
	Endogenous enzymes × NSP enzymes	0.003	0.001

^{a–c}Means within a row lacking a common superscript letter are different ($P < 0.05$).

¹The values represent mean of five replicates per sample.

²IVDMD = (sample dry matter weight – defatted residue dry matter weight)/sample dry matter weight; IVED = ([sample dry matter weight × sample gross energy] – [defatted residue dry matter weight × defatted residue gross energy])/([sample dry matter weight × sample gross energy]).

“+” and “–” mean that the NSP enzymes were supplemented with or without 40,500 U xylanase A/kg diet and 2,000 U β-glucanase X/kg diet. SEM, standard error of means.

and CF of corn, sorghum, and wheat (Jaworski et al., 2015). Gutiérrez-Alamo et al. (2008) pointed out that the increase of total and NSP contents had negative effects on wheat energy digestibility of monogastric animals, but little effect was induced by INSP level. Supplementation of NSP enzymes could reduce the variability of energy digestibility (Choct et al., 1995) and digesta viscosity (Barasch and Grimes, 2021), contributing to the enhanced performance of birds (Choct et al., 1999; Smeets et al., 2018). Xylanase and β-glucanase have been proved to be effective in eliminating the adverse effects of NSP on wheat effectively both in vitro and in vivo studies (Malathi and Devegowda, 2001). In this study, the net amount of reducing sugar released by different xylanase sources (A > B and C) and β-glucanase (X > Y) sources was different after the whole phase of in vitro digestion of wheat 1. The generation of reducing sugar amounts can be the result of the degradation of relevant NSP-fractions, as well as cellulose hydrolysis of wheat. Previous studies have shown that in vitro digestion with a single xylanase and xylanase and glucanase preparations in combination resulted in a degradation rate of about 20% of the NSP-fraction (Meng et al., 2005; Smeets et al., 2014). In addition, the degree of hydrolysis of xylanase A and β-glucanase X to wheat displayed a quadratic dose effect on the released total sugars contents. The in vitro efficiencies of xylanase A and β-glucanase X reached the maximum at 40,500 U/kg and 2,000 U/kg, respectively.

From a commercial point of view, the consistency of beneficial responses to NSP enzyme is one of the key considerations from a commercial. Studies have shown that dietary NSP-degrading enzymes can reduce the negative effects of NSP and improve animal performance, and sometimes the enzymatic

Table 5. Effects of NSP enzyme additions on in vitro dry matter and energy digestibility on two different wheat basal diets (%)¹

Endogenous enzymes treatment	NSP enzymes treatment	IVDMD ²	IVED ²
Corn–wheat–SBM diet	Control	74.03	74.85 ^b
	NSP enzymes	76.46	79.18 ^a
Wheat–CSM–RSM diet	Control	76.64	77.50 ^a
	NSP enzyme	77.35	78.88 ^a
	SEM	0.71	0.68
Wheat basal diets	Corn–wheat–SBM diet	75.25 ^b	75.34
	Wheat–CSM–RSM diet	76.99 ^a	76.90
	SEM	0.50	0.50
NSP enzymes	No NSP enzymes	77.01 ^b	76.10
	NSP enzymes	78.02 ^a	78.90
	SEM	0.48	0.48
P-value	Wheat basal diets	0.03	0.17
	NSP enzymes	0.047	0.002
	Wheat basal diets × NSP enzymes	0.25	0.03

^{a–b}Means within a row lacking a common superscript letter are different ($P < 0.05$).

¹The values represent mean of five replicates per sample.

²IVDMD = (sample dry matter weight – defatted residue dry matter weight)/sample dry matter weight; IVED = ([sample dry matter weight × sample gross energy] – [defatted residue dry matter weight × defatted residue gross energy])/([sample dry matter weight × sample gross energy]). The NSP enzymes were provided 40,500 U xylanase A/kg diet and 2,000 U β-glucanase X/kg diet.

SEM, standard error of means.

effects can not be observed (Adeola and Cowieson, 2011). The continued benefit of NSP enzymes depends upon the magnitude of any challenge to the digestive process and chemical conditions of the digestive tract, such as endogenous digestive enzymes and bile acid secretion (Aftab and Bedford, 2018). Therefore, it is necessary to predict the nutritional value and response of diets supplemented with NSP-degrading enzymes using in vitro models. According to the results of IVDMD and IVED tests on wheat, the addition of NSP enzyme had a significant enzymatic effect on nutritional value under in vitro digestion conditions without endogenous enzyme. The previous studies demonstrated that the NSP degradations of xylanase and enzyme mixture were 18.1% and 20.7%, while the INSP degradations of xylanase, and enzyme mixture were 12.7% and 20.6% (Smeets et al., 2013). Thus, the degradation of relevant NSP-fractions of wheat could partly be attributed to the greater values of IVDMD and IVED in wheat by the NSP enzyme addition. In contrast, NSP enzyme did not significantly improve IVDMD and IVED on wheat supplemented with an endogenous enzyme. It is noteworthy that the degradation of endogenous enzymes in the digestive tract may negatively affect the effectiveness of NSP enzymes in optimizing substrate decomposition. Although many studies have been reported beneficial effects of enzymes on wheat–SBM based broiler diets (Annison, 1993; Gutiérrez-Alamo et al., 2008), a common practice in the feed industry is to partially substitute CSM and RSM for SBM in poultry diets to cost effectiveness. However, few studies have compared the

efficacy of enzyme on the nutritive value of wheat-based diets formulated with different plant protein ingredients *in vitro*. In the current study, the interactions between wheat basal diets and the addition of NSP enzyme demonstrated that the addition of NSP enzyme has achieved a significant improvement in IVED of the corn–wheat–SBM diet, but failed to produce a favorable effect on IVED of wheat–CSM–RSM diet. The beneficial effect of adding NSP enzymes to corn–wheat–SBM diet implied that the efficacy of NSP enzyme may depend on dietary types. On the one hand, studies have shown that wheat SNSP could increase intestinal viscosity, which further slows down the diffusion rate of substrates, digestive enzymes, and digestion end products, thereby affecting digestion and utilization of nutrients (Johansen et al., 1996), but the level of INSP level had little effect on it (Choct et al., 1995). Therefore, xylanase and β -glucanase presented more effective on nutritional value in corn–wheat–SBM diet containing more SNSP than in wheat–CSM–RSM diet. On the other hand, the wheat–CSM–RSM diet contains more cellulose and pentosan than corn–wheat–SBM diet, as well as the antinutritional factors of gossypol and glucosinolates. Dietary types have different substrate compositions and structural action modes, therefore, it is impossible to synergize, thereby enhancing the effectiveness of NSP degradation (Bach Knudsen, 2011). In summary, this study provided information for narrowing the investigation scope of effective commercial enzymes considering their substrate specificity.

In conclusion, xylanase and β -glucanase additions could effectively eliminate the adverse effects on wheat and wheat basal diets at the optimal pH levels of 3.5 and 7.0 by simulating the proventriculus and small intestine parts, respectively. The efficacy of NSP enzymes was influenced by the enzyme sources, dietary type, and the interaction of endogenous enzymes.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Conflict of Interest Statement

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Effects of dietary iron sources on growth performance, iron status, Fe-containing enzyme activity and gene expression related to iron homeostasis in tissues of weaned pigs

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The aim of this study is to evaluate the effects of dietary iron sources on growth performance, iron status and activities of Fe-containing enzymes and gene expression related to iron homeostasis in tissues of weaned pigs. A total of 480 piglets at d 28 (Duroc X Landrace) were allotted to four groups as a factorial arrangement of treatments with 30 pigs/pen (male: female = 1:1) and 4 replicate pens/treatment. The treatments for iron in the diets were: control basal diet (Con); Con + 150 mg Fe/kg as inorganic Fe (iFe); Con + 75 mg Fe/kg as inorganic Fe + 75 mg Fe/kg as organic Fe-peptide complex (iFe+oFe) and Con + 150 mg of Fe/kg as organic Fe-peptide complex (oFe). The feeding trial lasted for 36 days. There were no significant differences in final body weight, ADG, ADFI, and G/F as well as blood hemoglobin and MCHC contents between piglets fed the control and iron-supplemented groups ($P > 0.05$). The iron supplemented groups exhibited increased iron content in the liver, kidney and spleen as well as the CAT and SDH activities in liver compared to the control group ($P < 0.05$), while piglets in oFe group experienced greater Fe accumulation and activities of CAT and SDH in the liver than piglets in the iFe group. Compared with the control group, dietary supplementation of iron increased the *NCOA4* mRNA expression and decreased the *TfR1* mRNA expression in liver of piglets. The *TfR1*, *NCOA4* and *Ferritin* mRNA expressions of bone marrow in both iFe and iFe+oFe groups were greater than both in the Con and oFe groups. These results suggest that dietary supplementation of iron does not influence the growth performance and hematological parameters in weaned pigs fed a corn-soybean meal basal diet (75.8 mg/kg) from d 28 to d 70, but increased tissue iron status and activities of Fe-containing enzymes at d 70. The addition of organic Fe-peptide complexes presents greater beneficial effects on enhancing tissue Fe accumulation and Fe-containing enzyme activities, which may be involved in different gene expression patterns related to iron intake and transport in tissues of weaned pigs.

KEYWORDS

weaned pig, iron sources, growth performance, iron status, gene expression

Introduction

The phenomenon of piglet anemia in production has been paid more and more attention. Piglets are prone to iron deficiency anemia for several reasons, including low iron stores at birth, inadequate sow milk supply, and rapid growth rate (1, 2). For decades, pigs have been selected for large litters, high birth weights and fast growth, which has resulted in greater body blood volume, red blood cells (RBC) count, and iron demands (3). Additionally, weaned piglets have a limited ability to utilize dietary iron (4). Therefore, various iron supplementation strategies have been shown to be beneficial in reducing anemia, as evaluated by measuring the RBC indices and iron status (5). Several attempts have been made to enhance iron status of piglets by iron injection (dosage of 200 mg of iron dextran) or dietary supplementation (100 mg Fe/kg of a corn-soybean meal basal diet as FeSO₄, Fe-Gly or Heme) without significant effect on growth performance and hematologic variables (6, 7). These results may be attributed to the inability of iron sources and dietary supplemental levels in providing sufficient iron uptake to meet iron requirements. For example, the use of inorganic iron sources is known to affect the absorption and utilization of iron, showing lower bioavailability in animal diets. Organic iron, as an alternative to inorganic iron, presents the advantages of good bioavailability, high absorbability, excellent stability and high safety (8, 9). Among the different types of organic iron supplements, Fe-peptide chelating complexes have been received increasing attention in improving iron bioavailability, with regard to biological effects and intestinal iron absorption properties (10). Organic Fe-peptide chelating complexes bind with iron to form soluble chelate that avoids iron precipitation, and are absorbed through the intestinal peptide absorption pathway, while regulating the proliferation and differentiation of enterocytes through the interaction with the intestinal iron absorption pathway. However, data concerning the effects of Fe-peptide chelating complexes dietary supplementation on iron uptake and transport in piglets are relatively limited. Therefore, the objective of the current study is to evaluate the effects of replacing inorganic Fe with organic Fe-peptide complex on growth performance, hematological parameters, Fe status and activities of enzymes and gene expression related to the iron intake and transport in tissues of weaned pigs.

Materials and methods

Animals and diets

The animal care and use protocol was approved by the Animal Care and Use Committee of South China Agricultural University (SCAU-10564), and the study was conducted following the Regulations for the Administration of Affairs Concerning Experimental Animals.

A total of 480 weaned pigs (Duroc X Landrace) at 28 d with an average initial body weight of 8.14 ± 0.71 kg were allotted to four treatments (approximately 0.4 m² of floor space/pig) as a factorial arrangement of treatments, with 30 pigs/pen (male: female = 1:1) and 4 replicate pens/treatment. Four treatments of Fe in the diet were performed: control basal diet (Con, Table 1); Con + 150 mg Fe/kg as inorganic Fe (iFe); Con + 75 mg Fe/kg as inorganic

Fe + 75 mg Fe/kg as organic Fe-peptide complex (iFe+oFe), and Con + 150 mg of Fe/kg as organic Fe-peptide complex(oFe). The corn-soybean meal basal diet was formulated to meet nutrient requirements other than iron according to the recommendation of NRC (11). The supplemental Fe level added to the basal diet was considered industry standards, thus exceeding the NRC (11) requirements. Lysine and methionine, as the first and second limited amino acid (AA) in weaned pig diets, could eliminate the effect of additional AA produced by iron-peptide complexes and were considered to AA content in the diets. Therefore, lysine and methionine levels in the control diet or diets supplemented with inorganic iron were balanced by adding synthetic lysine-HCl and DL-methionine depending on the amount of lysine and methionine supplementation from the source of the Fe-peptide complex. Each pen contained two stainless steel nipple drinkers and a four-hole, stainless steel feeder. The ambient temperatures were initially set at 28°C but were adjusted as needed to remain within pigs' comfort zone. Pigs had *ad libitum* access to diets and water during the 42 experimental days from d 28 to d 70. The body weight and feed intake of pigs were measured by pens on d 28 and d 70 of the trial, and then average daily gain (ADG), average daily feed intake (ADFI) and the ratio of feed intake to gain (F/G) were calculated as following.

$$\text{ADG} = (\text{off-test pen weight} - \text{allotment pen weight}) / (\text{pigs placed} \times \text{d on-test})$$

The feed intake was calculated by subtracting the residual feed from the feed provided, after making corrections for dry matter content of the feed $\text{ADFI} = (\text{Total feed eaten}) / (\text{pigs placed} \times \text{d on-test})$. F/G is calculated by dividing the kg of feed eaten daily, by the kg of live weight gained daily.

After 12 h of fasting, the same two piglets per replicate pen were bled *via* cannulation of the ear veins puncture for repeat sampling of small volumes (< 4 mL) at birth before dietary iron treatment, and on d 42, 56 and 70, respectively. At d 70, these selected pigs were anesthetized with an intravenous injection of pentobarbital sodium (50 mg/kg, Tc-P8411, Toscience Biotechnology, Shanghai, China) and sacrificed for tissue samples collection. Samples of their liver, kidney, and spleen were collected and a subsample was frozen at -20°C for analyses of iron content and catalase (CAT) and succinate dehydrogenase (SDH) activities. Another subsample of liver and bone marrow tissues were frozen in liquid nitrogen for gene expression assays. At the end of the experiment, the rest of piglets were removed and were fed with a commercial feed to meet the nutrients requirements. Lighting and feeding management were performed according to the instructions of piglets' management guidelines. The recovery performance standards and normal behaviors were done to evaluate the optimum welfare weekly.

Sample collections and analyses

Blood samples (2 to 3 mL) were placed on ice and transported to the laboratory for hematological measurements. Liver, kidney, and spleen samples were homogenized in ice-cold 10% (wt/vol) physiological saline for 1 min using a tissue grinder (T 18 D S25; IKA Group, Staufen, Germany) and then processed for 1 min using an ultrasonic wave cell grinder (JY92-11; Ningbo Scientz

TABLE 1 Composition and nutrient levels of the basal diets (as-fed basis).

Item	Post-weaning period (d 28-70)
Ingredient, g/kg	
Corn	391.5
Extruded corn	186.0
Soybean meal	100.0
Fermented soybean meal	50.0
Extruded soybean	100.0
Grease powder	20.0
Whey powder	12.5
Brown sugar	20.0
Grease powder	10.0
Fish meal	12.0
Fish peptide	10.0
Limestone	6.15
Dicalcium phosphate	9.8
Salt	3.5
DL-Met (98%)	1.0
L-Lys sulphate ^a	5.6
Micronutrients ^a	2.0
Nutrient composition, %	
DE, MJ/kg	3,450
CP ^b	18.2
Lys	1.35
Met	0.60
Met + Cys	0.90
Ca ^b	0.67
Nonphytate P ^b	0.35
Fe ^b , mg/kg	75.8

^aSupplied the following per kilogram of diet: vitamin A, 2200 IU; vitamin D₃, 220 IU; vitamin E, 16 IU; vitamin K₃, 0.5 mg; vitamin B₁, 1.5 mg; vitamin B₂, 4.0 mg; vitamin B₆, 3.0 g; vitamin B₁₂, 0.02 mg; pantothenate, 12 mg; nicotinic acid, 30 mg; Fe, 0 mg; Cu, 6 mg; Zn, 100 mg; Mn, 4 mg; Se, 0.3 mg; I, 0.14 mg; colistin, 40 mg; 50% olaquinox, 20 mg. ^bAnalyzed values based on triplicate determinations.

Biotechnology Co., Ltd., Ningbo, Jiangsu, P. R. China) for 1 min (1 s, with 2 s interval). Then, the homogenates were centrifuged at $1,000 \times g$ for 15 min at 4°C to harvest the supernatants for immediately analyzing total protein content and CAT and SDH activities.

Iron contents in the diets and tissues were determined by inductively coupled plasma emission spectroscopy after wet digestions with HNO₃ and HClO₄ as described by Zhang et al. (12). The iron analysis was validated with bovine liver powder as a standard reference material (GBW [E] 080193; National Institute of Standards and Technology, Beijing, China). Concentrations of Ca and CP in feed ingredient or diet samples were determined as described by the AOAC (13). Lysine and methionine contents of organic iron were analyzed by an automatic amino acid analyzer L-8800 (Hitachi, Tokyo,

TABLE 2 The information of PCR primers.

Genes	Primer sequences (5'-3')	Product size(bp)	Accession No.
<i>β-actin</i>	F: CCAGCACCATGAAGATCA AGATC	102	NM_001614.5
	R: ACATCTGCTGGAAGGTG GACA		
<i>TfR1</i>	F: GGCTGTATTCTGCTCGT GGA	195	NM_214001.1
	R: AGCCAGAGCCCCAGA AGATA		
<i>NCOA4</i>	F: CCTGAGCCTGAGAAACAT	134	NM_001006.5
	R: TAAAGGGACACCACGAAG		
<i>Ferritin</i>	F: GCCAAATACTTCTTCAC CA	142	NM_213975.1
	R: CAGTCAGCCCATTCTCCC		
<i>Tf</i>	F: GCGGGTTTGGTATTTGA GGC	153	NM_212787.1
	R: GGTGTGGATTATCTTTC TGCCC		

Tf, transferrin; TfR1, transferrin receptor 1; NCOA4, nuclear receptor coactivator 4.

Japan). Hemoglobin (HGB) and mean corpuscular hemoglobin concentration (MCHC) levels in blood were determined using an automated AVIDIA 2010 analyzer (Siemens, Germany). CAT activities were analyzed by Goth's colorimetric method (14) with a commercial available test kit (A022-1-1; Jiancheng Bioengineering Institute, Nanjing, China). The activities of SDH were analyzed using a colorimetric method with a commercially available test kit (A002; Jiancheng Bioengineering Institute, Nanjing, China). Total protein concentrations in the supernatants of tissue homogenates were assayed by a BCA Protein Assay kit (23225; Thermo Scientific, Rockford, IL, USA) as per the manufacturer's instruction. The activities of these enzymes were expressed on a per mg protein basis.

Total RNA in the liver and marrow were isolated using TRIZOL reagent (1596018; Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. Reverse transcription was performed using the PrimeScript RT reagent Kit with gDNA Eraser (RR047A; Takara Bio Inc., Otsu, Japan) according to the manufacturer's protocols. The obtained cDNA was used to determine the gene mRNA expression of *Ferritin*, *transferrin* (*Tf*), *transferrin receptor 1* (*TfR1*), and *nuclear receptor coactivator 4* (*NCOA4*) by ABI 7500 RT-PCR detection system (Applied Biosystems, Merelbeke, Belgium, USA) using Fast SYBR Green Master Mix. Information of primers was summarized in Table 2. The relative gene expression was quantified by normalizing to the mRNA expression of *β-actin* as per the $2^{-\Delta\Delta C_t}$ method (15).

Statistical analyses

Data were analyzed by one-way ANOVA using the PROC GLM procedure of the SAS (SAS Inst. Inc., Cary, NC) and all data are presented as mean \pm SD. The number of replicate served as the

TABLE 3 Effects of different iron sources on growth performance of weaned piglets from d 28 to d 70.

Items	Dietary Fe sources				P-value
	Con	iFe	iFe+ oFe	oFe	
Final weight, kg	26.5 ± 1.68	26.7 ± 1.55	25.9 ± 2.31	26.2 ± 2.38	0.74
Average daily gain, g/d	430.5 ± 45.3	460.7 ± 35.1	443.9 ± 50.3	421.6 ± 50.2	0.71
Average feed intake, g/d	833.3 ± 82.9	850.9 ± 45.6	816.4 ± 64.4	888.3 ± 77.0	0.56
Feed/gain	1.90 ± 0.11	1.89 ± 0.14	1.86 ± 0.12	1.95 ± 0.11	0.82

All data were presented as mean ± SD. Mean represented the average value of 4 replicates (n = 4). Con, control basal diet; iFe, Con + 150 mg Fe/kg as inorganic Fe; iFe+ oFe, Con + 75 mg Fe/kg as inorganic Fe + 75 mg Fe/kg as organic Fe-peptide complex; oFe, Con + 150 mg of Fe/kg as organic Fe-peptide complex.

TABLE 4 Effects of different dietary iron sources on hemoglobin and MCHC levels of weaned piglets from d 28 to d 70.

Items	Sampling day	Dietary Fe sources				P-value
		Con	iFe	iFe+ oFe	oFe	
Hemoglobin, g/L	d 28	115.3 ± 9.4	116.3 ± 6.3	113.3 ± 10.4	112.0 ± 9.6	0.93
	d 42	97.5 ± 9.3	109.0 ± 6.7	103.0 ± 8.2	113.0 ± 3.7	0.38
	d 57	98.5 ± 9.0	112.0 ± 10.2	101.8 ± 8.6	96.8 ± 4.1	0.41
	d 70	95.5 ± 11.0	99.3 ± 8.4	100.5 ± 9.6	97.5 ± 4.0	0.93
	d 28	446.2 ± 56.7	404.6 ± 69.4	438.4 ± 87.9	433.6 ± 67.2	0.69
MCHC, g/L	d 42	353.9 ± 28.0	395.7 ± 38.5	373.9 ± 37.0	410.2 ± 36.1	0.38
	d 56	215.4 ± 28.8	251.3 ± 17.2	218.3 ± 15.5	217.1 ± 11.9	0.28
	d 70	310.2 ± 67.2	322.4 ± 36.1	326.5 ± 23.4	316.7 ± 26.9	0.93

All data were presented as mean ± SD. Mean represented the average value of 4 replicates (n = 4). Con, control basal diet; iFe, Con + 150 mg Fe/kg as inorganic Fe; iFe+oFe, Con + 75 mg Fe/kg as inorganic Fe + 75 mg Fe/kg as organic Fe-peptide complex; oFe, Con + 150 mg of Fe/kg as organic Fe-peptide complex.

experimental unit. Differences among means were tested by the Fisher’s Least Significance Difference test method, and statistical significance was set at $P \leq 0.05$.

Results

Growth performance and blood hemoglobin and MCHC levels

No significant differences ($P > 0.05$) were observed between pigs fed the control diet and those fed the iron-supplemented diets in terms of final body weight, ADG, ADFI, and F/G (Table 3). There were also no significant differences ($P > 0.05$) in hemoglobin and MCHC contents at all sampling time points between the pigs fed the control diet and those fed Fe-supplemented diets (Table 4).

Tissue iron content and activities of fe-containing enzymes

Compared with the control piglets, piglets in Fe supplemented groups had greater iron concentrations in the liver, kidney and spleen regardless of the iron source ($P < 0.05$; Table 5), while piglets in oFe group had greater tissue iron concentrations than iFe or iFe+oFe groups ($P < 0.05$). Piglets in either iFe+oFe or oFe groups showed greater CAT and SDH activities in liver than Con group ($P < 0.05$; Table 5), while piglets in oFe group had greater CAT and SDH activities in liver than those in iFe group ($P < 0.05$). There

were no significant differences between Con and iFe groups as well as between iFe+oFe and oFe groups. CAT and SDH activities were higher in the oFe than in the Con group in kidney of piglet ($P < 0.05$), but there were no significant differences between the other groups. There was no difference in the activities of CAT and SDH in spleen among different groups.

Gene expression related to iron homeostasis

Dietary iron supplementation increased ($P < 0.05$) the expression of *NCOA4* mRNA and decreased ($P < 0.05$) the expression of *TfR1* mRNA expression in liver. However, there were no significant differences ($P > 0.05$) in above gene mRNA expressions in the liver of piglets fed the diets with different iron sources. The *TfR1*, *NCOA4* and *Ferritin* mRNA expressions of bone marrow were greater ($P < 0.05$) than that of the control and oFe groups. There were no significant differences ($P > 0.05$) in the above gene mRNA expressions of bone marrow between the piglets fed the control diet and those fed oFe-supplemented diets.

Discussion

In the present study, no significant effects were observed for growth performance in piglets from d 28 to d 70 after dietary 150 mg Fe/kg supplementation originated by different sources in the corn-soybean meal basal diet. On the one hand, both hepatic

TABLE 5 Effects of different dietary iron sources on Fe contents and Fe-containing enzyme activities in tissues of weaned piglets at d 70.

Tissues	Indices	Dietary Fe sources				P-value
		Con	iFe	iFe+ oFe	oFe	
Liver	Fe content, mg/kg	147.8 ± 23.4 ^d	238.7 ± 13.8 ^c	285.8 ± 19.9 ^b	398.6 ± 12.4 ^a	< 0.0001
	CAT, U/ mg protein	74.8 ± 3.2 ^c	75.6 ± 2.1 ^{bc}	82.4 ± 2.7 ^{ab}	89.0 ± 4.7 ^a	< 0.001
	SDH, U/ mg protein	82.8 ± 1.3 ^c	86.2 ± 1.9 ^{bc}	94.0 ± 2.8 ^{ab}	99.9 ± 4.6 ^a	0.004
Kidney	Fe content, mg/kg	90.3 ± 5.3 ^d	110.4 ± 7.4 ^c	118.8 ± 4.6 ^b	127.6 ± 3.1 ^a	< 0.0001
	CAT, U/ mg protein	34.5 ± 5.1 ^b	36.3 ± 5.5 ^{ab}	37.2 ± 6.8 ^{ab}	43.3 ± 1.1 ^a	0.04
	SDH, U/ mg protein	56.3 ± 3.6 ^b	59.7 ± 3.2 ^{ab}	65.9 ± 5.2 ^{ab}	69.7 ± 1.3 ^a	0.005
Spleen	Fe content, mg/kg	60.1 ± 3.6 ^d	76.0 ± 2.5 ^c	94.2 ± 3.9 ^b	130.8 ± 2.0 ^a	< 0.0001
	CAT, U/ mg protein	16.4 ± 3.3	12.4 ± 1.6	15.3 ± 2.8	12.7 ± 2.4	0.14
	SDH, U/ mg protein	46.8 ± 11.7	49.7 ± 9.2	53.9 ± 7.9	46.4 ± 9.5	0.42

All data were presented as mean ± SD. Mean represented the average value of 4 replicates (n = 4). Lacking common letters (a–c) significant differences at P < 0.05. Con, control basal diet; iFe, Con + 150 mg Fe/kg as inorganic Fe; iFe+oFe, Con + 75 mg Fe/kg as inorganic Fe + 75 mg Fe/kg as organic Fe-peptide complex; oFe, Con + 150 mg of Fe/kg as organic Fe-peptide complex.

TABLE 6 Effects of different dietary iron sources on gene expression related to iron homeostasis in tissues of weaned piglets at d 70.

Tissues	Gene	Dietary Fe sources				P-value
		Con	iFe	iFe+ oFe	oFe	
Liver	<i>Tf</i>	1.52 ± 0.62	1.14 ± 0.52	1.66 ± 0.70	1.86 ± 0.64	0.51
	<i>TfR1</i>	3.39 ± 0.92 ^a	1.67 ± 0.96 ^b	1.42 ± 0.40 ^b	1.41 ± 0.38 ^b	0.02
	<i>NCOA4</i>	0.87 ± 0.24 ^b	2.03 ± 0.92 ^a	2.26 ± 1.24 ^a	2.60 ± 0.65 ^a	0.04
	<i>Ferritin</i>	1.94 ± 0.28	1.67 ± 0.74	1.29 ± 0.59	1.47 ± 0.28	0.61
Bone marrow	<i>Tf</i>	1.32 ± 0.70	1.14 ± 0.77	1.26 ± 0.66	1.46 ± 0.87	0.67
	<i>TfR1</i>	0.41 ± 0.25 ^c	7.14 ± 1.60 ^a	3.48 ± 1.33 ^a	1.70 ± 0.68 ^b	0.002
	<i>NCOA4</i>	0.61 ± 0.19 ^b	5.74 ± 1.26 ^a	4.20 ± 1.04 ^a	0.75 ± 0.46 ^b	0.03
	<i>Ferritin</i>	0.87 ± 0.28 ^b	2.51 ± 0.66 ^a	3.68 ± 1.27 ^a	1.14 ± 0.18 ^b	0.03

All data were presented as mean ± SD. Mean represented the average value of 4 replicates (n = 4). Lacking common letters (a–c) significant differences at P < 0.05. The GAPDH expression was used to normalize the expressions of the targeted genes. Con, control basal diet; iFe, Con + 150 mg Fe/kg as inorganic Fe; iFe+oFe, Con + 75 mg Fe/kg as inorganic Fe + 75 mg Fe/kg as organic Fe-peptide complex; oFe, Con + 150 mg of Fe/kg as organic Fe-peptide complex; Tf, transferrin; TfR1, transferrin receptor 1; NCOA4, nuclear receptor coactivator 4.

iron reserves and the sow's milk are sufficient to maintain growth after weaning. On the other hand, dietary supplementation with no supplementation of iron could provide sufficient iron uptake to meet the iron requirements. As reported previously, the corn-soybean meal basal diet supplemented with 100 mg Fe/kg as FeSO₄, Fe-Gly or Heme did not affect the growth performance of weanling pigs aged from d 25 to d 53 (6). However, the inconsistent result showed that the iron-deficient diet (25.8 mg/kg) supplemented with 100 mg/kg ferrous glycine significantly improved growth performance in weanling pigs during the 21-day trial period (16). The discrepancy between the studies may depend on the differences in the supplemental Fe levels, Fe sources, Fe content in basal diets and Fe depletion periods of the piglets. Recently, larger and faster growing pigs have shown signs of anemia earlier because of their increasing tissue growth and the corresponding need for a greater blood volume (3). Therefore, the aged effect of iron sources in hematological parameters was determined in the present study. Several studies have focused on the level of hemoglobin and the cut-off values for anemia in piglets (17, 18). Supplementation of purified or semi-purified diets with different iron contents and sources (~20.2 mg Fe/kg), below requirements, resulted in

higher concentrations of hemoglobin and hematocrit in piglets (19) and chick broilers (20). In the present study, there were no significant differences in hematological indices of hemoglobin and MCHC levels between the control diet and iron-supplemented diets during the experimental period, suggesting that hematological indices of piglets may not be sufficient sensitivity required to detect differences of iron status in piglets when a commercial corn-soybean meal diet containing a higher iron content of 75.8 mg Fe/kg is adopted, which are consistent with the results of the previous studies in piglets (7, 17, 21) and broilers (12). Iron depletion using a practical basal diet for 36 days after weaning may be not enough to present a subclinical form of iron deficiency in micro erythrocytes.

Target tissue accumulations of iron have always been considered to be sensitive criteria to assess the iron status and relative bioavailability of iron sources (12, 22). In the present study, supplementation with 150 mg Fe/kg, irrespective of the source of iron deficiency diet, significantly increased iron content in liver, kidney and spleen. In particular, iron retention in tissue increased as the organic iron peptide complexes replaced inorganic iron levels. Iron amino acid complex (23) and iron glycine chelate (24) were more effective in improving iron status of weanling pigs

than iron sulfate. Collectively, these results imply that organic Fe-peptide complex may have better bioavailability than inorganic iron salts fed to lactating pigs, due to its high absorption and good stability. Firstly, Organic Fe-peptide complexes are more stable in the digestive tract and less prone to interactions and antagonisms due to binding to organic molecules, and as a result, improve iron efficiency by intestinal iron absorption pathway (25). Zhang et al. (12) and Cao et al. (22) indicated that the relative bioavailability of organic iron sources was closely related to their chelation strength, and organic iron sources with greater chelation strengths showed higher iron bioavailability. Secondly, Fe-peptide complexes have been reported to be absorbed by intestinal peptide absorption pathway (24). Iron is an essential element required for the functions of numerous enzymes, such as CAT, SDH, and so on (26). The activities of enzymes are affected by Fe level (27), and they are used as indices to evaluate iron supplementation efficiency in pigs. In the present experiment, compared with control group, CAT activities in the liver and kidney of pigs increased with dietary Fe supplementation regardless of Fe sources, and the same tendency of SDH activity in liver was observed in broilers (12) and piglets. In addition, piglets fed with oFe diet had greater CAT and SDH activities in kidney than those fed Con diet, with no significant differences observed among other groups. These results indicate the sensitivity of Fe status and the activities of Fe-containing enzymes in tissues over indicators of growth performance and mature erythrocytes in detecting the bioavailability of iron sources in weaned piglets.

Iron uptake, transport and retention are indicators of the efficiency of absorption and bioavailability of supplemental Fe level and sources (4). Hepatic iron stores represent the primary source of iron in response to the metabolic demands of the organism. Ferritin, a ubiquitous and highly conserved iron-chelating protein, is considered as the major iron storage protein that maintains a large iron core in its cavity and has ferroxidase activity (28). However, no significant effect of on hepatic *ferritin* mRNA expression was observed in pigs between the control and Fe-supplemented groups in the current study, while *ferritin* mRNA expression in bone marrow was increased in either iFe or iFe+oFe group compared with the control and oFe groups (Table 6). This is because transferrin is saturated, and the highly available free iron form induces ferritin expression. It was speculated that low levels of free iron or high levels of chelated iron were present in bone marrow of piglets fed the control and oFe diets, respectively. In addition, delivery of ferritin to lysosomes required NCOA4 protein to enable cells to use stored iron, which recruits ferritin as a cargo molecule (29). Our results show that there is a decrease in the NCOA4 mRNA expression in liver and marrow under iron depletion. *In vitro* study revealed that NCOA4-deficient cells were unable to degrade ferritin, leading to a decrease in bioavailable intracellular iron (30). However, NCOA4 mRNA expression was observed to be significantly decreased in the bone marrow of piglet fed oFe diet. This finding could be possibly attributed to the degradation of soluble NCOA4 and ferritin by the macro autophagy pathway in an effort to prevent relative excessive iron storage under prolonged iron repletion by Fe-peptide complex with high bioavailability. Our results suggest that the NCOA4-ferritin axis may be involved in modulating intracellular iron

homeostasis in accordance with cellular iron availability. Under physiological circumstances, the major iron uptake route utilized by most cells involves Tf-bound iron, which is bound to TfR1 and then internalized through receptor-mediated endocytosis (31). In the present study, dietary iron supplementation regardless of sources failed to affect *Tf* mRNA expression in liver and marrow. Translation of *TfR1* mRNA in both liver and marrow was increased under iron deficient conditions, which was consistent with the increased tissue Fe accumulation due to the resultant reduction in cellular iron export. The transferring receptor serves as a sensitive indicator of iron deficiency and can be used to provide a reliable index of iron stores.

Conclusion

In conclusion, the levels and sources of dietary iron supplementation do not influence growth performance and hematological parameters in weaned piglets fed a corn-soybean meal basal diet supplemented with 150 mg Fe/kg from d 28 to d 70. Supplementation of 150 mg Fe/kg in the basal diet regardless of sources increases tissue iron status and activities of Fe-containing enzymes and the addition of organic Fe-peptide complex presents greater beneficial effects on iron bioavailability of weaned pigs at d 70. In addition, the iron homeostasis regulated by iron sources may involve different gene expression patterns related to iron intake and transport in tissues.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by the Animal Care and Use Committee of South China Agricultural University (SCAU-10564) and the study was conducted following the Regulations for the Administration of Affairs Concerning Experimental Animals.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

JY, JL, Y-HJ, and R-QH were employed by the Guangdong Guangken Animal Husbandry Group Co., Ltd.

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Dietary vitamin D₃ requirement of magang goslings from 1–21 days of age

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ABSTRACT

The present study was conducted to investigate the effect of dietary vitamin D₃ levels (0, 200, 400, 800, 1600, and 3200 IU/kg) on growth performance, calcium-phosphorus metabolism, and tibia development of goslings from 1 to 21 days of age. A total of 720 one-day-old Magang male goslings with similar initial body weight were randomly divided into 6 treatments with 6 replicates per treatment and 20 geese per replicate. The goslings fed with 400 IU vitamin D₃/kg of diet obtained the maximum average daily gain, while vitamin D₃ supplementation significantly improved the feed/gain of goslings aged from 1 to 21 days ($P < 0.05$). The vitamin D₃ requirement of gosling aged from 1 to 21 days for optimal feed/gain ratio and average daily gain were estimated from three regression models (one-slope straight, two-slope straight, and quadratic broken-line model) to be 246–352 IU/kg diet and 400–518 IU/kg diet, respectively. Serum calcium and phosphorus contents, parathyroid hormone level, and 25-hydroxycholecalciferol level responded to increasing dietary vitamin D₃ levels ($P < 0.05$) and reached a plateau at 800, 0, and 200 IU vitamin D₃/kg diet, respectively. The mRNA expressions of target genes related to calcium homeostasis such as vitamin D₃ receptor, Na⁺/Ca²⁺ exchanger 1, and plasma membrane calcium ATPase 1b in the duodenum and kidney ($P < 0.05$) and parathyroid hormone receptor mRNA expression in the kidney ($P < 0.05$) were increased linearly and quadratically with the increasing dietary vitamin D₃ levels, respectively. Graded doses of dietary vitamin D₃ from 0 to 3200 IU/kg produced linear responses in tibial density ($P < 0.05$). The vitamin D₃ requirement of gosling aged from 1 to 21 days ranged from 519 to 698 IU/kg diet when tibial density as an evaluation criterion. In conclusion, the vitamin D₃ requirement of gosling aged from 1 to 21 days for optimal growth performance (feed/gain and average daily gain) and tibial density was estimated to be 246–352 IU/kg of diet, 400–518 IU/kg of diet, and 519–698 IU/kg of diet based on three regression models (one-slope straight, two-slope straight, and quadratic broken-line model).

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; ALP, alkaline phosphatase; BW, body weight; Ca, calcium; CT, calcitonin; ER, estimated VD₃ requirement; F/G, feed/gain; NCX1, Na⁺/Ca²⁺ exchanger 1; NPP, non-phytate phosphorus; P, phosphorus; PMCA1b, plasma membrane calcium ATPase 1b; PTH, parathyroid hormone; PTHR, parathyroid hormone receptor; R², coefficient of determination; SEM, standard error of the mean; UV, ultraviolet; VD₃, vitamin D₃; VDR, vitamin D₃ receptor; 1, 25(OH)₂D₃, 1,25-hydroxycholecalciferol; 25OHD₃, 25-hydroxycholecalciferol.

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respectively. The most appropriate vitamin D₃ requirement was recommended at 602 IU/kg of diet of young geese based on the mean estimated values of three regression models for the tibial density with the highest coefficient of determination and lowest mean square error.

1. Introduction

Vitamin D₃ (VD₃) is an essential fat-soluble vitamin that plays a critical role in several physiological processes of the homeostasis of Ca-P metabolism (Fleet, 2017; Shao et al., 2019) and bone mineralization (Nordin, 2010; Hayakawa et al., 2019) in poultry. Previous studies have shown that the chickens fed with a VD₃-deficient diet were characterized by an increased incidence of rickets (Itakura et al., 1978; Itakura et al., 1978; Oviedo-Rondon et al., 2006) and a reduction in the mRNA expression of the calcium-binding protein (Liu, 2017). Many studies have been conducted for the optimum VD₃ requirements for broiler chickens (Baker et al., 1998; Atencio et al., 2006; Jiang et al., 2015; Leyva-Jimenez et al., 2019) and ducks (Wang, 2010; Shi, 2012), but limited information was available for geese (Wang et al., 2013). The requirements of a 200 IU VD₃/kg diet for geese from NRC (1994) were also estimated based on the recommended value of chicken. Goose is a kind of waterfowl with a relatively more developed paired cecum compared to other poultry breeds such as chicken and duck (Abecia et al., 2005; Li et al., 2018), in which microorganisms involved in some physiological processes such as the absorption and synthesis of vitamins (DeGoulier et al., 1999). Therefore, the VD₃ requirement needs to be reevaluated in geese due to the huge differences in digestive physiology and vitamin digestibility between chickens and geese. For example, Wang et al. (2013) reported that the VD₃ requirement of Qingnonghui geese aged from 0 to 4 weeks has been estimated to be 472 IU/kg diet based on the maximal ADG, which was twice as high as that from NRC (1994). In addition, geese rearing in the litter floor system were changed to the plated or caged systems with limited space (Rodenburg et al., 2005). The VD₃ requirement should be paid more attention to bone development and mineralization of geese (Wang et al., 2020). Therefore, the VD₃ requirement based on maximal performance may not apply to achieve the full potential of bone development and mineralization in modern geese breeds. The study aimed to evaluate the effects of dietary VD₃ levels on growth performance, serum biochemical parameters, tibia development, and Ca-P metabolism to reevaluate the VD₃ requirement of goslings aged from 1 to 21 d.

2. Materials and methods

2.1. Animals, diets, and experiment design

All experimental procedures in this study were approved by the Institutional Animal Care and Use Committee of South China Agricultural University, and the study was performed following the Regulations for the Administration of Affairs Concerning Experimental Animals.

A total of 720 one-day-old male Chinese native Magang goslings with similar body weight (BW) were randomly divided into 6 treatments with 6 replicate pens per treatment and 20 birds for each pen. The basal diet was a corn-soybean meal-based diet formulated to meet or exceed the nutritional requirements of goslings at the starter period except for VD₃. The metabolizable energy of the basal diet could be calculated by summing the metabolizable energy value for geese of each feedstuff included in the diet (NRC, 1994). The six dietary treatments were supplemented with different VD₃ levels of 0 (as the control group), 200, 400, 800, 1600, and 3200 IU VD₃/kg of diets on top of the basal diet, respectively. The composition and nutrient levels of the basal diet were presented in Table 1. The measured VD₃ values in experimental diets were 0, 210, 420, 780, 1560, and 3180 IU/kg of diets, respectively. Goslings were rearing in the caged systems and the initial brooding temperature of 33 °C during the first week was decreased each week until

Table 1
Composition and nutrient level of the basal diet (DM basis).

Ingredient, g/kg		Nutrient composition and level	
Corn	480	Metabolizable energy, ^a MJ/kg	12.2
Wheat bran	155	Crude protein, ^b %	20.1
Soybean meal	320	Calcium, ^b %	0.84
Limestone	10	Total phosphorus, ^b %	0.75
Dicalcium phosphate	12	Non-phytate phosphorus, ^a %	0.42
Sodium chloride	10	Lysine, ^a %	1.12
Lysine	1	Methionine, ^a %	0.48
Methionine	2	Methionine + Cysteine, ^a %	0.78
Premix ^c	10	Vitamin D ₃ , ^d IU/kg	ND

^a Values were calculated. $ME_{\text{diet}} = \sum ME_{\text{ingredients}} \times \text{the proportion of the ingredient in the complete diet}$.

^b Measured values.

^c Provided per kilogram of diet: vitamin A, 10,000 IU; vitamin E, 20 IU; vitamin K₃, 4.0 mg; thiamin, 6.0 mg; riboflavin, 2.0 mg; pyridoxine, 4.0 mg; vitamin B₁₂, 0.025 mg; calcium pantothenate, 20 mg; nicotinic acid, 85 mg; folate, 2.5 mg; D-biotin, 0.15 mg; choline chloride, 1800 mg; Cu (CuSO₄•5 H₂O), 2.5 mg; Fe (FeSO₄•7 H₂O), 80 mg; Zn (ZnSO₄), 50 mg; Mn (MnSO₄•H₂O), 100 mg; Se (Na₂SeO₃), 0.10 mg; I (KI), 0.35 mg.

^d "ND" means that the content of vitamin D₃ in the basal diet was not detected.

the temperature reached approximately 20 °C at the end of the study (Zhai et al., 2019). The 12 h light per day was provided by a fluorescent 48-inch tube lamp covered with red plastic shields (Leyva-Jimenez et al., 2019). Feed and water were provided with ad libitum access during the experimental period. Feed consumption was recorded by each replicate pen and the average daily gain (ADG), average daily feed intake (ADFI), and feed/gain ratio (F/G) were calculated.

2.2. Sample collections and analysis

Two 21-day-old geese based on the average BW per pen were chosen for sampling after 12 h of feed withdrawal. The blood samples were collected via the brachial vein and centrifuged at 3,000g for 15 min at 4 °C to prepare serum and stored at – 30 °C. The geese were euthanized with carbon dioxide and the tibias were obtained by manually removing adhering tissue and meat for the determination of bone characteristics and composition. After washing in PBS, samples of approximately 1–2 cm of the middle portion of the liver and duodenum were collected and then snap-frozen in liquid nitrogen for mRNA extraction.

The VD₃ contents in diets were analyzed according to the classical procedures of the Association of Official Analytical Chemists (method 980.26; AOAC, 1990) with a liquid chromatographic. The crude protein content of the diets was determined with the Kjeldahl method (method 954.01; AOAC, 1990) on a Kjeltec™ 8400 apparatus (FOSS Inc., Eden Prairie, MN, USA). The right tibia was obtained to determine the tibial weight, length, ash, Ca, and P content, while the left tibia was used to determine the tibial density. The tibial length was measured by a vernier caliper with an accuracy of 0.001 cm. After being de-fatted by ethyl alcohol and diethyl ether and dried at 105 °C for 24 h, the dry tibia was weighed using an analytical balance with a weighing accuracy of 0.001 g. After being ashed in a muffle furnace for 18 h at 600 °C (Han et al., 2009) and dissolved with nitric and perchloric acids, the Ca and total P content of diets and tibias were determined with an atomic absorption spectrophotometer (Shimadzu, AAS-6300, Kyoto, Japan) and a spectrophotometer (Model UV-754, Shanghai Precision & Scientific Instrument Co., Ltd, Shanghai, P. R. China), respectively. Tibial density was determined using a dual-energy X-ray absorptiometry system (DEXA, Lexxos-2000, Medlink, France) (Tang et al., 2019).

Serum Ca (C004–2–1), total P (C006–1–1), and alkaline phosphatase (ALP, A059–2–2) contents and the concentrations of 25-hydroxycholecalciferol [25(OH)D₃, H191–1], parathyroid hormone (PTH, H207), and calcitonin (CT, H153) were determined using the commercial kits and Elisa kits respectively from Nanjing Jiancheng Institute of Bioengineering (Nanjing, China) by a microplate reader (Multiskan GO Microplate Reader, Thermo Scientific, Finland).

Total RNA was isolated from liquid nitrogen-frozen kidneys and duodenum using the RNA Purification Kit (B0004DP, EZBioscience Co., Ltd., Beijing, China). The quantity and purity of RNA were assessed using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and agarose gel electrophoresis was used to test the quality of RNA. Total RNA was reverse transcribed into cDNA by the Color Reverse Transcription Kit (A0010CGQ, EZBioscience Co., Ltd., Beijing, China), and a 2 × Color SYBR Green qPCR Master Mix kit (A0012-R2, EZBioscience Co., Ltd., Beijing, China) was used for subsequent RT–PCR amplification on a Bio-Rad C1000 Touch Thermal Cycler (Bio-Rad, Pleasanton CA, USA). The primers were designed and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China), and the sequences were as follows:

VDR: 5'GGAAGCGGAGATGATAC3' and 5'GAGGATGTCGATGACTTTCTG3'.

PTHr: 5'AGGAGATGGAGCGGATCTCT3' and 5'TGCCATGAAGATGAGGCTGT3'.

NCX1: 5'ACAGCTGGAGAGAACAGTTC3' and 5'AGGACCTTCCAGAAGACAGT3'.

PMCA1b: 5'TTCAGGTACTCATGTGATGGAAGG3' and 5'CAGCCCCAAGCAAGGTAAAG3'.

β-actin: 5'CCATTGGCAATGAGAGGTTTC3' and 5'TGGATACCGCAGGACTCCATA3'.

All amplicons were 200–500 bp and were sequenced to confirm the specificity of amplification. Throughout real-time PCR analysis, product identities were confirmed by melting curve analysis. The quantification of each gene was relative to a standard curve generated from a serially diluted sample. The relative amount of each experimental gene was then normalized to the abundance of the housekeeping gene β-actin.

2.3. Statistical analysis

The experimental data were subjected to one-way ANOVA by using the General Linear Model procedure of SAS (SAS Institute, Cary, NC). The treatment comparisons for significant differences were tested by the LSD method. And all data were presented as mean values with SEM. Orthogonal polynomials were applied for linear and quadratic responses of dependent variables to dietary VD₃ levels. Each replicate served as the experimental unit for all statistical analyses, and statistical significance was set at $P < 0.05$. There was no particular nonlinear model that is necessarily best for all nutritional response data (Vedenov and Pesti, 2008). Therefore, three regression models were used to estimate the dietary VD₃ requirement of geese from 1 to 21 d of age for different response data (Robbins et al., 2006). The one-slope straight broken line model was described as $y = L + U \times (R - x)$, and $(R - x)$ is defined as zero when $x > R$. The two-slope straight broken line model was described by $y = L + U \times (R - x) + V \times (x - R)$, where $(R - x)$ is zero when $x > R$, otherwise, $(x - R)$ is zero. The quadratic broken-line model can be written as $y = L + U \times (R - x)^2$. In the three models, L is defined as the y value at breaking point ($x > R$), U is the slope ratio of the line when $x < R$ and R is defined as the VD₃ requirement of gosling from 1 to 21 d of age. Mean square error (smaller values indicate a better model) and R^2 (larger values indicate a better model) were used to assess the goodness of fit for the different models.

3. Results

3.1. Growth performance

The ADG of goslings aged from 1 to 21 d increased with dietary VD₃ levels from 0 to 400 IU VD₃/kg diet and then decreased with dietary VD₃ levels from 400 to 3200 IU/kg of diet and reached a peak at 400 IU VD₃/kg diet (Table 2, $P < 0.05$), while F/G was decreased in response to increased dietary VD₃ levels from 0 to 3200 IU/kg of diet (Table 2, $P < 0.05$). However, no difference was observed between treatments for final BW and ADFI (Table 2, $P > 0.05$). In addition, dietary VD₃ supplementation from 200 to 800 IU/kg diet decreased the mortality of goslings aged from 1 to 21 d (Table 2, $P < 0.05$).

3.2. Serum biochemistry parameters

Serum Ca concentration was increased with increasing dietary VD₃ levels from 0 to 3200 IU/kg of diet, while serum P concentration in 800 IU VD₃/kg diet treatment was higher than that in 0, 200, and 400 IU VD₃/kg diet treatments (Table 3, $P < 0.05$). 200 IU VD₃/kg diet treatment had a higher serum 25(OH)D₃ concentration than 0 and 3200 IU VD₃/kg diet treatments (Table 3, $P < 0.05$). Serum PTH level of 21 d-old goslings was decreased with increasing dietary VD₃ levels from 0 to 3200 IU/kg of diet (Table 3, $P < 0.05$). There was no significant difference in serum ALP and CT levels of 21-d-old goslings among treatments with different dietary VD₃ levels (Table 3, $P > 0.05$).

3.3. Tibia parameters

The tibial length was significantly affected by dietary VD₃ levels and the shortest tibia was observed on geese fed a VD₃-free diet. The tibial density of 21-d-old geese increased linearly and quadratically with increasing dietary VD₃ levels from 0 to 3200 IU/kg of diet and the tibial density in 0 IU VD₃/kg diet treatment was lower than that in other treatments (Table 4, $P < 0.05$). No difference was observed in tibial weight, Ca, P, and ash contents of 21-d-old geese among treatments with different dietary VD₃ levels (Table 4, $P > 0.05$).

3.4. mRNA expression

The mRNA expressions of *VDR*, *NCX1*, and *PMCA1b* in the kidney and duodenum increased linearly in response to increasing dietary VD₃ levels from 0 to 3200 IU/kg of diet (Table 5, $P < 0.05$), while the *PTHr* mRNA expression increased linearly and quadratically in the kidney, but not in the duodenum (Table 5, $P > 0.05$), with increasing dietary VD₃ levels from 0 to 3200 IU/kg of diet and reached a peak at 800 IU VD₃/kg diet (Table 5, $P < 0.05$).

3.5. Regression model to estimated VD3 requirement

Three regression models for estimating VD₃ requirements using growth performance and tibial parameters as sensitive indicators were presented in Table 6. The VD₃ requirement for optimal F/G, ADG, tibial length, and tibial density of goslings aged from 1 to 21 d was estimated from the three regression models (one-slope straight, two-slope straight, and quadratic broken line regression models) to be 246–352, 400–518, 243–344, and 519–698 IU VD₃/kg diet. Three regression models for tibial density had higher R² and lower mean square error, and the most appropriate vitamin D₃ requirement of young geese was 602 IU/kg of diet based on the mean value of three estimated VD₃ values.

Table 2

Effects of dietary VD₃ levels on growth performance (BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; F/G, feed/gain ratio) of goslings aged from 1 to 21 d¹.

Dietary VD ₃ level, IU/kg	0	200	400	800	1600	3200	SEM	P value		
								treatment	linear	quadratic
Initial BW, g/bird	89.0	89.3	89.8	89.7	89.1	89.0	0.93	0.977	0.705	0.813
Final BW, g/bird	718	731	745	750	751	746	15.2	0.629	0.340	0.213
ADG, g/bird/day	31.5 ^b	31.2 ^b	36.5 ^a	33.0 ^b	33.1 ^b	32.9 ^b	0.95	0.028	0.287	0.375
ADFI, g/bird/day	65.5	60.5	64.4	61.3	64.5	059.7	3.23	0.720	0.401	0.727
F/G, g/g	2.14 ^a	1.85 ^b	1.70 ^b	1.73 ^b	1.83 ^b	1.80 ^b	0.831	0.025	0.260	0.124
Mortality, %	11.1 ^a	5.79 ^b	5.66 ^b	5.47 ^b	8.10 ^{ab}	11.9 ^a	1.477	0.021	0.031	0.031

SEM, standard error of the mean;

¹Each value represents the mean of 6 replicates.

^{a-b} Means within a row without a common superscript differ significantly ($P < 0.05$).

Table 3

Effects of dietary VD₃ levels on serum biochemical parameters (Ca, calcium; P, phosphorus; ALP, alkaline phosphatase; 25(OH)VD₃, 25-hydroxycholecalciferol; PTH, parathyroid hormone; CT, calcitonin) of 21-d-old geese ¹.

Dietary VD ₃ level, IU/kg	0	200	400	800	1600	3200	SEM	P value		
								treatment	linear	quadratic
Ca, mmol/L	2.49 ^b	2.91 ^a	2.99 ^a	3.10 ^a	2.97 ^a	3.08 ^a	0.134	0.045	0.053	0.101
P, mmol/L	2.13 ^b	2.03 ^b	2.00 ^b	2.85 ^a	2.36 ^{ab}	2.42 ^{ab}	0.164	0.019	0.106	0.103
ALP, U/L	518	476	466	457	418	427	65.1	0.914	0.353	0.515
25(OH)VD ₃ , ng/mL	61.9 ^c	130 ^a	111 ^{ab}	106 ^{ab}	97.1 ^{abc}	83.2 ^{bc}	12.08	0.036	0.384	0.166
PTH, ng/dL	120 ^a	114 ^a	105 ^{ab}	86.7 ^{ab}	71.8 ^b	71.0 ^b	12.33	0.043	0.121	0.110
CT, ng/L	335	358	343	340	261	230	50.5	0.557	0.088	0.887

SEM, standard error of the mean;

¹Each value represents the mean of 6 replicates.

^{a-c} Means within a row without a common superscript differ significantly ($P < 0.05$).

Table 4

Effects of dietary VD₃ levels on tibia parameters (tibial weight; tibial length; tibial ash; tibial Ca; tibial P; tibial density; tibial Ca + P content content) of 21-d-old geese ¹.

Dietary VD ₃ level, IU/kg	0	200	400	800	1600	3200	SEM	P value		
								treatment	linear	quadratic
Tibial weight, g	2.13	2.23	2.30	2.14	2.15	2.27	0.126	0.869	0.704	0.667
Tibial length, mm	82.8 ^b	86.0 ^{ab}	88.6 ^a	85.3 ^{ab}	87.0 ^a	85.9 ^{ab}	1.18	0.048	0.487	0.125
Tibial ash, g/kg	485	461	479	473	475	478	9.3	0.601	0.832	0.706
Ca ² , g/kg	166	167	171	181	181	177	8.0	0.581	0.270	0.188
P ² , g/kg	49.4	50.7	50.7	52.0	50.8	45.5	3.25	0.871	0.338	0.339
Tibial density, g/m ²	0.155 ^b	0.169 ^a	0.169 ^a	0.175 ^a	0.178 ^a	0.171 ^a	0.0034	0.002	0.018	0.003

SEM, standard error of the mean;

¹Each value represents the mean of 6 replicates.

²Ca and P content of tibial ash.

^{a-b} Means within a row without a common superscript differ significantly ($P < 0.05$).

Table 5

Effect of dietary VD₃ levels on mRNA expression of *VDR* (vitamin D₃ receptor), *PTHr* (parathyroid hormone receptor), *NCX1* (Na⁺/Ca²⁺ exchanger 1), and *PMCA1b* (plasma membrane calcium ATPase 1b) in the duodenum and kidney ¹.

Dietary VD ₃ levels		0	200	400	800	1600	3200	SEM	P value		
									treatment	linear	quadratic
Duodenum	VDR	1.00 ^b	0.94 ^b	1.22 ^b	1.32 ^b	1.32 ^b	2.01 ^a	0.161	0.020	< 0.001	0.694
	PTHr	1.00	0.98	1.06	1.51	1.65	1.44	0.352	0.565	0.181	0.210
	NCX1	1.00 ^b	1.04 ^b	0.94 ^b	2.30 ^a	3.28 ^a	2.90 ^a	0.354	< 0.001	< 0.001	0.002
	PMCA1b	1.00 ^b	0.95 ^b	1.29 ^b	1.16 ^b	1.40 ^b	3.05 ^a	0.346	0.004	< 0.001	0.153
Kidney	VDR	1.00 ^b	0.91 ^b	1.23 ^{ab}	1.10 ^b	1.02 ^b	1.84 ^a	0.202	0.047	0.007	0.200
	PTH1R	1.00 ^{ab}	1.10 ^{ab}	1.06 ^{ab}	1.69 ^a	1.55 ^a	0.40 ^b	0.291	0.043	0.025	0.033
	NCX1	1.00 ^b	1.85 ^b	1.64 ^b	1.42 ^b	2.13 ^b	3.80 ^a	0.462	0.046	0.002	0.438
	PMCA1b	1.00 ^c	0.96 ^c	1.06 ^{bc}	1.04 ^{bc}	1.41 ^{ab}	1.74 ^a	0.135	0.004	< 0.001	0.915

SEM, standard error of the mean;

¹Each value represents the mean of 5 replicates.

^{a-c} Means within a column without a common superscript differ significantly ($P < 0.05$).

4. Discussion

Birds could obtain VD₃ from the vitamin premix, endogenous production from exposure to ultraviolet (UV) light, and animal byproducts in diets (Tian et al., 1994). Modern indoor husbandry has resulted in an environment where birds receive almost no UV light, making dietary VD₃ has become the sole source (Jiang et al., 2015). In the absence of UV light in our study, the maximum ADG of geese fed a 400 IU VD₃/kg diet and the improved F/G by VD₃ supplementation were consistent with the positive VD₃ effects on broiler growth performance confirmed in previous studies (Browning and Cowieson, 2014; Jiang et al., 2015; Kheiri and Landy, 2019). However, dietary VD₃ levels from 400 to 3200 IU/kg decreased ADG of goslings from 1 to 21 d of age. These adverse effects on performance were in line with the broiler chickens fed diets with VD₃ supplementation levels above 20,000 IU/kg (Browning and Cowieson, 2014). It was suggested that excessive dietary VD₃ supplementation could induce a toxic effect on growth performance in growing poultry (Liu et al., 2022). The VD₃ requirement for the optimal F/G (246–352 IU/kg diet) and ADG (400–518 IU/kg diet) in Magang goslings from 1 to 21 d of age was higher than the NRC (1994) recommendation of 200 IU/kg diet. Similar results were also

Table 6
Estimated VD₃ requirement and model comparison.

Items	Model		ER	R ²	P value	Mean square error
F/G	One-slope straight broken line	$Y = 1.79 + 0.00142 (246 - X)$	246	0.395	0.005	0.0286
	Two-slope straight broken line	$Y = 1.74 + 0.00143 (278 - X), X < 278$ $Y = 1.74 + 0.000033 (X - 278), X \geq 278$	278	0.417	0.012	0.0290
ADG	Quadratic broken line	$Y = 1.78 + 2.83E-6 (352 - X)^2$	352	0.410	0.037	0.0275
	One-slope straight broken line	$Y = 33.3 + 0.00579 (400 - X)$	400	0.183	0.080	6.13
	Two-slope straight broken line	$Y = 33.7 + 0.00576 (400 - X), X < 400$ $Y = 33.7 + 0.00002 (X - 400), X \geq 400$	400	0.191	0.158	5.70
	Quadratic broken line	$Y = 33.6 - 9.37E-6 (518 - X)^2$	518	0.159	0.116	5.70
Tibial length	One-slope straight broken line	$Y = 87.7 - 0.0163 (243 - X)$	243	0.250	0.027	7.41
	Two-slope straight broken line	$Y = 87.3 - 0.0163 (282 - X), X \geq 282$ $Y = 87.3 - 0.0052 (X - 282), X < 282$	282	0.276	0.048	7.45
	Quadratic broken line	$Y = 87.6 - 0.00003 (344 - X)^2$	344	0.259	0.057	6.55
Tibial density	One-slope straight broken line	$Y = 0.175 - 0.00003 (519 - X)$	519	0.528	< 0.001	0.000048
	Two-slope straight broken line	$Y = 0.177 - 0.00003 (589 - X), X \geq 589$ $Y = 0.177 - 1.9E-6 (X - 598), X < 589$	589	0.550	0.001	0.000045
	Quadratic broken line	$Y = 0.174 - 3.81E-8 (698 - X)^2$	698	0.544	0.002	0.000043
	Quadratic broken line	$Y = 0.174 - 3.81E-8 (698 - X)^2$	698	0.544	0.002	0.000043

F/G, feed: gain ratio; ADG, average daily gain; ER, estimated VD₃ requirement; R², coefficient of determination.

The one-slope straight broken line model was described as that $y = L + U \times (R - x)$; The two-slope straight broken line model was described as $y = L + U \times (R - x) + V \times (x - R)$; The quadratic broken-line model was described as $y = L + U \times (R - x)^2$.

found in Chinese yellow-feathered broilers aged from 1 to 63 d (Jiang et al., 2015), broiler breeders aged from 37 to 66 weeks (Atencio et al., 2006), and Peking ducklings aged from 0 to 13 d (Rush et al., 2005). The above results indicated that the VD₃ requirements need to be urgently re-evaluated for modern meat birds as with the improvement of genetic evolution and upgrade of rearing system in poultry production (Wang et al., 2020).

Vitamin D₃ is involved in the regulation of Ca-P metabolism and Ca homeostasis (Crew et al., 2009) by enhancing the absorption of Ca and P (St-Arnaud, 2008; Haussler et al., 2013). 25(OH)D₃ is the major circulating VD₃ metabolite and the best measure of VD₃ status in vivo (Zerwekh, 2008; McCullough et al., 2009). Studies showed that serum 25(OH)D₃ concentration increased in response to dietary VD₃ levels and was accompanied by increases in serum Ca and P levels in broiler chickens (Whitehead et al., 2004; Rao et al., 2006; Rama Rao et al., 2009; Khan et al., 2010). It was consistent with our results in geese that the serum concentrations of 25(OH)D₃, Ca, and P increased in response to increased dietary VD₃ levels from 0 to 800 IU/kg diet. In general, dose-response curves of nutrient status markers normally follow a quadratic or curvilinear function, with the curve flattening or declining after reaching a transient plateau when the nutrient requirement is covered (Kiourtzidis et al., 2020). Thus, serum 25(OH)D₃ level reached a transient plateau from 200 to 800 IU VD₃/kg diet and then decreased when dietary VD₃ levels increased from 800 to 3200 IU/kg diet in our study. It was also confirmed our results that estimated VD₃ requirements based on growth performance and tibial parameters were both lower than 800 IU/kg of diet in geese aged from 1 to 21 d. In addition, Ca homeostasis was mainly regulated by PTH and CT, the secretion of PTH was provoked in response to hypocalcemia and the major function of CT is to reduce blood Ca levels (Huebner et al., 2006; Khanal and Nemere, 2008). In our study, dietary VD₃ levels decreased the serum PTH levels of geese, reflecting an increase in serum Ca levels, consistent with results conducted in broiler chickens (Jiang et al., 2015). The serum CT secretion was regulated mainly in response to serum Ca level (Naveh-Many and Silver, 1988). There was no significant change in serum CT level when the increased serum Ca level remained within the normal range regardless of dietary VD₃ supplementation (Chen et al., 2020).

The loads exerted on the skeleton might be up to 32 times higher due to the rapidly increased body weight of modern poultry breeds, requiring rapid skeletal adaption (Yair et al., 2012), therefore VD₃ deficiency could lead to impaired bone mineralization and rickets (Atencio et al., 2006). Onyango et al. (2003) suggested that tibial density might be a more sensitive indicator than the shear force for evaluating the VD₃ requirement. Bone density refers to the mass of material per volume of bone and was considered to reflect bone mineral content (Rath et al., 2000). In the current study, geese fed a VD₃-free diet had the shortest tibia and the lowest tibial density, which was in agreement with the results reported in broiler chickens by Whitehead et al. (2004) and Zhang et al. (2019) respectively. However, no VD₃ effect on tibia ash content in geese aged from 1 to 21 d was consistent with the results conducted in drakes aged from 0 to 13 d that no response to increasing concentrations of VD₃ from 826 to 8260 IU/kg on bone ash contents (Rush et al., 2005). It might be attributed to the fact that the dietary Ca-NPP ratio might be the primary factor affecting bone ash content compared to VD₃ (Rama Rao et al., 2009). In addition, no VD₃ effects on tibial Ca and P contents were found in geese fed a sufficient Ca-non-phytate phosphorus (NPP) ratio (0.84% Ca vs 0.42% NPP) diet in our study, which was consistent with the results reported in ducks aged from 1 to 21 d by Wang (2010). It could be due to that the positive effect of VD₃ on Ca-P metabolism is closely related to Ca and P contents in diets as well as the nutritional status of Ca and P in geese (Wang et al., 2020). Compared with the optimal VD₃ levels (246–518 IU/kg of diet) for growth performance, the tibial density response to higher VD₃ levels (519–698 IU/kg of diet) implied that the existing VD₃ requirements values based on growth performance of geese might not apply to achieving the full potential of bone development and mineralization. Furthermore, the three regression models for tibial density had the highest R² and lowest mean square error, the mean of three estimated VD₃ requirements of 602 IU/kg of diet might be the most appropriate VD₃ level for young geese. In addition, tibial density but not growth performance of Chinese yellow-feathered broilers also was used as sensitive indicators for estimating VD₃ requirements (Jiang et al., 2015) using the regression models with the higher R².

Vitamin D₃ is involved in regulating calcium absorption from the gut and interacted with PTH in the maintenance of calcium homeostasis (Steingrimsdottir et al., 2005). *NCX1* and *PMCA1b* are considered to be essential for intestinal transcellular Ca absorption (Christakos et al., 2003; Hoenderop et al., 2005; Lee et al., 2007). In our study, linear responses to increasing dietary VD₃ levels were demonstrated for mRNA expression of *VDR*, *NCX1*, and *PMCA1b* in the kidney and duodenum (Table 5, $P < 0.05$), reflecting an increase in serum Ca levels, which was in agreement with the results of previous studies (Glendenning et al., 2000; Centeno et al., 2011; Agrawal et al., 2012). The *PTHr* mRNA expression was linear and quadratic responded to increasing dietary VD₃ levels in the kidney (Table 5, $P < 0.05$), however, but not in the duodenum (Table 5, $P > 0.05$), demonstrating that the regulation of *PTHr* by dietary VD₃ is tissue-specific. Moreover, the response of *PTHr* mRNA and serum PTH levels to dietary VD₃ levels of geese was similar in our study.

5. Conclusion

Dietary VD₃ levels within the physiological range positively improved growth performance, Ca-P metabolism, and tibia development of goslings aged from 1 to 21 d. The VD₃ requirement of gosling aged from 1 to 21 d for the optimal F/G, ADG, and tibial density was estimated to be 246–352 IU/kg diet, 400–518 IU/kg diet, and 519–698 IU/kg diet, respectively. The most appropriate VD₃ requirement of young geese was 602 IU/kg of diet based on the mean estimated values of the three regression models for tibial density with the highest R² and lowest mean square error.

Ethical approval

All animal experiments in the present study were approved by the South China Agricultural University Experimental Animal Welfare Ethics Committee.

Statement on animal welfare

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

CRediT authorship contribution statement

H. Wang and X. Y. Wang: designed research and analyzed data; H. Wang, X. Y. Wang, Y. C. Zhan, B. Peng, and H. Q. Zhang: performed the research; W. C. Wang, L. Yang, and Y. W. Zhu: investigation; methodology; H. Wang and Y. W. Zhu: wrote the manuscript. All Authors have read the revised manuscript and have agreed to submit it in its current form for consideration for publication in Animal Feed Science and Technology.

Declaration of Competing Interest

There is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Effect of Dietary Zinc Level on Egg Production Performance and Eggshell Quality Characteristics in Laying Duck Breeders in Furnished Cage System

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Abstract

In order to investigate the effect of dietary Zn levels on laying performance, eggshell quality, and eggshell microstructure in Muscovy duck breeders under furnished cages. Firstly, the effects of age (35 weeks vs 40 weeks) and rearing system (littered floor vs furnished cage) on eggshell quality of laying duck breeders were studied (Exp. 1). Then, a total of 324 30-week-old Muscovy duck breeders were allotted into 3 dietary Zn groups with 6 replicates (18 ducks per replicate), including 0 mg Zn/kg (control-Zn group, C-Zn), 40 mg Zn/kg (normal-Zn group, N-Zn), and 140 mg Zn/kg (high-Zn group, H-Zn). The experimental period for 6 weeks was divided into 3 periods of 30–32, 32–34, and 34–36 weeks of age (Exp. 2). In Exp. 1, duck breeder eggs in the furnished cage system had lower the average shell thickness than birds in the littered floor system at 40 weeks of age ($P < 0.05$), not at 35 weeks of age. In Exp. 2, N-Zn and H-Zn groups had greater egg weight, egg production, and egg to feed ratio of duck breeders than C-Zn group ($P < 0.05$). Additionally, H-Zn group had higher laying rate, qualified egg ratio, and Haugh unit as well as lower mammillary cone width than C-Zn group ($P < 0.05$), with no differences between C-Zn and N-Zn groups ($P > 0.05$). Diet supplemented with 140 mg Zn/kg increased shell thickness and palisade layer thickness of duck breeders at 36 weeks of age ($P < 0.05$), but not at 32 and 34 weeks of age. In conclusion, diets with 40 or 140 mg Zn/kg improved egg production performance and egg quality of laying duck breeders during 30–36 weeks of age in a furnished cage system. Dietary supplementation of 140 mg Zn/kg level increased the ultrastructural palisade layer thickness contributing to greater eggshell thickness of duck breeders at 36 weeks of age.

Keywords Duck breeders · Eggshell microstructure · Eggshell thickness · Zinc

Introduction

With the development of duck industry the traditional free-range rearing system resulted in the low nutrient efficiency high incidence of diseases and severe water pollution of waste in duck production [1, 2]. The duck housing system is

being gradually shifted from the non-caged system to the caged system to ensure the better health status and maximal economic benefit in China. However one major issue was the fact that the caged system provided a restricted physical and psychological space and prevented birds from performing natural behaviors compared with the non-caged system [3].

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Moreover the caged system exerted some negative effects on the productivity of laying birds due to limited movement such as poor bone strength [4] and egg quality [5, 6]. Poor eggshell quality characteristics could reduce the shelf life and safety of eggs and egg products [7]. For example the decreased eggshell thickness could increase the risk of the bacterial contamination in hatching eggs [8] and then impair hatchability performance of laying breeders [9]. However there is limited systematic information available on eggshell quality of duck breeders in alternative furnished cage system. Some researchers have proposed that the effects of different housing systems on eggshell quality were also dependent on the layer age [10, 11]. Therefore we firstly investigated the effects of bird age and rearing system (floor pen vs furnished cage) on eggshell quality of laying duck breeders

Zinc (Zn) as a component of carbonic anhydrase (CA) enzyme plays a role in eggshell formation [11, 12]. Many studies have showed that dietary Zn supplementation enhanced eggshell quality by increasing eggshell thickness and strength of hen layers and duck layers [13, 14]. Dietary Zn requirement recommendations for Peking duck breeders in NRC (1994) [15] and Muscovy duck breeders in INRA (1989) [16] were sourced from the data based on the non-caged systems. However, caged system that allows less movement of birds could influence feed intake and energy expenditure of birds [17]. Thus, nutrient requirements should be modified as with the changes of physical activity and energy intake of birds under the different housing systems [6]. So far, there has been little research on dietary Zn nutrition for improving eggshell quality of duck breeders reared in furnished cage system. Therefore, the effects of dietary Zn levels on laying performance, egg quality, and eggshell microstructure were investigated in Muscovy duck breeders under furnished cages in the present study.

Materials and Methods

Animals and Diets

All procedures of our experiments were approved by animal care and welfare committee institute of South China Agricultural University. Firstly, the eggshell quality of laying Muscovy duck breeders reared in two rearing technologies, a cage-free system and a furnished cage system, was compared (Exp. 1). Sixty Muscovy ducklings were selected and housed in the plastic floor system (from 0 to 12 weeks of age) and caged-free littered system (from 13 to 27 weeks of age) at the early laying period. After that, sixty laying duck breeders were divided into two groups consisting of thirty birds, which were placed into littered floor system (LF) and furnished cage system (FC), respectively. The littered system had an indoor and outside dirt

lot, which both had perches (15 cm² of area per duck) and nest box (4 ducks per nest). The floor in the indoor area was covered with wood shavings as a litter material. The outside area without grass or forage had a shelter and was enclosed by wire fences to keep out predators. A total of seven and eight ducks per square meter were provided in the indoor and outside areas, respectively. The furnished cage met the requirement of the EU Directive 1999/74/EC. The furnished cage units were made of a wire mesh floor, galvanized metal partitions between the cages, and full-opening fronts at each long side of the cages consisting of spaced horizontal bars. All laying duck breeders' ducks were fed restrictively with a commercial feed at the same nutritional level (11.82 MJ ME/kg, 180 g CP/kg, 8.0 g lysine/kg, 7.2 g methionine + cysteine/kg, 33.0 g Ca/kg, 3.8 g available P/kg). Water was available ad libitum. The droppings were automatically removed on manure belts. The 20 eggs were collected from the laying birds between at the end of 35 and 40 weeks of age, respectively. A total of 40 eggs from each rearing system were analyzed for eggshell quality. In Exp. 2, a total of 370 26-week-old Muscovy duck breeders obtained from a commercial duck breeder farm (WENS Group, Yunfu, Guangdong) were housed in furnished cages for 4 weeks of an adaptation period. During the early laying period, all ducks were fed restrictively with a commercial feed at the nutritional level (11.32 MJ ME/kg, 180 g CP/kg, 7.0 g lysine/kg, 7.2 g methionine + cysteine/kg, 24.0 g Ca/kg, 3.8 g available P/kg, 40 mg Zn/kg). Water was provided ad libitum. At 30 weeks of age, 324 laying duck breeders were selected, balanced for laying rate, and then randomly allotted into 3 dietary Zn groups with 6 replicates (18 ducks per replicate). The experimental diets included base diet without Zn supplementation (control-Zn group, C-Zn), basal diet + 40 mg Zn/kg (normal-Zn group, N-Zn), and basal diet + 140 mg Zn/kg (high-Zn group, H-Zn) as ZnSO₄·7H₂O source. The basal diet was formulated to meet or exceed the nutritional requirements of laying duck breeders except Zn. The composition and nutrient level of the basal diet were shown in Table 1. The analyzed values of Zn contents in C-Zn, N-Zn, and H-Zn diets were 29.2, 63.4, and 163.4 mg/kg, respectively. The experimental period lasting for 6 weeks was divided into 3 periods of 30–32, 32–34, and 34–36 weeks of age. All ducks had diets restrictively and access to water ad libitum according to the operation manual and guideline of Muscovy duck breeders. The ducks received 16 h of daily lighting from 04:30 am to 20:30 pm. Room temperature and humidity were controlled by the air conditioner and recorded daily. Manure was removed through an automatic belt system daily. All eggs were collected from each replicate daily and egg production (number of total laid eggs, defective eggs, and average qualified egg weight) were recorded daily. Feed consumption and

Table 1 Composition and nutrient levels of the basal diets for laying duck breeders during 30–36 weeks of age (as-fed basis)

Item (%)	Laying period
Corn	51.67
Soybean meal	17.70
Corn gluten meal	7.75
Wheat middlings	8.97
Lard	1.84
Dicalcium phosphate	1.80
Limestone	8.50
Sodium chloride	0.30
DL-Methionine	0.27
L-lysine·HCl	0.20
Vitamin and mineral premix ¹	1.00
Total	100.00
Nutrient composition	
Calculated value (%)	
ME, MJ/kg	11.63
Crude protein	18.51
Calcium	3.70
Total phosphate	0.60
Non-phytin phosphorus	0.44
Lysine	0.91
Methionine	0.57
Methionine + cysteine	0.84
Zinc ²	29.2

¹ Provided per kilogram of diet without Zn addition: vitamin A, 5000 IU; vitamin D₃, 800 IU; vitamin E, 20 IU; thiamine, 2.0 mg; riboflavin, 15 mg; pyridoxine, 4.0 mg; vitamin B₁₂, 0.02 mg; calcium pantothenate, 10 mg; folate, 0.15 mg; niacin, 60 mg; biotin, 0.20 mg; choline (choline chloride), 1500 mg; Cu (CuSO₄·5H₂O), 8 mg; Fe (FeSO₄·7H₂O), 80 mg; Mn (MnSO₄·H₂O), 100 mg; Se (NaSeO₃), 0.3 mg; I (KI), 0.4 mg

² Analyzed values based on triplicate determinations

egg weight were measured weekly. Feed intake was calculated by dividing total feed consumed by numbers of ducks per replicate per day. Four eggs based on the average egg weight each replicate were collected at the last day of each period for the measurements of egg quality and eggshell microstructure.

Sample Analyses

The Zn concentrations in diets were measured using atomic absorption spectrophotometry (model Shimadzu AA-6800, Tokyo, Japan). At the end of 32, 34, and 36 weeks of age, four eggs per replicate were used to measure the related indices of egg quality. The color of the yolk and albumen height from the collected eggs were determined using an egg multitest (model EMT-5200, Touhoku Rhythm Ltd., Tokyo, Japan). Haugh unit was calculated based on egg weight and albumen height (Haugh, 1937). Shell strength

was determined with an Egg Force Reader (model EFR-01, Orka, Ramat HaSharon, Israel). Eggshell was separated from albumen and yolk, washed to remove residual albumen, and cleaned by a paper towel and then weighed. Eggshell thickness was measured without inner and outer shell membranes at the blunt end (bottom), sharp end (top), and middle using a peacock dial pipe gauge (model P-1, Ozaki MFG Ltd., Tokyo, Japan). Shell thickness was calculated as the average thickness at the three points of eggshell. Eggshell microstructures were measured by scanning electron microscopy as described by Berwanger et al. (2018) [17]. Average values of the mammary cone width and the thickness of mammary layer, palisade layer, and eggshell membrane were estimated from 3 measurements (μm) in each photograph.

Statistical Analysis

Data were analyzed by 2-way ANOVA using the PROC GLM procedure of the SAS 9.2 (SAS Inst. Inc., Cary, NC). The models included rearing system and age, and their interactions for Exp. 1, as well as dietary Zn, period, and their interactions for Exp. 2. The replicate served as the experimental unit. Differences among means were tested by the LSD method, and statistical significance was set at $P \leq 0.05$.

Results

Eggshell Quality

Shell weight, shell strength, and shell thickness were affected by age ($P < 0.01$), not by rearing system ($P > 0.05$). The eggs from laying duck breeders at 40 weeks of age had higher shell weight and weaker shell strength and thickness than those from birds at 35 weeks of age. The interaction between age and system affected the average shell thickness ($P = 0.05$) and had no effect on other measured indices ($P > 0.05$). The rearing system did not influence the average shell thickness of duck breeder eggs at 35 weeks of age, whereas FC system had a lower average shell thickness of duck breeder eggs at 40 weeks of age compared with the LF system (Table 2).

Egg Production Performance

Egg weight, laying rate, egg production, egg to feed ratio, and qualified eggs ratio were affected by age ($P < 0.0001$) and dietary Zn ($P < 0.05$), not by their interaction ($P > 0.13$). Egg weight, laying rate, egg production, egg to feed ratio, and qualified eggs ratio were increased as age and dietary Zn level increased ($P < 0.04$). Egg weight, laying rate, egg production, egg to feed ratio, and qualified eggs ratio of duck breeders during 32–34 weeks and 34–36 weeks of age were greater than birds during 30–32 weeks of age ($P < 0.0001$),

Table 2 Effects of age and rearing system on eggshell quality of laying duck breeders at 35 and 40 weeks of age

Age (week)	System	Shell weight (g/egg)	Shell strength (kg/N)	Shell thickness (cm)			
				Top	Middle	Bottom	Average
35 ¹	LS	9.51	5.77	0.423	0.427	0.418	0.423 ^a
	FC	9.31	6.19	0.421	0.431	0.42	0.424 ^a
40 ¹	LS	10.43	4.37	0.347	0.367	0.359	0.358 ^b
	FC	11.28	4.15	0.315	0.336	0.329	0.329 ^c
System ²	SEM	0.29	0.31	0.01	0.01	0.01	0.008
	LS	9.97	5.07	0.39	0.397	0.389	0.391
	FC	10.30	5.17	0.37	0.384	0.375	0.377
Age ³	SEM	0.27	0.25	0.01	0.01	0.01	0.006
	35 weeks	9.41	5.98	0.42	0.429	0.419	0.424
	40 weeks	10.86	4.26	0.33	0.352	0.344	0.344
P value	SEM	0.23	0.20	0.01	0.01	0.01	0.006
	System	< 0.0001	< 0.001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Age	0.32	0.46	0.86	0.28	0.22	0.27
	Age*System	0.59	0.10	0.17	0.17	0.20	0.05

^{a-c} Means within a column lacking a common superscript differ ($P < 0.05$)

¹ Values represented the means of 20 replicates ($n = 20$)

² Values represented the means of 40 replicates ($n = 40$)

³ Values represented the means of 40 replicates ($n = 40$)

with no differences between the periods of 32–34 weeks and 34–36 weeks of age ($P > 0.05$). Compared with C-Zn group, N-Zn and H-Zn groups increased egg weight, egg production, and egg to feed ratio of duck breeders ($P < 0.02$), with no differences between N-Zn and H-Zn groups ($P > 0.36$). Duck breeder from H-Zn group had higher laying rate and qualified egg ratio than C-Zn group ($P < 0.01$), and no differences were observed between C-Zn and N-Zn groups as well as N-Zn and H-Zn groups ($P > 0.24$) (Table 3).

Egg Quality

Shell thickness, Haugh unit, and yolk color were affected by duck age ($P < 0.03$). Dietary Zn and the interaction between age and dietary Zn influenced ($P < 0.04$) Haugh unit and shell thickness, respectively. Age, dietary Zn, and their interaction had no effects ($P > 0.05$) on the shell weight and shell strength of duck breeders. Eggs from ducks at 34 weeks old had higher Haugh unit and lower shell thickness than birds at 32 and 36 weeks old ($P < 0.03$), while egg yolk color from ducks at 34 and 36 weeks old was higher than birds at 32 weeks old ($P < 0.01$). Haugh unit in H-Zn group was higher ($P < 0.02$) than C-Zn group, with no differences between C-Zn and N-Zn groups. Dietary Zn level had no effect on shell thickness of duck breeders at 32 and 34 weeks of age ($P > 0.27$), whereas dietary supplementation of 40 and 140 mg Zn/kg increased

shell thickness of duck breeders at 36 weeks of age compared with the control group ($P < 0.05$) (Table 4).

Eggshell Microstructure

The mammillary cone width and thickness were affected ($P < 0.02$) by bird age. Dietary Zn level had an effect ($P < 0.02$) on mammillary cone width and did not affect ($P > 0.05$) mammillary cone width, palisade layer thickness, and membrane thickness. The interaction between age and dietary Zn influenced ($P = 0.05$) palisade layer thickness of ducks. Eggs from ducks at 32 and 34 weeks old had greater mammillary cone width than birds at 36 weeks old, while H-Zn group had a lower mammillary cone width and a greater membrane cross-linking structure than C-Zn and N-Zn groups ($P < 0.01$; Figs. 1 and 2). Dietary Zn supplementation had no effect on palisade layer thickness of duck breeders at 32 and 34 weeks of age ($P > 0.48$), whereas H-Zn group increased palisade layer thickness of duck breeders at 36 weeks of age compared with C-Zn group ($P < 0.04$), with no differences between C-Zn and N-Zn groups ($P > 0.72$) (Table 5).

Discussion

Broken egg problem is a large financial loss to the poultry industry. The eggshell quality is one of the most important

Table 3 Effects of age and dietary Zn on egg production performance of duck breeders at 30 to 36 weeks of age under furnished cage system

Period (week)	Dietary Zn (mg/kg)	Egg weight (g)	Laying rate (%)	Egg production (g/bird/d)	Feed intake (g/bird/d)	Egg to feed ratio	Qualified egg ratio (%)
30–32 ¹	0	74.3	73.4	53.4	153.6	0.35	69.4
	40	76.2	77.1	58.4	154.4	0.38	73.8
	140	75.6	81.7	61.7	154.2	0.40	80.0
32–34 ¹	0	75.7	84.2	65.7	156.3	0.42	83.3
	40	76.6	88.0	69.1	155.5	0.44	86.0
	140	77.1	90.4	70.3	156.4	0.45	89.5
34–36 ¹	0	76.1	88.2	67.5	157.8	0.43	87.1
	40	77.3	90.9	70.9	157.6	0.45	89.6
	140	78.6	92.6	73.0	156.8	0.47	91.8
	SEM	0.39	2.65	2.00	0.53	0.01	2.67
Age ²	30–32	75.4 ^b	77.4 ^b	57.8 ^b	154.1 ^c	0.38 ^b	74.4 ^b
	32–34	76.5 ^a	87.5 ^a	68.3 ^a	156.1 ^b	0.44 ^a	86.3 ^a
	34–36	77.3 ^a	90.5 ^a	70.5 ^a	157.4 ^a	0.45 ^a	89.5 ^a
	SEM	0.24	1.58	1.19	0.29	0.007	0.22
Zn ³	0	75.4 ^b	81.9 ^b	62.2 ^b	155.9	0.40 ^b	79.9 ^b
	40	76.7 ^a	85.3 ^{ab}	66.1 ^a	155.8	0.42 ^a	83.2 ^{ab}
	140	77.1 ^a	88.2 ^a	68.3 ^a	155.8	0.44 ^a	87.1 ^a
	SEM	0.22	1.53	1.16	0.28	0.007	1.54
	Age	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Zn	< 0.0001	0.03	0.004	0.96	0.001	0.01
	Age × Zn	0.14	0.96	0.93	0.25	0.81	0.87

^{a–b} Means within a column lacking a common superscript differ ($P < 0.05$)

¹ Values represented the means of 6 replicates ($n = 6$)

² Values represented the means of 18 replicates ($n = 18$)

³ Values represented the means of 18 replicates ($n = 18$)

parameters for maintaining the shelf life and the safety of eggs and egg products [7]. For poultry breeders, good eggshell quality could prevent the hatching eggs from bacterial contamination [8] to maintain hatchability performance [9]. Several studies have proved that eggshell characteristics could be affected by bird age and different housing systems in laying hens and breeder hens [10, 18]. In the current study, eggshell parameters such as weight, strength, and thickness were decreased with the increased age of duck breeders at 35–40 weeks of age regardless of rearing systems. Moreover, significant interaction between age and housing system on eggshell thickness demonstrated that no differences were observed on the average shell thickness of duck breeder eggs at 35 weeks of age under two rearing systems, whereas FC system had a lower average shell thickness than LF system in duck breeders at 40 weeks of age. It was suggested that shell thickness of a duck breeder in the FC system was decreased much greater as the age was increased. The declined eggshell quality of duck breeders in the FC system was confirmed by the comparison of eggshell quality in laying hens under the battery cage and deep litter systems [19]. It is probably due to the lack of exercise and poor bone mineral mobilization of birds in the FC

system contributing to the poor mineralization during eggshell formation [4]. Another factor was that providing insufficient light intensity for laying birds at the lower tiers in multitier cage systems could induce the less calcium mobilization for eggshell formation resulting in the lower shell thickness [20]. Additionally, compared with the non-caged system, the caged system was characterized with the restricted space that allowed less movement of birds [21], which further affected physical activity, energy expenditure, and nutrient requirement of birds [6]. Therefore, it is very important to change the nutritional strategies to improve eggshell quality when duck breeders were shifted from the non-caged systems to FC systems.

Egg weight, egg production, and egg quality could be influenced by bird age [22, 23]. As the age was increased from 30 to 36 weeks old, egg production performance and yolk color of female breeders were increased linearly. The enhancement in egg production with advancing age was exhibited by the lower ovulation rate and shorter ovulation and oviposition sequence from laying early period to laying peaking period [23, 24]. Zn is a cofactor of more than 200 enzymes functioning in diverse physiological processes to maintain the

Table 4 Effects of age and dietary Zn on egg quality of duck breeders at 32, 34, and 36 weeks of age under furnished cage system

Age (weeks)	Dietary Zn (mg/kg)	Shell weight (g/egg)	Shell strength (kg/N)	Shell thickness (cm)	Haugh unit	Yolk color
32 ¹	0	9.01	5.92	0.386 ^{ab}	72.2	8.28
	40	9.07	6.20	0.387 ^{ab}	73.2	8.06
	140	9.29	5.96	0.374 ^{bc}	76.1	8.31
34 ¹	0	9.15	6.17	0.363 ^c	80.3	8.60
	40	9.01	6.08	0.360 ^c	77.6	8.67
	140	9.13	6.20	0.369 ^{bc}	83.1	8.32
36 ¹	0	8.85	6.00	0.355 ^c	72.1	8.46
	40	8.97	5.99	0.390 ^a	71.0	8.41
	140	9.19	6.11	0.395 ^a	74.4	8.40
	SEM	0.14	0.14	0.008	1.9	0.13
Age ²	32	9.13	6.03	0.383	73.8 ^b	8.21 ^b
	34	9.09	6.15	0.364	80.4 ^a	8.53 ^a
	36	9.00	6.03	0.380	72.5 ^b	8.42 ^a
	SEM	0.08	0.08	0.005	1.1	0.07
Zn ³	0	9.00	6.03	0.368	74.9 ^b	8.44
	40	9.02	6.09	0.379	73.9 ^b	8.38
	140	9.20	6.09	0.379	77.9 ^a	8.34
	SEM	0.08	0.08	0.005	1.1	0.07
	Age	0.51	0.48	0.02	< 0.0001	0.01
	Zn	0.17	0.81	0.15	0.03	0.59
	Age × Zn	0.73	0.59	0.03	0.88	0.23

^{a-b} Means within a column lacking a common superscript differ ($P < 0.05$)

¹ Values represented the means of 6 replicates ($n = 6$)

² Values represented the means of 18 replicates ($n = 18$)

³ Values represented the means of 18 replicates ($n = 18$)

optimum productive performance of poultry breeders [25]. The observations that dietary Zn deficiency impaired egg production and egg quality in layer and breeder birds were well established [26]. Our results showed that dietary Zn supplementation increased egg weight, egg production, egg to feed ratio, and Haugh unit in duck breeders housed in the FC system during 30–36 weeks old. The positive effects of Zn addition on laying performance were confirmed in laying hens [27–29] and laying ducks [14]. Dietary Zn nutrition could improve the epithelium quality via increasing the protein synthesis deposition of albumen, resulting in greater values of egg production and Haugh unit. However, other studies reported that adding Zn to the diets had no effects on the characteristics of egg production of laying hens and broiler breeders [30, 31]. The differences between the studies might depend on the differences in dietary Zn levels, Zn content in basal diets, experimental periods as well as the breed differences, ages, and physiological states of birds under different rearing systems. Moreover, the diets with 140 mg Zn/kg improved laying rate and qualified egg ratio compared with the control diet, whereas no differences were observed between the control diet and diet with 40 mg Zn/kg. It is implied that dietary higher Zn

level revealed the greater beneficial effect on egg production and egg quality in laying duck breeders in the FC system. Abd El-Hack et al. (2018) also stated that laying hens fed diet supplemented with 100 mg Zn/kg as Zn-Met source had greater egg production compared with birds fed basal diet supplemented with 50 mg Zn/kg as Zn-Met source [29]. One possible explanation for improvement in egg production may be the interaction of Zn with the secretion of reproductive hormones of the endocrine system during egg formation [32, 33].

Zinc is part of the CA enzyme which is essential in supplying carbonate ions during eggshell formation [34]. Numerous studies have shown that dietary Zn supplementation enhanced eggshell quality by increasing eggshell thickness and strength in laying birds [11, 12]. However, eggshell quality of laying ducks were not affected by dietary Zn level in the present study, which does not agree with results reported in laying hens [13]. The inconsistent results may be due to the differences in the Zn content and supplemental Zn levels in basal diets, experimental periods, age, and rearing systems of birds. In the present study, the basal diet containing about 29 mg Zn/kg diet seem to be sufficient for maintaining the eggshell quality in a shorter period during 30–34 weeks, according to

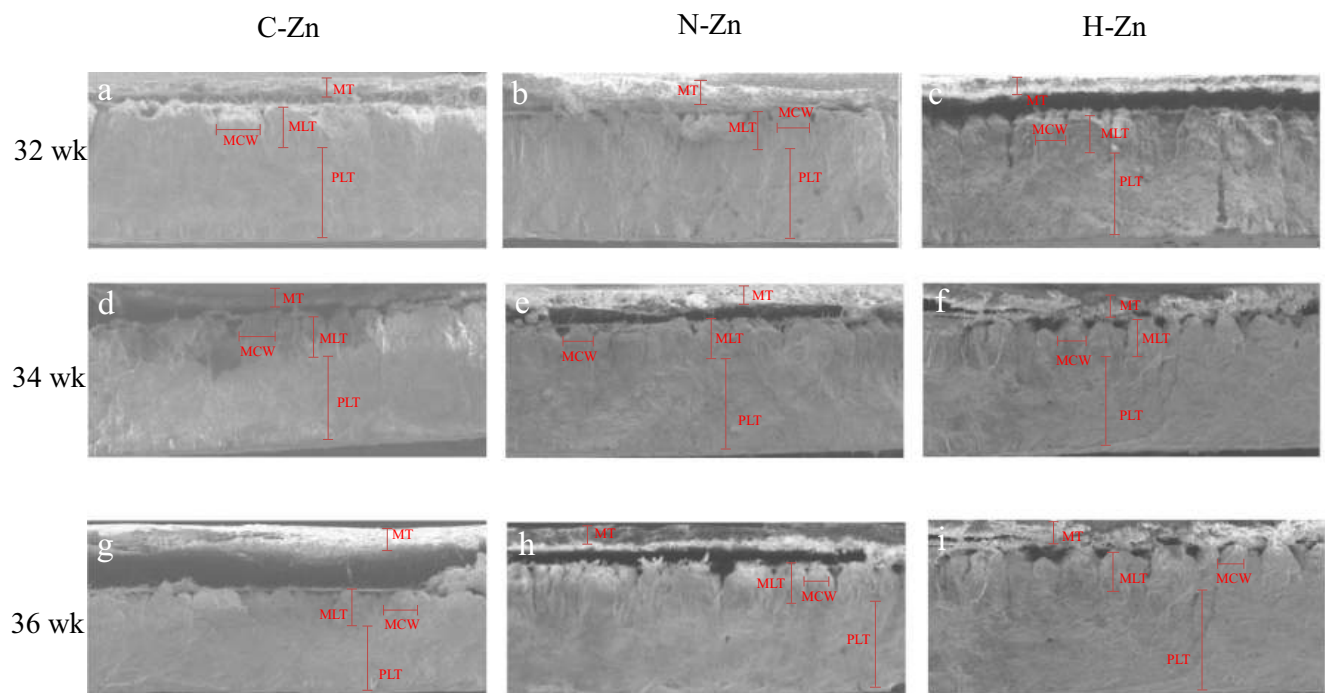


Fig. 1 Scanning electron cross-sections of eggshells from duck breeders fed C-Zn (A, D, G), N-Zn (B, E, F), and H-Zn (C, F, I) diets at 32, 34, and 36 weeks of age ($\times 200$). C-Zn, control Zn group with 0 mg Zn/kg diet; N-

Zn, normal Zn group with 40 mg Zn/kg diet; H-Zn, high Zn group with 140 mg Zn/kg diet; MT, membrane thickness; MCW, mammillary cone width; MLT, mammillary layer thickness; PLT, palisade layer thickness

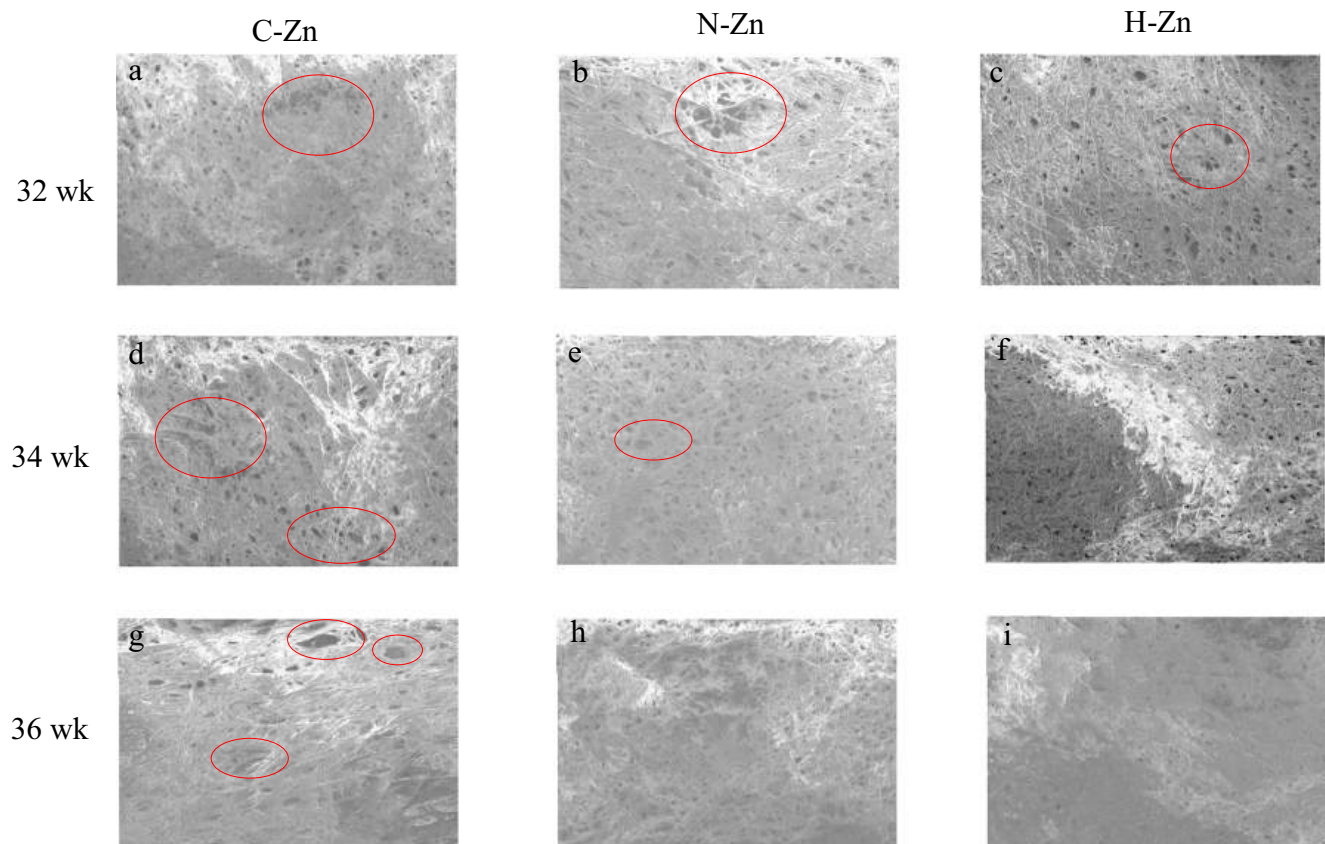


Fig. 2 Scanning electron fiber cross-linking structure of eggshell membranes from duck breeders fed C-Zn (A, D, G), N-Zn (B, E, F), and H-Zn (C, F, I) diets at 32, 34, and 36 weeks of age ($\times 600$). C-Zn, control Zn group with

0 mg Zn/kg diet; N-Zn, normal Zn group with 40 mg Zn/kg diet; H-Zn, high Zn group with 140 mg Zn/kg diet. The representative defective fiber cross-linking structure of eggshell membranes was indicated in red circle

Table 5 Effects of age and dietary Zn on eggshell microstructure of duck breeders at 32, 34, and 36 weeks of age under furnished cage system

Age (weeks)	Dietary Zn (mg/kg)	Mammillary cone width (μm)	Mammillary layer thickness (μm)	Palisade layer thickness (μm)	Membrane thickness (μm)
32 ¹	0	92.8	111	276 ^{ab}	60.7
	40	85.1	114	276 ^{ab}	59.6
	140	79.3	102	269 ^{ab}	62.5
34 ¹	0	85.6	125	249 ^b	66.2
	40	78.0	117	257 ^{ab}	60.7
	140	74.4	124	254 ^{ab}	51.6
36 ¹	0	77.5	112	244 ^b	64.9
	40	69.4	110	253 ^{ab}	75.0
	140	61.3	106	289 ^a	58.3
	SEM	5.4	6	15	6.7
Age ²	32	85.7 ^a	109 ^b	274	60.9
	34	79.3 ^a	122 ^a	253	59.5
	36	69.4 ^b	110 ^b	262	66.0
	SEM	3.1	3	8	3.8
Zn ³	0	85.3 ^a	116	256	62.8
	40	77.5 ^{ab}	114	262	67.3
	140	71.7 ^b	111	271	60.4
	SEM	3.1	3	8	3.8
	Age	0.003	0.01	0.76	0.46
	Zn	0.01	0.49	0.36	0.33
	Age \times Zn	0.99	0.56	0.05	0.50

^{a-b} Means within a column lacking a common superscript differ ($P < 0.05$)

¹ Values represented the means of 6 replicates ($n = 6$)

² Values represented the means of 18 replicates ($n = 18$)

³ Values represented the means of 18 replicates ($n = 18$)

the INRA (1989) recommendation of 20–40 mg Zn/kg of laying Muscovy duck breeders. The lacking effect of Zn addition on eggshell quality was consistent with the previous results in laying ducks fed a corn-soybean meal basal diet containing 37 mg Zn/kg during laying early and peak periods [14]. Although dietary Zn level had no effect on shell thickness of duck breeders at 32 and 34 weeks of age, whereas Zn supplementation in diets improved shell thickness of duck breeders at 36 weeks of age compared with the control diet. The interaction between age and dietary Zn on shell thickness implied that dietary Zn nutrition has a prolonged and accumulative beneficial effect on maintaining eggshell thickness of laying duck breeders. As the feeding time was increased, feeding Zn non-supplemented diet plus the lack of exercise of birds in the FC system resulted in the more severe body Zn depletion and insufficient bone Zn mobilization and then revealed the negative effect on shell thickness. Zhang et al. (2017) also indicated that Zn deficiency impaired eggshell

thickness associated with the decreased CA activity during eggshell formation for the 6 weeks of experimental period [11].

The eggshell is structurally combined by organic and inorganic components [35]. The organic components of eggshell consisted of ultrastructural layers divided into shell membrane, mammillary knob, palisade, and cuticle [36]. The assembly and calcitic mineralization of different eggshell compartments are thought to be guided by organic molecules, including non-collagenous proteins and proteoglycans [37]. It has been shown that the enzymatic activity as trace cofactors influenced the formation of the mammillary layer in the region of the oviduct termed the tubular shell gland [12]. In the present study, scanning electron photographs results showed that 140 mg Zn/kg addition in diet reduced the mammillary cone width of eggshell compared with the control diet, which was in agreement with laying hens fed Mn-supplemented diet [38]. The decreased size of mammillary cones due to Zn intake implied that the number of mammillary cones per unit area on the internal shell membrane was increased. It was thought that more mammillary buttons grew into adjacent palisade column by calcitic mineralization and then resulted in a greater resistance of breakage [39]. The palisade layer accounts for about two-thirds of the thickness of the calcified eggshell. Diet supplemented with 140 mg Zn/kg increased the palisade layer thickness of duck breeders at 36 weeks of age compared with the control diet, with no effects observed in duck breeders at 32 and 34 weeks of age, which coincided exactly with the results of shell thickness in the present study. It is suggested that Zn nutrition could contribute to eggshell quality via improving the ultrastructural features. Previous study has demonstrated that proteoglycans plays a key role in the crystallization of the eggshell [40]. The Zn addition in diets could stimulate the proteoglycans synthesis to promote the eggshell nucleation and crystalline palisade growth [41, 42]. In addition, the greater Zn addition could enlarge the negative effect of the presence of high Ca concentration (3.7%) and phytate in basal hen diet due to the formation of an insoluble Ca-phytate-Zn complex [43, 44]. Zn also plays a role in deposition of albumen in the magnum and production of eggshell membranes in the isthmus [26]. Although the outer shell membrane thickness is not affected by dietary Zn levels, the poor membrane fiber formation or cross-linking structure occurred in dietary Zn deficiency and was characterized by abnormal compactness and distribution uniformity of fibers. It is implied that the enhancement of ultrastructural structure of shell membrane may produce resistant eggshells and then prevent the moisture loss of inner egg components due to Zn addition, which contributed to the qualify egg ratio and Haugh unit score in the present study.

In conclusion, dietary Zn supplementation improved the egg production performance and Haugh unit of laying duck breeders reared in the FC system during 30–36 weeks of age,

displaying the greater beneficial effect for duck breeders fed a diet containing 140 mg Zn/kg. In addition, dietary high level of 140 mg Zn/kg increased ultrastructural palisade layer thickness contributing to the greater eggshell thickness of duck breeders at 36 weeks of age.

Authors' Contributions YWZ, LH, and JJS were responsible for the planning of the study, sample collections, analyses, and the manuscript writing. DQL, WCW, and YF were involved in the sample collections, biological analysis, and statistical analyses. LY and YWZ were involved in the experimental design and data interpretations. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

All procedures of our experiments were approved by the animal care and welfare committee institute of South China Agricultural University.

Competing Interests The authors declare that they have no competing interests.

Abbreviations *FC*, furnished cage; *LF*, littered floor; *CA*, carbonic anhydrase; *Zn*, zinc; *C-Zn*, control Zn group with 0 mg Zn/kg diet; *N-Zn*, normal Zn group with 40 mg Zn/kg diet; *H-Zn*, high Zn group with 140 mg Zn/kg diet

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ORIGINAL ARTICLE

Effect of dietary *Moringa* stem meal level on growth performance, slaughter performance and serum biochemical parameters in geese

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Abstract

Moringa stem meal (MSM) with a high level of crude fibre (CF) might be developed and utilized in herbivorous geese as an unconventional feedstuff. The aim of this study was to investigate the effect of the MSM level in the diet on the growth performance, slaughter performance, breast meat quality and serum biochemical parameters in geese from 22 to 70 days of age. A one-factor completely randomized design was adopted in our study. A total of one thousand eight 21-day-old geese were randomly divided into six groups, with six replicates per group and 28 birds per replicate. The geese were fed diets containing MSM levels of 0, 20, 40, 60, 80 or 100 g/kg during day 22–70. The dietary MSM level had no effect ($p > .05$) on the final body weight (BW), average daily gain (ADG) or average daily feed intake (ADFI). The feed/gain ratio (F/G) increased linearly ($p < .001$) as the dietary MSM level increased. No differences ($p > .05$) were observed in the slaughter performance, meat quality and the relative organ weight (except for thymus) of the geese ($p > .05$). The relative weight of the thymus in the geese fed diets with supplementation of MSM was higher than that in the non-supplemented MSM control group ($p < .05$). In addition, 100 g MSM/kg of diet decreased the serum glucose (GLU) level ($p < .05$) and increased the alanine transaminase (ALT) enzyme activity ($p = .03$). Dietary MSM levels of no more than 60 g/kg had no effects on the growth performance and slaughter performance, whereas diets with 100 g MSM/kg increased the F/G and serum ALT enzyme activity, as well as decreasing the serum GLU level. Therefore, MSM provided at a reasonable level could be developed as an unconventional feedstuff for geese at the finisher period.

KEYWORDS

geese, growth performance, *Moringa* stem meal, serum parameters, slaughter performance

1 | INTRODUCTION

In developing countries, the global poultry industry is expected to continue to grow because the demand for meat and eggs is driven by growing populations, rising incomes and urbanization (Mottet & Tempio, 2017). Along with the rapid development of poultry

husbandry, a shortage of conventional feed, and soaring prices for the raw material for feed have become severe problems. Therefore, the development of unconventional feedstuff resources for poultry feed formulation could not only reduce the feed cost but also prevent environmental pollution (Khatun & Khan, 2015).

Moringa (*Moringa oleifera*), which originated in India, is a slender, deciduous and perennial evergreen tree. *Moringa* has high

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biomass yield and crude protein (CP, 25%) and a balance of other nutrients, such as neutral detergent fibre (NDF, 21.9%), acid detergent fibre (ADF, 11.4%) and lipid (5.4%), in the leaves (Makkar & Becker, 1996). In broilers, diets supplemented with 80 g/kg and 110 g/kg *Moringa oleifera* leaves increased the final body weight and body weight gain (Melesse, Getye, Berihun, & Banerjee, 2013; Ramadan, 2017). Additionally, dietary supplementation of 15% and 20% *Moringa oleifera* leaves improved the morphology of the bursa, thymus and spleen of broilers (Alnidawi, Ali, Abdelgayed, Ahmed, & Farid, 2017). Moreover, *Moringa oleifera* is rich in water-soluble polysaccharides and Moringa flavonoids, thereby displaying antioxidant and anti-inflammation abilities (Al-Malki & El, 2015; Liang & Zhen, 2013). Broilers fed diets with *Moringa oleifera* peduncle (Yan, Ren, Li, Yu, & He, 2008) and 500–1,000 mg/kg *Moringa oleifera* leaf meal (Rama Rao, Raju, Prakash, Rajkumar, & Reddy, 2018) showed enhanced immunity and decreased liver lipid peroxidation. In rats, *Moringa oleifera* leaf meal and methanol extract display anti-inflammatory abilities (Helmy, Nfs, Elaby, & Maa, 2017; Omodanisi, Aboua, & Oguntibeju, 2017) and could alleviate the harmful effects of a high-fat diet on lipid metabolism (Teixeira, Carvalho, Neves, Silva, & Arantes-Pereira, 2014). Compared to *Moringa* leaves, *Moringa* stem meal (MSM) had a lower nutrient value with high crude fibre (CF, 17.41% vs. 53.7%) and low CP level (25.37% vs. 7%) (Alnidawi et al., 2017; Sobhy et al., 2016). However, geese have higher roughage utilization and adaptability with a bigger gizzard compared to poultry breeds of duck and chicken (Jin et al., 2014; Wang, Yang, Lu, Li, & Zou, 2014). CF is an essential nutrient for maintaining the productive performance of geese. For example, a low dietary CF level (2.5%) decreased the body weight, body size and slaughter yield in geese compared with higher CF levels (4.3% and 6.1%) (Li et al., 2017). A previous study has shown that CF also increased the relative weight and length of the caecum in geese on 112 days (He, Meng, Li, Zhang, & Ren, 2015). So far, research has mainly focused on the *Moringa oleifera* leaves and *Moringa oleifera* stem + leaves, which have a lower CF level than the MSM. However, limited information on MSM utilization in geese diets has been available. MSM has been hypothesized for development as a good unconventional feedstuff in feed formulation, as well as for having beneficial effects on the productive performance of geese. Therefore, the aim of this study was to evaluate the effect of the dietary MSM level on the growth performance, slaughter performance and serum parameters of geese from 22 to 70 days of age.

2 | MATERIALS AND METHODS

2.1 | Preparation and nutrient analysis in MSM

Firstly, collect the lateral branches of *Moringa oleifera* tree, planting base in South China Agricultural University, when it grows to

about 60 cm height. Then, the tender stems were carefully separated manually for air-drying. The dried stems were crushed using feed grinder to make *Moringa oleifera* meal. All the dried stems meal from different trees were well mixed to prepare the MSM samples. The ether extract (EE) and CF content in the MSM samples were analysed according to the classical procedures of the Association of Official Analytical Chemists (AOAC, 1995). The CP content in the MSM samples was determined by the Kjeldahl method. The NDF and ADF contents in the MSM samples were determined as described by Van Soest, Robertson, and Lewis (1991). Total flavonoids were determined according to Wang and Zhang (2016). Water-soluble polysaccharide (WSP) content was determined as described by Feng, Pan, Guo, and Liu (2008). The average nutrient values of the MSM were calculated based on the values of three parallel determinations.

The MSM sample was hydrolyzed with 6 N HCl at 110°C for 24 hr to measure the amino acids contents using an Amino Acid Analyzer (Hitachi L-8900), as described by Li et al. (2018). Methionine and cysteine were determined as methionine sulphone and cysteic acid after cold performic acid oxidation overnight and hydrolysis with 7.5 N HCl at 110°C for 24 hr using an Amino Acid Analyzer (Hitachi L-8800). Tryptophan was determined after LiOH hydrolysis for 22 hr at 110°C using high-performance liquid chromatography (HPLC) (Agilent 1,200 Series). The apparent metabolizable energy (AME) of the MSM, corn, soybean meal and corn gluten meal provided to the geese was measured by the emptying-force-feeding method, mainly as described by Sibbald (1976). Geese were fasted and emptied for 24 hr before force-feeding. Force-feeding was accomplished by inserting a stainless steel tube (5.5 mm, internal diameter) into the crop via the oesophagus. The feed, which was provided as cold-pressed pellets (4.76 mm, diameter), was placed in the tube and pushed into the crop with a stainless steel rod. After feeding, the birds were returned to their cages. A plastic bag was fixed in the cloaca to collect excreta (Zuo et al., 2018). The excreta was collected after 24 hr forcing feeding of geese. The excreta was dried for 10 min at 120°C to inactivation micro-organisms and enzymes and then for 24 hr at 65°C in a forced-air oven, and pooled within a pen for analysis. Care was taken to avoid contamination from feathers, scales and debris. The dried excreta were allowed to equilibrate to atmospheric conditions before being weighed. Representative samples were taken and ground to pass through a 0.5 mm sieve. The gross energy of diet and the excreta samples were determined using an adiabatic bomb calorimeter (Gallenkamp, British) standardized with benzoic acid. The AME values of the corn, soybean meal, corn gluten meal and MSM were calculated using the following formula. Appropriate corrections were made for differences in moisture content.

$$\text{AME (MJ/kg)} = \frac{(\text{Feed intake} \times \text{Feed gross energy}) - (\text{Excreta output} \times \text{Excreta gross energy})}{\text{Feed intake}}$$

TABLE 1 Composition and nutrient levels of the experimental diets for geese at day 22 to 70 (Air-dry basis, g/kg)

Item	Dietary MSM level (g/kg)					
	0	20	40	60	80	100
Ingredient (g/kg)						
Corn	530	540	560	570	570	580
MSM	0	20	40	60	80	100
Soybean meal	130	140	140	160	160	170
Fish meal	67	60	50	45	45	41
Corn gluten meal	22	22	32	30	30	36
Wheat bran	0	11	20	11	28	0
Chaff	172	137	96	56	15	0
Fat meal	46	35	21	11	4	0
Limestone	6	6	6	6	6	6
Calcium hydrogen phosphate	13	13	14	14	13	13
Salt	4	4	4	4	4	4
L-Lys HCL	1.5	1.5	1.5	1.5	1.5	1.5
DL-Met	2	2	2	2	2	2
Choline chloride	1.4	1.4	1.4	1.4	1.4	1.4
Bentonite	4.1	6.1	11.1	27.1	39.1	44.1
Premix ^a	1	1	1	1	1	1
Nutrient composition ^b						
ME (MJ/kg)	12.11	12.08	12.03	12.03	11.98	12.04
Crude protein (g/kg) ^c	165	171	168	172	168	165
Crude fibre (g/kg)	63	65	65	65	65	65
EE (g/kg)	53.3	47.5	40.2	34.5	31.4	28.7
Calcium (g/kg)	9	9	9	9	9	9
Nonphytate P (g/kg)	5	5	5	5	5	5
Lysine (g/kg)	9.50	9.56	9.26	9.54	9.68	9.69
Methionine (g/kg)	4.89	4.87	4.85	4.87	4.90	4.93

Abbreviations: ME, metabolizable energy; MSM, *Moringa* stem meal.

^aProvided per kilogram of diet: 9,000 IU of vitamin A, 1,400 IU of vitamin D₃, 15 mg of vitamin E, 1.5 mg of vitamin K₃, 2 mg of thiamin, 2 mg of riboflavin, 2 mg of vitamin B₆, 12 µg of vitamin B₁₂, 75 mg of niacin, 20.5 mg of folic acid, 0.1 mg of biotin, 1,400 mg of choline chloride, 13.5 mg of pantothenic acid, 60 mg Zn (ZnSO₄), 66 mg of Mn (MnSO₄), 96 mg of Fe (FeSO₄·7 H₂O), 5 mg of Cu (CuSO₄·5H₂O), 0.42 mg of I (KI) and 0.1 mg of Se (Na₂SeO₃·5H₂O).

^bNutrient levels are calculated values.

^cMeasured value.

2.2 | Animals, diets and experimental design

A one-factor completely randomized design was adopted in our study. A total of 1,008 one-day-old Qingyuan goslings were obtained from a local goose breeding farm, and the goslings were fed the same corn-soybean meal basal diet for an adjustment period during 1–21 days of age. On day 21, based on the similar average weight of geese per replicate, all geese were weighed and allotted to one of six treatments, with six replicates per treatment and 28 birds per replicates. The geese were fed the experimental diets at the six MSM inclusion levels of 0 (control group), 20, 40, 60, 80 or 100 g/kg (Table 1). The AME values of the corn (14.5 MJ/kg), soybean meal

(13.2 MJ/kg), corn gluten meal (13.0 MJ/kg) and MSM (3.9 MJ/kg) in the geese were measured by emptying-forcing feed. The experimental diets were formulated to be isocaloric and isonitrogenous, with CP and ME levels as well as other nutrient levels meeting or exceeding the NRC for geese (1994). Geese were raised on free-range and had an indoor area and stadium area with a pool. Three geese per m² were maintained for the indoor area. Each stadium area was enclosed by walls to prevent other geese from entering the area. Diets and water were provided ad libitum. All diets were given in pellet form. Natural light was provided throughout the experimental period during days 22–70. At 70 days of age, after a 12 hr feed withdrawal, which was according to the previous studies (Liu et al., 2011;

Sun et al., 2016), the birds each replicate were weighed by electronic balance (Zhejiang Yongkang Yinhe Electrical Appliance), and the accuracy of the measurement was to 50 g. Feed consumption was recorded by each replicate pen. The average daily gain (ADG), average daily feed intake (ADFI) and feed/gain ratio (F/G) were calculated.

2.3 | Sampling

Based on the average body weight of the birds in each pen, six geese were taken for blood sampling. After 12 hr feed withdrawal, approximately 10 ml of blood was collected via the jugular vein from the selected geese at 70 day of age. Serum was prepared by centrifuging the blood at 1,824 g for 10 min and then stored at -20°C .

2.4 | Calculations

After the blood collection, birds were euthanized by cervical dislocation, and then, the carcass of geese without the giblets was weighed and expressed as a percentage of its live body weight to determine the carcass yield. The birds were then eviscerated, and the carcass weight (carcass weight was calculated by subtracting the weight of feathers and blood from the live weight), half-eviscerated weight (half-eviscerated weight was calculated by subtracting the weight of the trachea, oesophagus, intestines, spleen, pancreas and reproductive organs from the carcass weight) and eviscerated weight (the eviscerated weight was calculated by subtracting the weight of glandular stomach, gizzard, liver, liver, lung, heart and abdominal fat from the half-eviscerated weight) were recorded. The breast muscle and thigh muscle were removed from the carcass, trimmed of adipose tissue and weighed. The carcass weight, the percentage of half-eviscerated yield and the percentage of eviscerated yield were then expressed as a percentage of the live body weight. The percentages of breast and thigh muscle were expressed as a percentage of the eviscerated weight. The weights of the liver (without the gall bladder), gizzard, proventriculus, heart, spleen, small intestine, the caeca and large intestine were recorded, and their relative weights were expressed as a percentage of the live body weight.

2.5 | Sample analysis

The values of pH, lightness (L^*), redness (a^*) and yellowness (b^*) of the breast were measured for the meat quality. The pH of the breast muscle at 45 min, 24 hr and 48 hr for 36 geese from each treatment was determined using a calibrated pH meter (FE-20, Mettler-Toledo Instruments, Zurich, Switzerland). Each muscle sample was measured in triplicate with a spear-type electrode at the bone side (cranial end) of the Pectoralis major. The meat colour was measured for 36 geese from each treatment in triplicate on the bone side (medial surface) with a Minolta Chroma Meter CR-400 (Minolta,). The CIE Lab system values of L^* , a^* and b^* were recorded at 1 hr postmortem. The serum samples were thawed and analysed for the contents of glucose (GLU), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and activities of aspartate transaminase (AST) and alanine transaminase (ALT) using commercial diagnostic kits (Jiancheng Bioengineering Institute).

2.6 | Statistical analysis

All values were subjected to a one-way ANOVA by using the general linear model procedure of SAS (SAS Institute, 2010). The treatment comparisons for significant differences were tested by the LSD method. Orthogonal polynomials were applied for linear and quadratic effects of dependent variables to independent variables. Each replicate served as the experimental unit for all statistical analyses. Significant differences were established as $p \leq .05$, with a trend at $.05 \leq p \leq .10$.

3 | RESULTS

3.1 | Nutrient levels of the MSM sample

As shown in Table 2, the MSM had low CP level and high levels of CF, NDF and ADF, which were 8.7%, 27.1%, 78.3% and 58.0% respectively. The MSM also contained some kinds of functional materials: 15.1 g/kg water-soluble polysaccharide and 337 mg/kg total flavonoids.

TABLE 2 Nutrient levels of MSM sample (dry matter basis)

Analysed composition	Content	AA	AA content (%)	AA	AA content (%)
Ash (%)	7.60	Aspartate	0.61	Tyrosine	0.11
CP (%)	8.70	Threonine	0.33	Phenylalanine	0.34
EE (%)	2.36	Serine	0.35	Histidine	0.18
CF (%)	27.10	Glutamine	0.92	Lysine	0.38
NDF (%)	78.30	Glycine	0.33	Arginine	0.30
ADF (%)	58.00	Alanine	0.42	Proline	0.33
Ca (%)	1.11	Valine	0.41	Total AA content	5.88
TP (%)	0.15	Methionine	0.04		
WSP (g/kg)	15.10	Isoleucine	0.31		
TF (mg/kg)	337	Leucine	0.52		

Abbreviations: AA, amino acid; ADF, acid detergent fibre; Ca, calcium; CF, crude fibre; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; TF, total flavonoid; TP, total phosphorus; WSP, water-soluble polysaccharide.

3.2 | Growth performance

The dietary MSM level had an effect ($p < .05$) on the F/G and had no effect ($p > .10$) on the BW, ADFI and ADG of geese from 22 to 70 days of age (Table 3). The F/G showed an increasing linear trend ($p < .0001$) as the dietary MSM level increased. The geese fed the diets with 80 and 100 g/kg MSM had higher F/G compared with birds fed the diets with 0, 20 and 40 g/kg MSM.

3.3 | Slaughter performance

The dietary MSM level did not affect ($p > .10$) the slaughter yield, semi-eviscerated carcass yield and breast muscle yield and tended to affect the thigh and abdominal fat yield geese from 22 to 70 days of age (Table 4). The breast muscle yield increased linearly with increases in the dietary MSM level ($p = .03$), whereas the abdominal fat yield was the lowest when the diet contained 100 g/kg MSM (Table 4).

The effects of the MSM level on the relative weight of the organs in geese at day 70 are shown in Tables 5 and 6. No difference ($p > .10$) was observed in the heart, liver, spleen, pancreas, bursa, proventriculus, duodenum, jejunum, ileum, caecum and rectum of geese among the groups. The relative weights of the kidney and thymus were affected ($p < .05$) by the dietary MSM level. The geese fed the diet with 100 g/kg MSM had lower relative kidney weight compared with birds fed the diets with 0, 20 and 80 g/kg MSM ($p = .03$). Diets containing 40, 80 and 100 g/kg MSM of geese increased the relative thymus weight compared with the control group ($p < .05$). The relative weight of the gizzard showed a trend of linear increase with the increased dietary MSM level ($p < .05$). The dietary MSM level had no influence on the breast muscle pH 45 min and 24 hr postmortem or on the breast muscle L^* , a^* and b^* values ($p > .10$, Table 7).

3.4 | Serum biochemical parameters

Dietary MSM levels had no effects on the TG, TC, HDL and LDL levels or on the LDH activities in serum in geese at day 70 ($p > .10$). The level of dietary MSM affected ($p < .05$) the serum GLU level and ALT activity of the geese at day 70 (Table 8). The diet with 100 g/kg MSM had a lower GLU level ($p = .03$) and higher ALT activity ($p < .01$) of serum in geese compared with other groups. Serum ALT

was increased linearly with an increase in the level of dietary MSM ($p < .01$). The serum AST activity increased quadratically as the level of dietary MSM increased ($p < .01$).

4 | DISCUSSION

The measured contents of the CF, NDF, ADF and CP of the MSM sample used in the present study were 27.1%, 78.3%, 58.0% and 8.70%, respectively, indicating that MSM is a kind of roughage, which was similar to the role of *Moringa* stems as reported in rabbit by Sobhy et al., (2016). As an herbivore, geese have a well-developed gizzard and caecum and could make good use of the unconventional feedstuff of MSM in their diets. In the present study, dietary MSM level of 100 g/kg had no effect on the BW, ADG and ADFI of geese from 22 to 70 days of age, which agreed with the results found when 15% MSM was added to the diets of rabbits, another herbivore (Sobhy et al., 2016). As reported in laying hens (34–50 weeks old), diets supplemented with 10% *Moringa* (leaves + stem) meal had no effect on the laying performance (Valdivi , Mesa, & Rodr guez, 2016). However, F/G was significantly increased in the diets containing 80 and 100 g/kg MSM of geese. These results indicate that, within a reasonable range, MSM could be developed as an unconventional feedstuff roughage resource for geese at a later growth stage. For one reason, *Moringa* gum from the stem of the plant *Moringa oleifera* could increase the viscosity of digestion and reduce the nutrient digestibility (Singhal, Jarald, Showkat, & Daud, 2012). In addition, high levels of ADF and NDF in 80 and 100 g/kg MSM diets could accelerate the emptying speed and reduce the nutrient availability per unit time. However, depressed growth was observed in Koekoek chickens fed diets with 50, 80 and 110 g/kg *Moringa* leaves meal (Melesse et al., 2013) and broilers under heat stress fed diets with 0.2% *Moringa* dried leaves (Gouda et al., 2018). The inconsistent results may be due to the differences in the breed, age and tolerance for the *Moringa* meal. Based on the results of growth performance, the recommended optimum MSM level in diets of no more than 60 g/kg had no effect on the growth performance, whereas diets containing higher MSM levels of 80 or 100 g/kg had negative effects on the F/G of geese from 22 to 70 days of age.

TABLE 3 Effect of dietary MSM level on the growth performance of geese from 22 to 70 days of age^a

Dietary MSM level (g/kg)	0	20	40	60	80	100	SEM	p-value		
								Treatment	Linear	Quadratic
Final BW (g/bird)	3,454	3,413	3,443	3,414	3,400	3,345	105	.98	.30	.93
ADFI (g/d/bird)	222	220	220	226	230	232	7.90	.84	.22	.59
ADG (g/d/bird)	52.0	51.2	51.8	51.2	50.9	50.0	1.90	.98	.48	.80
F/G	4.28 ^c	4.32 ^c	4.26 ^c	4.41 ^{bc}	4.53 ^{ab}	4.65 ^a	0.05	<.001	<.0001	.017

Note: Values within a row with no common letters differ significantly ($p < .05$).

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; F/G, feed/gain ratio; MSM, *Moringa* stem meal; SEM, standard error of mean.

^aEach value represents the mean of six replicates.

TABLE 4 Effect of dietary MSM level on slaughter performance of geese at 70 days of age^a

Dietary MSM level (g/kg)	0	20	40	60	80	100	SEM	p-value		
								Treatment	Linear	Quadratic
Slaughter yield (%) ^c	90.8	89.2	89.6	90.0	88.8	89.5	0.53	.17	.28	.80
Percentage of eviscerated carcass (%) ^b	67.1	62.1	65.9	66.1	66.0	66.0	1.27	.08	.05	.17
Percentage of half-eviscerated carcass (%) ^b	82.3	78.1	81.4	81.3	81.0	81.2	1.21	.28	.89	.56
Breast muscle yield (%) ^c	8.36	8.62	9.62	9.33	9.20	9.68	0.48	.38	.03	.89
Thigh muscle yield (%) ^c	9.22	10.10	10.00	10.70	11.00	10.90	0.44	.07	.49	.40
Abdominal fat yield (%) ^c	2.65	2.63	2.68	2.43	2.19	1.60	0.28	.08	.01	.12

Abbreviations: MSM, *Moringa* stem meal; SEM, standard error of mean.

^aEach value represents the mean of six replicates.

^bCalculated as a percentage of live body weight.

^cCalculated as a percentage of eviscerated carcass weight.

TABLE 5 Effect of dietary MSM level on the relative weight of organ of geese at 70 days of age^a

Dietary MSM level (g/kg) ^b	0	20	40	60	80	100	SEM	p-value		
								Treatment	Linear	Quadratic
Heart (g/kg)	6.96	7.16	7.17	7.37	7.64	6.98	0.31	.68	.51	.28
Liver (g/kg)	17.7	15.3	15.7	17.0	16.2	17.2	0.68	.16	.85	.07
Kidney (g/kg)	6.73 ^a	6.31 ^a	5.56 ^{ab}	5.89 ^b	6.38 ^a	4.55 ^b	0.49	.03	.01	.63
Spleen (g/kg)	0.72	0.60	0.46	0.58	0.62	0.61	0.06	.12	.44	.03
Pancreas (g/kg)	3.25	3.00	2.68	3.16	3.39	2.68	0.26	.30	.58	.95
Bursa (g/kg)	1.00	0.51	0.88	0.96	0.67	0.90	0.15	.30	.98	.52
Thymus (g/kg)	1.33 ^b	2.13 ^{ab}	2.58 ^a	1.90 ^{ab}	2.93 ^a	2.43 ^a	0.32	.03	.01	.18

Note: Values within a row with no common letters differ significantly ($p < .05$).

Abbreviations: MSM, *Moringa* stem meal; SEM, standard error of mean.

^aEach value represents the mean of six replicates.

^bCalculated as a relative weight of live BW.

Slaughter performance is one of the important indicators of the economic benefits of meat poultry. In the present study, dietary MSM supplementation had no effect on the slaughter performance and the relative organ weight (except kidney and thymus) of the geese. These results were in accordance with those reported in broiler chickens fed diets with aqueous *Moringa oleifera* leaf extracts (Alabi, Malik, Ng Ambil, Obaje, & Ojo, 2017). However, the geese that received diets with 100 g/kg MSM showed decreases in relative kidney weight and increases in relative thymus weight. Similarly, Jaiswal, Kumar, Kumar, Mehta, and Watal (2009) found that the crude leaf extract of *Moringa oleifera* decreased the relative weight and cholesterol level in the kidneys of rats. Additionally, the 80 and 100 g MSM/kg diets increased the relative weight of the thymus in the geese. As reported previously in Japanese quail, dietary supplementation with *Moringa oleifera* leaf meal (0.2%, 0.4% and 0.6%) increased the bursa and thymus percentages (Elkloub, Riry, Mousa, Alghonimy, & Youssef, 2015). The improvement in the immune organs could be

related to the immunomodulatory properties of functional flavone in MSM, which could improve the immune-enhancing activity in animals (Fan et al., 2014; Hager-Theodorides, Goliomytis, Delis, & Deligeorgis, 2014). The main function of the gizzard is in grinding of the feed to make good use of CF by the goose. In our study, the relative weight of the gizzard showed a linear trend of increasing with the increased dietary MSM level in the present study, which agreed with a previous study in broilers fed with 15% coarse hulls (Sacranie et al., 2012), whereas broilers fed with 0.5%, 1.5% and 2.5% *Moringa oleifera* leaf meal with a lower CF level showed no effect on the relative weight of their gizzards (Nkukwana et al., 2014). These results indicate that MSM with a high level of CF promoted gizzard development to obtain greater digestion and absorption of roughage.

Meat water-holding capacity, pH and colour as important meat quality characteristics affect consumer preferences (Fanatico, Cavitt, Pillai, Emmert, & Owens, 2005). Lipid peroxidation and microorganism contamination may generate changes in meat quality

TABLE 6 Effects of dietary MSM on the relative weights of digestive organ of geese at 70 days of age^a

Dietary MSM level (g/kg) ^b	0	20	40	60	80	100	SEM	p-value		
								Treatment	Linear	Quadratic
Gizzard (g/kg)	30.8 ^b	35.19 ^{ab}	33.0 ^{ab}	33.7 ^{ab}	33.4 ^{ab}	37.2 ^a	1.41	.06	.03	.73
Proventriculus (g/kg)	3.17	3.29	3.36	3.23	3.37	3.46	0.16	.89	.26	.96
Duodenum (g/kg)	2.72	2.39	2.58	2.64	2.67	2.97	0.18	.38	.07	.48
Jejunum (g/kg)	5.70	5.14	5.61	5.77	5.40	5.82	0.30	.58	.14	.67
Ileum (g/kg)	4.37	4.63	4.40	5.41	4.96	5.05	0.43	.50	.54	.57
Caecum (g/kg)	1.05	1.02	1.16	1.25	1.17	1.21	0.09	.39	.17	.14
Rectum (g/kg)	1.79	1.82	1.70	2.22	1.79	2.37	0.20	.13	.48	.85

Note: Values within a row means a trend of linear increase with the increased dietary MSM level ($p < .10$).

Abbreviations: MSM, *Moringa* stem meal; SEM, standard error of mean.

^aEach value represents the mean of six replicates.

^bCalculated as a percentage of live BW.

parameters such as colour, flavour, odour, texture and even nutritional values (Doulgeraki, Ercolini, Villani, & Nychas, 2012; Kolakowska, 2003). The increase in lipid oxidation is potentially due to the depletion of meat redox capacity during storage and the formation of secondary products that interact with fatty acids, which can cause a chain reaction and intensify lipid oxidation (Chamorro et al., 2015). In general, the colour stability of meat appears related to lipid oxidation (Viana, Canto, Costa-Lima, Salim, & Conte-Junior, 2016). In addition, the micro-organisms produce undesirable quality changes in meats, especially in relation to lactic acid bacteria, a major bacterial group associated with meat spoilage (Doulgeraki et al., 2012). Studies have shown that *Moringa oleifera* or its extract, acting as a potential antioxidant (Lin, Zhang, & Chen, 2018) and antimicrobial agent (Onyuka, Kakai, Arama, & Ofulla, 2013), might be helpful in improving the meat quality and prolonging the meat storage time (Zhang, Wu, & Guo, 2016). Many studies have demonstrated that diets supplemented with *Moringa oleifera* leaf meal or extract improved the meat quality by, for example, increasing the meat pH and water-holding capacity in goats, broilers and pork (Hazra, Biswas, Bhattacharyya, Das, & Khan, 2012; Mukumbo, 2013; Qwele, 2011). Rabbit fed with 10% *Moringa oleifera* leaf meal could decrease the meat drip loss and the shear force of the longissimus dorsi (Sun et al., 2017). This

decrease might be attributed to the quercetin in *Moringa* by assisting in the prevention of meat degradation by oxidation (Lin et al., 2018; Moyo, Masika, Hugo, & Muchenje, 2013). Quercetin has been reported to inhibit the lipid peroxidation by chelating transition metal ions and scavenging free radicals such as H_2O_2 and ROS (Das et al., 2013; Terao & Piskula, 1999). In the present study, no effect of MSM was observed on the meat pH and colour, possibly because antioxidant compounds such as the flavones in *Moringa oleifera* stem meal were lower than in the *Moringa oleifera* leaf meal (Vongsak et al., 2013).

Alanine transaminase and AST, as a part of a diagnostic evaluation of hepatocellular injury, are commonly measured clinically to determine the health states of the liver and other tissues (Wang, Chang, Yao, Wang, & Chou, 2012). In the present study, significant increases were observed in the serum ALT and AST activity in geese fed the diet with 100 g MSM/kg, which indicate that higher MSM level diets might lead to tissue damage in geese. However, the possibility of these harmful effects from MSM contradicts previous observations made on *Moringa oleifera* leaf meal or *Moringa oleifera* leaf extract, which indicated hepatoprotective effects in broilers and rats (Akpert, Ibekwe, & Onyeama, 2014; Ogbunugafor et al., 2012). The exact reason for these effects needs to be further studied. In addition, dietary 100 g MSM/kg showed a significant decrease in the serum GLU level

TABLE 7 Effect of dietary MSM level on breast meat colour and pH value of geese at 70 days of age^a

Dietary MSM level (g/kg)	Items	0	20	40	60	80	100	SEM	p-value		
									Treatment	Linear	Quadratic
pH	45 min	6.13	5.95	6.03	5.93	6.08	6.09	0.07	.29	.84	.57
	24 hr	6.08	6.05	6.04	6.09	6.04	6.11	0.03	.30	.42	.18
	48 hr	6.03	6.01	6.01	6.01	6.00	6.02	0.04	.99	.86	.07
Meat colour	L [*]	44.60	48.10	46.10	46.30	44.70	45.60	1.92	.80	.73	.87
	a [*]	19.60	18.20	17.70	19.30	18.50	18.50	0.63	.31	.54	.33
	b [*]	1.14	0	0.78	1.05	1.03	0.69	0.73	.73	.78	.53

Abbreviations: a^{*}, redness; b^{*}, yellowness; L^{*}, lightness; MSM, *Moringa* stem meal; SEM, standard error of mean.

^aEach value represents the mean of six replicates.

TABLE 8 Effect of dietary MSM level on serum biochemical parameters of geese at 70 days of age^a

Dietary MSM level (g/kg)	0	20	40	60	80	100	SEM	p-value		
								Treatment	Linear	Quadratic
GLU (μmol/mL)	13.7 ^a	11.4 ^{bc}	11.7 ^{abc}	13.2 ^{ab}	11.4 ^{bc}	10.9 ^c	0.64	.03	.12	.11
TG (μmol/mL)	0.75	0.85	0.76	0.72	0.85	0.71	0.11	.93	.03	.76
TC (μmol/mL)	4.02	4.23	4.52	4.10	4.10	3.79	0.21	.31	.79	.23
HDL (μmol/mL)	2.64	2.76	2.94	2.46	2.59	2.17	0.18	.12	.23	.38
LDL (μmol/mL)	1.28	1.45	1.69	1.29	1.45	3.65	0.76	.31	.53	.34
AST (mIU/mL)	36.1	35.7	45.5	40.7	45.7	62.8	5.69	.07	.05	<.01
ALT (mIU/mL)	15.3 ^b	13.1 ^b	14.3 ^b	13.8 ^b	15.2 ^b	21.4 ^a	1.34	<.01	<.01	.01
LDH (mIU/mL)	456	417	456	380	474	425	55.30	.86	.06	.13

Note: Values within a row with no common letters differ significantly ($p < .05$).

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; GLU, glucose; HDL, high-density lipoprotein; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; MSM, *Moringa* stem meal; SEM, Standard error of mean; TC, cholesterol; TG, triglyceride

^aEach value represents the mean of six replicates.

in geese at day 70. Similar results were observed in alloxan-induced diabetic rat (El-Desouki, Tabl, Abdel-Aziz, Salim, & Nazeeh, 2015) and Wistar rats fed diets supplemented with *Moringa oleifera* extract (such as flavonoids) (Oyewo, Adeleke, Fakunle, & Iniaghe, 2013a). These results might be attributed to the flavonoids in MSM, such as quercetin, which could prevent the absorption of dietary glucose in the gastrointestinal tract (Oyewo, Adewale, Abdulfatai Ayoade, & Akanji, 2013b). Studies have shown that quercetin could decrease serum glucose by increasing the insulin activity and decreasing the hepatic glucose-6-phosphatase activity (Panda & Kar, 2007).

5 | CONCLUSION

In conclusion, dietary MSM levels of no more than 60 g/kg had no effects on the growth performance and slaughter performance, whereas diets with 100 g MSM/kg increased the F/G and serum ALT enzyme activity and decreased the serum GLU level. Therefore, within a reasonable range, MSM could be developed as an unconventional feedstuff roughage resource for geese from 22 to 70 days of age.

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CONFLICT OF INTEREST

There is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that the European Union (EU) standards for the protection of animals used for scientific purposes and feed legislation have been followed.

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BRIEF REPORT



The pattern of body growth and intestinal development of female Chinese native geese from 1 to 10 weeks of age

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ABSTRACT

The aim of this study was to investigate the pattern of body growth and intestinal development of female Chinese native geese from 1 to 10 weeks of age. At weekly intervals, from hatch through 10 weeks of age, ten geese were sampled to measure the absolute and relative weight and length of the intestinal segments and overall intestine of geese. The body weight of Bertalanffy ($R^2 = 0.989$), Gompertz ($R^2 = 0.990$), Logistic ($R^2 = 0.980$) and Richards ($R^2 = 0.982$) models showed an increase in the asymptotes at 1–10 weeks old, while the inflection points were at 30.53, 28.56, and 25.59 d, respectively. The relative weight and length of the intestinal segments and overall intestine were decreased linearly ($P < 0.01$), while the absolute weight and length of the intestinal segments and overall intestine were increased linearly ($P < 0.01$) during 1–7 weeks old, but significantly decreased ($P < 0.01$) during 8–10 weeks old, suggesting that the intestinal system was developing rapidly at the starter period based on the intestinal weight and length, but was declining at the later period. The knowledge of growth curve and intestinal development pattern could provide some useful information on the optimum management practices and breeding strategies in geese production.

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Introduction

To meet the requirement of animal protein due to the increase of population, the production of poultry other than chicken and ducks, such as geese, was increasing year by year in China (Kozák 2021). The size of the geese population in China increased to 639 million heads in 2020 (Hou and Liu 2021). In addition, the meat products of geese were regarded as one of the most valuable protein co-products and were increasingly popular in the Chinese market. Growth traits were important characteristics of both economic profitability and population dynamics in poultry production (Kenny et al. 2018). Growth curve models can be used for predicting body weight (BW) changes with age, which provide a visual assessment of growth as a function of time (Narinc et al. 2017). Therefore, knowledge about the growth pattern of meat goose was necessary to optimize the geese production system (e.g. by selection, feeding management and marketing strategies) (Boz et al. 2017). For example, it was unclear how much of an impact on growth production was the semi-intensive and intensive production systems, instead of the free-range backyard type (Boz et al. 2021). Therefore, the relationship between body weight and age depended on the different breeds and sexes (Uhlířová et al. 2018). To explain the growth curve of poultry, Logistic, Bertalanffy and Gompertz models were often used in male and female chicken, turkeys and ducks (Rogers 1987; Cigdem and Hulya 2001; Maruyama et al.

2001; Vitezica et al. 2010; Thinh et al. 2021). Moreover, precision feeding was separately performed for geese according to gender, in China. These models were applied to Chinese native geese of mixed gender (Zhao et al. 2007; Liu et al. 2017), but rarely to female geese. The intestinal system is the primary site to perform the functions of digestion, absorption, and protection (de Carvalho et al. 2021). The intestinal developmental patterns had positive effects on growth performance in poultry (González-Alvarado et al. 2008; Wang et al. 2014; Zhang et al. 2020). The knowledge of growth curve and intestinal development patterns could provide some useful information on the design of optimum management practices and breeding strategies in geese production. However, little information was available concerning the pattern of intestinal development in geese. Therefore, the purpose of this study was to investigate the developmental pattern of body weight and intestinal tract of female native Magang geese under the intensive production system, in favour of the breeding strategy to modify the trajectory of growth.

Methods and materials

Animals, management and housing

All procedures of this study were approved by the animal care and welfare committee institute of South China Agricultural

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University (SCAU-10564), and the study was performed following the Regulations for the Administration of Affairs Concerning Experimental Animals. The study was conducted at the South China Agricultural University Agricultural Faculty's Experimental Farm.

A total of one hundred and eighty female Magang goslings were assigned randomly to ten pens and eighteen birds per pen were raised in the environmental chambers under an intensive production system. Pens were separated by a wire mesh. Each pen contained one round feeder and one round drinker. The temperature started at $30 \pm 1^\circ\text{C}$ and was reduced by $3 \pm 1^\circ\text{C}$ each week until 21.1°C was attained at week 10. All birds were fed a starter diet from 0 to 4 weeks, and a grower diet from 4 to 10 weeks that met or exceeded the nutrient recommendations for geese (Table 1) by National Research Council (1994). Feed and water were provided *ad libitum* and economic white bulbs were used for lighting. The feeding and lighting programme were performed according to the guideline of management for Magang goose. The birds were individually weighed at 08:00 h and body weight (BW) was recorded at weekly intervals for 10 weeks. At the end of each week, one bird from each pen was slaughtered and the average BW of the remaining birds was used as the data point for the growth curve model.

Sample collections

One bird based on the average BW per pen was selected, weighed, and killed by carbon dioxide asphyxiation. The

gastrointestinal tract and organs were carefully excised and determined with a modified method (Amerah et al. 2008). In brief, the empty weights of digestive tract segments from the duodenum to the caeca of each bird were determined. The length of each intestinal segment was determined with a flexible tape on a glass surface to prevent inadvertent stretching. After division and freeing of each intestinal segment, separating all connective tissue and fat, and removing the content with ice-cold saline flushing, empty weights (± 0.01 g) were determined. The relative values were calculated as a ratio of live body weight.

Statistical analyses

The non-linear regression models of Logistic, Gompert, Bertalanffy, Richard, Brody and Weibull were fitted using the NLIN models of SAS software (SAS Institute 2000). The forms of equations (Table 2) for the Logistic, Gompert, Bertalanffy, Richard, Brody and Weibull models were: where W is the weight corresponding to age (t) with 3 parameters: A = asymptotic or maximum growth response, B = intercept, or weight when age (t) = 0 and K = rate constant. For digestive tract measurements, individual birds were considered as the experimental unit. All data were subjected to a one-way analysis of variance by using PROC GLM of SAS software. Orthogonal polynomials were applied for linear and quadratic effects on the parameters of intestinal development corresponding to age. Differences were considered to be significant at $P < 0.05$, and significant differences between means were separated by the least significant difference test.

Results and discussion

The development of the meat goose industry requires further knowledge of the overall growth patterns for different breeds and sexes (Uhlířová et al. 2018). However, there was limited information on the growth curve for some native geese lines (Nder et al. 2017). In the present study, the growth curve analysis for female Chinese native Magang goose showed an increase in the asymptotes during the selection period of 1–10 weeks of age, and the age to reach the inflection point was estimated at 30.53, 28.56, 25.59, and 29.2 d, using the Logistic, Gompertz, Bertalanffy and Richards models, respectively (Table 1). The ages to reach the inflection points provided the estimates of maturity of the growth processes. In the current study, estimation of the inflection points was close to that reported in Chinese Sichuang white geese with mixed gender using the Gompertz model (27.51 d) (Liu et al. 2017) and female Turkish native geese using the Logistic model (32.9 d) (Nder et al. 2017) and shorter than that reported in Lion-head geese with mixed gender using the Gompertz model (52.5 d) (Zhao et al. 2007). In the present study, the accuracy of Bertalanffy ($R^2 = 0.990$) and Gompertz ($R^2 = 0.989$) models fitted body weights was higher than the Logistic ($R^2 = 0.980$) and Richards ($R^2 = 0.982$) models, indicating that the Gompertz and Bertalanffy models with high coefficients of determination could be suitable models for female Chinese native Magang geese growth. Laird (1966) reported that the Gompertz function has been preferred over the logistic

Table 1. Composition and nutrient levels of the experimental diets (as-fed basis).

Ingredient, %	Starter (week 0–4)	Grower (week 5–10)
Corn	60.00	60.00
Soybean meal	18.50	9.39
Corn gluten meal	7.60	4.00
Wheat bran	8.10	20.58
Oil/Fat powder	1.25	1.85
Limestone	1.00	0.90
Dicalcium phosphate	2.19	2.35
Sodium chloride	0.35	0.35
DL-Methionine	0.12	0.13
L-lysine.HCl (98.5%)	0.78	0.35
Vitamin and mineral premix ¹	0.1	0.1
Total	100	100
Nutrient composition		
Calculated value, %		
ME, MJ/kg	12.37	11.84
Crude protein	19.8	15.3
Lysine	1.33	0.8
Methionine	0.44	0.36
Methionine + cysteine	0.75	0.61
Calcium	0.93	0.91
Total phosphorus	0.75	0.81
Non-phytate phosphorus	0.5	0.53

¹Provided per kilogram of diet for geese at week 0–4: vitamin A, 10,000 IU; vitamin D₃, 1,500 IU; vitamin E, 10 IU; thiamine, 1.8 mg; riboflavin, 3.6 mg; pyridoxine, 3.0 mg; vitamin B₁₂, 0.003 mg; calcium pantothenate, 10 mg; folate, 0.25 mg; niacin, 35 mg; biotin, 0.10 mg; choline (Choline chloride), 500 mg; Cu (CuSO₄·5H₂O), 8 mg; Fe (FeSO₄·7H₂O), 100 mg; Zn (ZnSO₄·7H₂O), 100 mg; Mn (MnSO₄·H₂O), 120 mg; Se (NaSeO₃), 0.3 mg; I (KI), 0.7 mg.

Provided per kilogram of diet for geese at week 5–10: vitamin A, 5,000 IU; vitamin D₃, 1,000 IU; vitamin E, 5 IU; thiamine, 1.3 mg; riboflavin, 1.8 mg; pyridoxine, 3.0 mg; vitamin B₁₂, 0.009 mg; calcium pantothenate, 15 mg; folate, 0.55 mg; niacin, 55 mg; biotin, 0.15 mg; choline (Choline chloride), 1300 mg; Cu (CuSO₄·5H₂O), 3 mg; Fe (FeSO₄·7H₂O), 80 mg; Zn (ZnSO₄·7H₂O), 50 mg; Mn (MnSO₄·H₂O), 50 mg; Se (NaSeO₃), 0.1 mg; I (KI), 0.35 mg.

Table 2. Growth curve parameters in different models for Magang geese ($n = 10$).

Model	Function	A	B	k	N	Weight at point of inflection (kg)	Age of maximum growth (d)	Maximum growth weight(kg)	R ²	Mean squared residue
Logistic	$Wt = A/(1 + Be^{-kt})$	3.425	12.092	0.071	-	1.712	35.070	0.061	0.980	0.171
Gompertz	$Wt = Ae^{-Be^{-(kt)}}$	3.840	3.352	0.041	-	1.413	29.199	0.059	0.989	0.114
Bertalanffy	$Wt = A(1 - Be^{-kt})^3$	4.181	0.745	0.031	-	1.239	25.856	0.058	0.990	0.099
Brody	$Wt = A(1 - Be^{-kt})$	7.406	1.038	0.009	-	-	-	-	0.984	0.152
Weibull	$Wt = A - Be^{-(kt)^n}$	5.043	5.159	0.006	1.217	-	-	-	0.984	0.150
Richards	$Wt = A/(1 + Be^{-kt})^n$	3.837	0.013	0.042	256.065	1.414	29.224	0.060	0.982	0.176

Notes: MSR, mean squared residue, calculated by dividing the sum of the squares of the residue by the number of observations; R², adjusted coefficient of determination.

function for fitting monophasic growth curves of chickens. When analysed by growth curves derived from the Bertalanffy model, the body weight of Magang geese during the periods of 2–4 weeks (521 g/week) and 7–8 weeks (483 g/week) of age was faster than the other growth period (Figure 1). The greatest body weight gain appeared at 4 weeks of age (581 g/week), which was similar to the data obtained from female Bohemian and Italian White geese (Knizetova et al. 1994). As reported in ducks, the age of the inflection point that remained constant was 26 d for Pekin ducks, 21 d for Mallard ducks and 37 d for Muscovy ducks (Gille and Salomon 1994). It was inferred that the geese were characterized by an early maturing rate, nearly as early as ducks (Knizetova et al. 1991b) and earlier than chickens (Knizetova et al. 1991a). It was apparent that the selection programme, without a delay in maturity, reduced the time to reach the market weight to achieve more efficient geese production.

The intestinal system was the primary site of entry for any orally administered compound, including dietary ingredients. There was a growing interest on the influence of diet on the development of the gastrointestinal tract in poultry (González-Alvarado et al. 2008; Jiménez-Moreno et al. 2009; Wang et al. 2014; Zhang et al. 2020). In the present study, the relative weight and length of the intestinal segments and overall intestine decreased linearly with the increased week of age (Tables 3 and 4). The greatest relative weight and length of the intestine were observed during the first three weeks. It was suggested that the intestinal system of a hatchling must undergo tremendous change before it was capable of efficiently digesting the dietary nutrients. Previous studies demonstrated that the growth of the intestinal system may exceed that of the rest of the body by as much as five-fold in chickens during the first 5–7 days of post-hatch (Uni et al. 1999; Sklan 2001). A previous study reported that avian species with high growth rate capacities at the starter period were also characterized by a rapid development of the digestive organs (Lilja 1983), which was confirmed in geese of the current study. The absolute weight and length of the duodenum, jejunum and ileum segments and overall intestine were increased quadratically in response to the increased age (Tables 5 and 6). The absolute length of the intestinal segments and overall intestine was increased as observed over the 1–5 week period in geese, which was in agreement with those reported in broilers from post-hatching to 21 days old (Uni et al. 1999). No significant changes were observed in the absolute length of the intestine during 6–10 weeks of age. It was suggested that the intestines tend to be mature by 5–6 weeks of age. Some interesting patterns were observed in the absolute weight of the digestive system during the growth period. The absolute weight and length of the intestinal segments and overall intestine increased linearly during 1–7 weeks and the age to reach the inflection points was at 7 weeks of age. However, there was a significant decrease in the absolute weight of the duodenum, jejunum and ileum segments and overall intestine during 8–10 weeks old. It was implied that the shrinking of gastrointestinal system development occurred during the later growth period, which resulted in the lower growth rate capacity in geese during 8–10 weeks. The reduced weight of the small intestine was in line with the results that the absolute mass of the small intestine

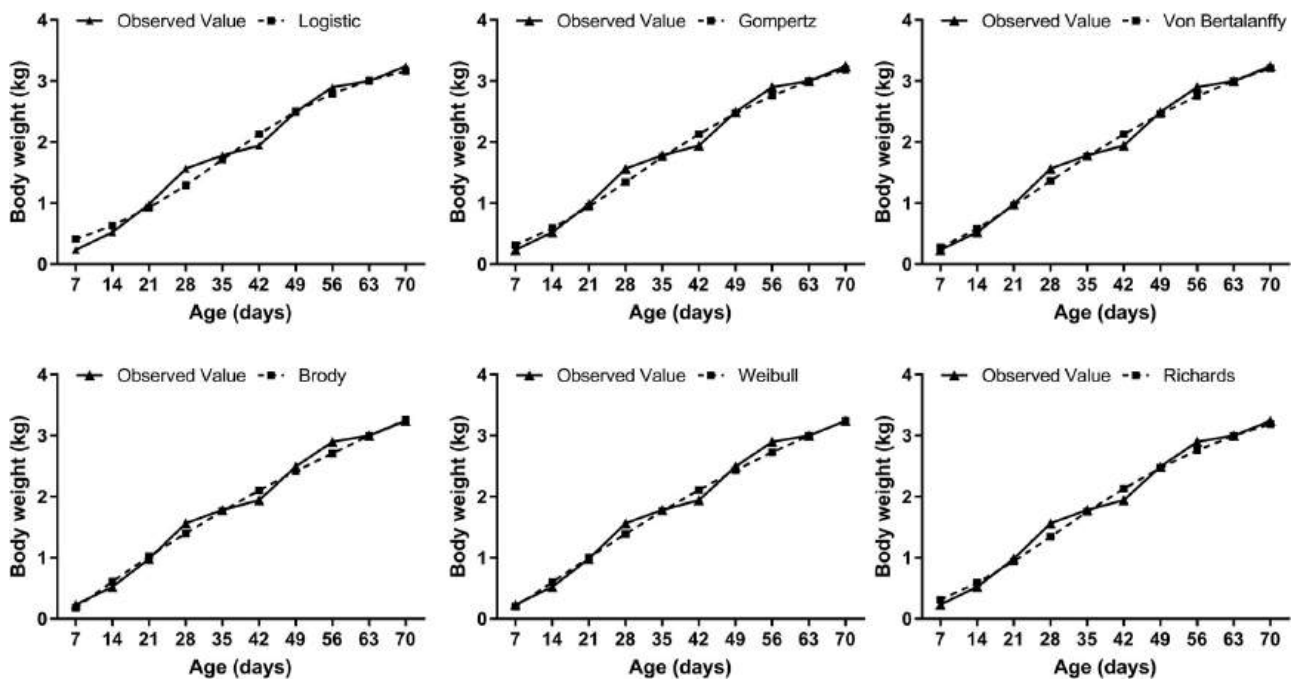


Figure 1. Predicted average body weight and observed growth curves for the Logistic, Gompert, Bertalanffy, Richard, Brody and Weibull models.

Table 3. Effect of age on the relative weight of intestine of Magang geese from 1 to 10 weeks of age (g/kg)^A.

Age (week) ^B	Duodenum	Jejunum	Ileum	Caecum	Rectum	Overall intestine
1	10.03 ^a	20.48 ^a	18.91 ^a	2.09 ^{cde}	4.82 ^a	56.33 ^a
2	7.61 ^b	18.24 ^b	16.47 ^b	3.11 ^{ab}	4.47 ^a	49.89 ^b
3	6.29 ^c	15.49 ^c	13.90 ^c	3.69 ^a	3.61 ^b	43.92 ^c
4	4.25 ^e	8.38 ^{gf}	9.09 ^{de}	2.17 ^{cde}	2.58 ^c	26.03 ^{ef}
5	5.45 ^d	13.48 ^d	10.25 ^d	2.71 ^{bc}	2.57 ^c	34.46 ^d
6	4.07 ^e	9.36 ^f	7.72 ^e	2.34 ^{bcd}	2.34 ^c	25.82 ^f
7	4.64 ^e	11.43 ^e	8.09 ^e	2.54 ^{bc}	2.66 ^c	29.35 ^e
8	3.16 ^f	7.24 ^{gh}	5.06 ^f	1.62 ^{de}	2.34 ^c	19.18 ^g
9	3.00 ^f	6.65 ^h	5.01 ^f	1.93 ^{cde}	1.54 ^d	18.02 ^g
10	2.76 ^f	6.91 ^h	4.89 ^f	1.44 ^e	1.42 ^d	17.42 ^g
SEM	0.25	0.44	0.65	0.25	0.14	1.14
P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	<0.01	<0.01	<0.01	0.039	<0.01	<0.01

^ALacking common letters (a or h) significant differences at $P < 0.05$.

^BMean represented the average value of 10 replicates ($n = 10$).

Table 4. Effect of age on the relative length of the intestine of Magang geese from 1 to 10 weeks of age (cm/kg)^A.

Age (week) ^B	Duodenum	Jejunum	Ileum	Caecum	Rectum	Overall intestine
1	86.44 ^a	177.2 ^a	162.08 ^a	32.35 ^a	23.63 ^a	481.72 ^a
2	44.34 ^b	100.0 ^b	90.98 ^b	20.35 ^b	12.41 ^b	268.08 ^b
3	27.68 ^c	62.33 ^c	53.95 ^c	13.14 ^c	7.91 ^c	161.40 ^c
4	23.09 ^d	43.61 ^d	45.36 ^d	10.80 ^{cd}	6.04 ^{cd}	126.24 ^d
5	18.34 ^e	43.78 ^d	37.43 ^e	6.89 ^{ef}	5.56 ^{de}	113.06 ^{de}
6	18.22 ^e	36.10 ^{de}	32.79 ^{ef}	9.31 ^{de}	4.34 ^{de}	102.21 ^{ef}
7	15.17 ^{ef}	31.65 ^{ef}	27.98 ^{fg}	7.51 ^{ef}	3.99 ^{de}	86.31 ^{fg}
8	12.34 ^f	26.39 ^f	23.81 ^{gh}	6.88 ^{ef}	3.81 ^e	73.22 ^{gh}
9	12.43 ^f	25.65 ^f	22.63 ^{gh}	7.29 ^{ef}	3.63 ^e	71.62 ^{gh}
10	11.05 ^f	23.78 ^f	19.97 ^h	5.74 ^f	3.44 ^e	64.16 ^h
SEM	1.43	2.80	2.05	0.97	0.63	5.83
P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	<0.01	<0.01	<0.01	0.039	<0.01	<0.01

^ALacking common letters (a or h) significant differences at $P < 0.05$.

^BMean represented the average value of 10 replicates ($n = 10$).

Table 5. Effect of age on the absolute weight of the intestine of Magang geese from 1 to 10 weeks of age (g/kg) ^A.

Age (week) ^B	Duodenum	Jejunum	Ileum	Caecum	Rectum	Overall intestine
1	2.33 ^f	4.75 ^g	4.37 ^e	0.48 ^f	1.11 ^e	13.03 ^f
2	3.96 ^e	9.51 ^f	8.59 ^{de}	1.62 ^e	2.33 ^d	26.01 ^e
3	6.14 ^d	15.22 ^{de}	13.63 ^{cd}	3.62 ^d	3.55 ^c	41.15 ^d
4	6.51 ^d	12.88 ^e	14.25 ^c	3.32 ^d	4.16 ^{bc}	50.48 ^d
5	9.58 ^b	22.34 ^b	18.33 ^{ab}	4.79 ^{bc}	4.53 ^b	61.12 ^b
6	7.51 ^c	17.19 ^{cd}	14.09 ^c	4.31 ^{cd}	4.26 ^{bc}	47.36 ^{de}
7	11.53 ^a	28.42 ^a	20.19 ^a	6.20 ^a	6.58 ^a	72.93 ^a
8	9.14 ^b	20.61 ^b	14.68 ^c	4.66 ^{bc}	6.45 ^a	55.53 ^{bc}
9	8.76 ^{bc}	19.45 ^{bc}	14.67 ^c	5.55 ^{ab}	4.45 ^b	51.96 ^{cd}
10	8.90 ^{bc}	20.61 ^b	15.75 ^{bc}	4.66 ^{bc}	4.59 ^b	56.22 ^{bc}
SEM	0.37	0.92	0.64	0.24	0.22	2.25
P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	<0.01	<0.01	<0.01	0.039	<0.01	<0.01

^ALacking common letters (a or f) significant differences at $P < 0.05$.^BMean represented the average value of 10 replicates ($n = 10$).**Table 6.** Effect of age on the absolute length of the intestine of Magang geese from 1 to 10 weeks of age (cm/kg) ^A.

Age (week) ^B	Duodenum	Jejunum	Ileum	Caecum	Rectum	Overall intestine
1	19.96 ^d	40.96 ^d	37.50 ^c	7.47 ^d	5.44 ^g	111.33 ^f
2	23.01 ^d	52.08 ^c	47.43 ^b	10.60 ^{de}	6.47 ^{fg}	139.65 ^e
3	27.18 ^c	61.20 ^b	49.10 ^b	12.91 ^{cd}	7.08 ^{ef}	158.50 ^d
4	35.75 ^{ab}	66.90 ^b	70.41 ^a	14.54 ^{bc}	9.02 ^{cd}	198.13 ^{bc}
5	34.37 ^{ab}	77.05 ^a	65.92 ^a	11.69 ^{de}	9.38 ^{bc}	198.40 ^{abc}
6	33.16 ^b	66.08 ^b	62.40 ^a	17.05 ^{ab}	7.96 ^{de}	186.65 ^c
7	37.67 ^{ab}	78.25 ^a	69.17 ^a	18.40 ^{ab}	9.82 ^{ab}	213.30 ^a
8	35.73 ^{ab}	76.33 ^a	68.97 ^a	19.79 ^a	11.08 ^a	211.91 ^{ab}
9	36.05 ^{ab}	74.37 ^a	65.55 ^a	21.08 ^a	10.42 ^{ab}	207.47 ^{ab}
10	36.67 ^a	76.67 ^a	64.33 ^a	18.50 ^{ab}	10.67 ^{ab}	206.83 ^{ab}
SEM	0.86	1.68	1.59	0.70	0.28	0.54
P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	<0.01	<0.01	<0.01	0.33	0.12	<0.01

^ALacking common letters (a or f) significant differences at $P < 0.05$.^BMean represented the average value of 10 replicates ($n = 10$).

in domesticated ducks declined by 38% at 5 weeks post-hatching (Watkins et al. 2004). This reduction of the intestinal segments can be due more to the changes in the intestinal thickness rather than the intestinal length, which could be associated with the alteration of bioavailability and utilization of nutrients. There is no obvious explanation for this decline because it does not appear to optimize digestion.

Conclusion

Considering that the R^2 values of the Gompertz and Bertalanffy models were higher than for the Logistic and Richards models, Gompertz and Bertalanffy models with high coefficients of determination could be suitable models for female Chinese native Magang geese growth, and the age of the inflection points was estimated at 30.53 and 28.56 d, respectively. It was suggested that the intestinal system was well developed during 1–7 weeks old in terms of the intestinal weight and length, but displayed a decline in geese during 8–10 weeks old. The knowledge of growth curve models and intestinal development pattern could provide some useful information on the design of optimum management practices and breeding strategies in geese production.

Disclosure statement

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Data availability

The data that support this study are available in the article.

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肠道菌群及其代谢产物调节动物线粒体功能的研究进展

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摘 要: 线粒体是动物细胞生产能量的主要场所,可参与三磷酸腺苷的产生、细胞线粒体 Ca^{2+} 稳态的维持,在调节动物机体能量代谢方面发挥重要的作用。目前,研究发现肠道微生物及其代谢产物可影响细胞线粒体代谢水平和功能,参与调节机体营养物质代谢周转速度,最终影响畜禽生长发育及饲料转化效率等。本文在总结线粒体生物学功能的基础上,重点阐述了肠道微生物及其代谢产物对线粒体功能的调节作用及影响因素,旨在为饲料营养手段介导肠道微生物-宿主线粒体途径调节动物生长发育和肠道健康提供理论参考。

关键词: 线粒体功能;肠道微生物;代谢产物;肠道健康

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Regulation of Mitochondrial Function by Gut Microbiota and Their Metabolites in Animal

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Abstract: Mitochondria are the primary energy-generating sites in animal cells, where they can participate in the production of triphosphate and the maintenance of a constant state of mitochondrial Ca^{2+} , which is crucial for animal health and energy metabolism. Studies have found that gut microflora and their metabolites can mediate the level and function of mitochondrial metabolism, which then affects the growth and development of the turnover of nutrient metabolism, feed conversion efficiency, etc. Based on the summary of the biological function of mitochondria, this review focuses on the influence of gut microflora and their metabolites on mitochondrial activity and the influencing factors, aiming to provide a theoretical reference for regulating growth and intestinal health of animals by the dietary nutrition-mediated intestinal microbial-host mitochondrial pathway.

Key words: mitochondrial function; intestinal microorganism; metabolites; intestinal health

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肠道微生物群作为动物最大的微生态系统,将 动物消化道无法消化的饲料成分降解成可吸收的成

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分和一些具有生理活性的小分子化合物。肠道菌群(如乳酸菌、链球菌、肠杆菌、梭杆菌)及其代谢产物(短链脂肪酸(short-chain fatty acids, SCFA)、胆汁酸、内毒素等)对宿主营养素消化吸收、免疫系统发育、肠道健康有重要作用^[1]。越来越多研究发现,肠道微生物及其代谢产物在调节线粒体功能方面产生影响^[2-3],进而影响畜禽机体的抗氧化能力、生长发育、营养代谢周转速度及饲料转化效率等^[4]。短链脂肪酸(乙酸、丙酸和丁酸等)可提高宿主细胞氧气消耗和烟酰胺腺嘌呤二核苷酸(NADH)、烟酰胺腺嘌呤二核苷酸磷酸(NADPH)水平,改善线粒体氧化磷酸化(oxidative phosphorylation, OXPHOS)水平和抗氧化性能^[5];肠道微生物有害代谢产物(如毒胆酸)可增加活性氧(reactive oxygen species, ROS)含量、抑制线粒体呼吸链复合体的活性^[6]。肠道微生物中间产物吲哚丙酸(Indole propionic acid, IPA)诱导线粒体 Ca^{2+} 超载以及 O_2 过度消耗,从而导致线粒体功能障碍^[7]。此外,肠道微生物区系及其代谢产物类型与饲料营养水平密切相关。研究表明,日粮纤维增加了肠道菌群多样性,并显著升高毛螺菌科丰度以及丁酸浓度^[8]。因此,期望通过饲料营养手段靶向调控肠道微生物菌群及其代谢物来调节畜禽线粒体功能,改善其生长与健康水平。

1 线粒体的生物学功能

线粒体是真核细胞中的一种重要的细胞器,能够产生三磷酸腺苷(ATP)、维持细胞 Ca^{2+} 稳态和调节 ROS 产生^[9]。细胞线粒体产生的 ATP,参与糖酵解中底物的氧化、三羧酸循环(TCA)、丙酮酸脱羧^[10-11]。其中,氧化磷酸化系统由 4 个多蛋白复合物(I ~ IV)和 ATP 合酶(复合物 V)组成,OXPHOS 是需氧产生 ATP 的主要来源,特别对神经元^[12]等需要高能量的细胞至关重要。作为电子传递链(ETC)的起始位点,复合物 I、II 促进电子的传递并与电子受体 O_2 结合,从而驱动 ATP 合成。钙是线粒体功能的调节因子,在细胞器内的多水平上促进 ATP 合成,而氧化应激下,细胞中钙相关通道功能障碍,大量 Ca^{2+} 聚集于线粒体,从而导致线粒体损伤和细胞凋亡^[13]。当细胞内 Ca^{2+} 超载时,线粒体内膜上的电子传递链产生 ROS,而线粒体内 ROS 的产生是细胞氧化应激的主要来源^[14]。畜禽体内 ROS 生成与清除的动态平衡被打破对畜禽健康有害,当 ROS 的浓度超过抗氧化剂的缓冲能力

时,ROS 可导致关键生物分子(如脂类、蛋白质或 DNA)过氧化损伤。机体线粒体 ROS 生成的增加可引起热休克反应、生长缓慢、免疫失调等代谢疾病^[15-17]。

2 肠道微生物及其代谢产物对线粒体功能的影响

胃肠道菌群是动物体内最复杂的共生微生物生态系统,微生物既可以通过代谢宿主的饲料成分产生氨基酸、SCFA 等,也可以通过自身的生物合成基因簇,产生独特结构和功能的代谢产物影响动物生长发育。近年来的大量研究发现,肠道菌群参与调节宿主的神经生理功能,并提出“微生物-肠-脑轴”^[18]概念。但这些研究多侧重于肠道微生物及其代谢产物对宿主组织和细胞的影响及其作用机制,对细胞器功能(如线粒体功能)调节作用的研究报道偏少。Saijara 等^[19]研究发现,小鼠肠道菌群可介导过氧化物酶体增殖物激活受体 γ 辅激活因子 1α (peroxisome proliferator-activated receptor γ coactivator- 1α , PGC- 1α) 通路促进动物线粒体生物合成。线粒体功能方面,益生菌鼠李糖乳杆菌激活过氧化物酶增殖物激活受体 α (peroxidase proliferators activate receptors- α , PPAR- α) 上调脂肪细胞因子表达,提高线粒体 OXPHOS 水平^[20]。此外,小鼠巨噬细胞中结核分枝杆菌抑制内毒素(LPS)介导的 Toll 样受体(Toll-like receptors, TLRs)信号通路,从而减少线粒体 ROS 的产生^[21]。上述研究提示,肠道微生物菌群及其代谢产物可维持肠道稳态或介导调节线粒体生物合成代谢途径,对宿主线粒体功能产生影响。

2.1 肠道微生物及其代谢产物调节线粒体能量代谢

SCFA 是膳食纤维在动物肠道中的乳酸菌和双歧杆菌等介导下发酵产生的代谢产物^[22],包括乙酸、丙酸和丁酸等。短链脂肪酸是结肠上皮细胞的主要能量来源^[23],其中丁酸作为首要能源物质,对维持肠道功能稳态以及调节细胞能量代谢具有重要作用。丁酸盐和乙酸盐可通过激活小鼠结肠细胞腺苷酸活化蛋白激酶(AMP-activated protein kinase, AMPK)通路调控线粒体功能,AMPK 可作为调节线粒体 OXPHOS 的能量感受器^[24]; Mollica 等^[25]发现,N-丁酸盐通过激活 AMPK-乙酰辅酶 A 羧化酶(acetyl-CoA carboxylase α , ACC) 通路改善了线粒体呼吸能力,从而促进了肝中的线粒体能量代谢。

目前,大量畜禽试验已证实丁酸及其衍生物在畜禽生长发育和免疫健康及细胞能量与组织稳态等方面具有显著改善作用。同时,丁酸也可以通过能量底物这一作用,提高结肠上皮细胞线粒体功能,进而调节肠上皮细胞功能。Donohoe 等^[26]的体外试验证实,丁酸盐进入线粒体,经过 β 氧化、TCA 循环后有助于提高 NADH 呼吸链酶活性及 ATP 合成,并促进肠细胞氧化代谢及抑制细胞自噬,当添加丁酸盐到无菌小鼠的结肠细胞中时,OXPHOS 水平增加,自噬情况得到改善。体内试验同样证实,饲料中添加丁酸钠可显著提高饲料转化效率^[27]和抗氧化能力^[28],一方面,丁酸激活 PGC-1 α 通路并增强线粒体功能来加快能量转化效率;另一方面,丁酸作为信号分子,可以通过抑制组蛋白去乙酰化酶(histone deacetylase,HDAC)参与线粒体能量代谢酶活性的调控。肝组织中产生的初级胆汁酸^[29],经过部分肠道厌氧菌的降解成为次级胆汁酸,如去氧胆酸和石胆酸。次级胆汁酸是脂质和能量代谢调节剂,可激活核花呢素 X 受体(farnesoid X receptor,FXR)和 G 蛋白偶联胆汁酸受体 1(G protein-coupled bile acid receptor 5,TGR5)信号通路与线粒体互作调控^[30]。胆汁酸具有促进脂肪消化吸收、抑制肠道有害菌群的生长繁殖以及提高能量代谢水平的作用^[31]。肠道中的胆汁酸与 FXR 受体结合产生纤维因子 19/15(fibroblast growth factor 19/15,FGF19/15)通过脑-肠轴到达下丘脑调节葡萄糖代谢,进一步研究发现,下丘脑中 FGF19 具有调节能量代谢的作用^[32]。

2.2 肠道微生物及其代谢产物调节线粒体 ROS 的产生

OXPHOS 产生 ATP 的过程中,线粒体内膜上的电子传递链产生 ROS,畜禽机体 ROS 的产生随着能量需要的增加呈上升趋势,其原因是线粒体 ETC 泄漏导致的^[33]。复合物 I 是 ROS 产生的主要来源,其不稳定的性质,将导致蛋白质、脂质和 DNA 发生氧化反应^[34]。肠道微生物及其代谢产物通过调节 ROS 的产生靶向调控线粒体,动物机体 ROS 的大量增加主要源于胆汁酸引起的线粒体膜通透性改变。体外试验证实,毒胆酸可增加人和小鼠肝细胞中 ROS 含量、破坏线粒体膜电位(mitochondrial membrane potential,MMP)和抑制线粒体呼吸链复合体的活性^[6]。研究表明脱氧熊胆酸(UDAC)浓度低于 $50 \mu\text{mol} \cdot \text{L}^{-1}$ 时可使线粒体膜

通透性增加^[35]。饲料添加高浓度脱氧胆酸钠(NaDOC)可能造成线粒体氧化损伤,NaDOC 作为氧化应激诱导剂在结肠以及肝细胞上得到广泛运用^[36],NaDOC 抑制肠道 Ca^{2+} 吸收以及增加 ROS 的产生和线粒体膜通透性的改变,导致线粒体氧化应激以及功能障碍^[37]。Xavier 等^[38]研究表明,一定浓度的 UDAC 可以降低神经干细胞线粒体 ROS 产量并提高细胞色素含量,进而保护线粒体的完整性和功能,可作为一种靶向线粒体的抗凋亡和抗氧化物质。虽然在畜禽生产上相关的研究较少,但小鼠模型研究表明,丙酸可干扰线粒体代谢,导致线粒体 ROS 的产生。丙酸与辅酶 A 结合后生成重要的中间代谢产物丙酰辅酶 A,进一步转化后以琥珀酰辅酶 A 的形式进入柠檬酸循环^[39]。琥珀酰辅酶 A 在正常生理浓度下可以提高线粒体基质功能水平,但高浓度的琥珀酰辅酶 A 抑制柠檬酸合成酶,直接导致线粒体 ROS 产生。在丙酸存在的情况下,柠檬酸循环的第一个步骤被抑制,NADH 的产量减少导致线粒体呼吸链复合物 I 效率下降,线粒体功能受到影响。有研究表明,添加较高浓度的丙酸,可加剧 ROS 的产生^[40]。其原因是 ROS 的存在导致细胞内产生活性氮,活性氮可与丙酸反应生成一种能有效抑制线粒体功能的化合物 3-硝基丙酸^[41]。饲料中 1%~2% 的色氨酸可被一些带有色氨酸酶的梭状芽孢杆菌、大肠杆菌代谢成五羟色胺和褪黑素^[23]。褪黑素的实质是一种内源性的吲哚胺,吲哚胺是强大的抗氧化剂和自由基清除剂,可以保护细胞膜、ETC 和线粒体 DNA 免受氧化损伤。在心血管相关研究中发现,褪黑素可通过 AMPK-PGC-1 α -SIRT3 信号通路,提高核因子(NRF)和线粒体转录因子 A(mitochondrial transcription factor A,TFAM)的表达,改善呼吸链功能,减少 ROS 产生,保护心血管。且相比于传统的抗氧化剂,褪黑素对线粒体的保护作用更具有靶向性^[42]。

2.3 肠道微生物及其代谢产物调节线粒体 Ca^{2+} 稳态

线粒体 Ca^{2+} 稳态与线粒体的能量代谢显著相关。当线粒体对 Ca^{2+} 转运能力降低时,可导致 MMP 降低、线粒体 Ca^{2+} 摄取减少,从而影响线粒体呼吸链的完整性^[43]。近年来,研究者们发现了线粒体和细胞钙稳态新的生理作用,即线粒体 Ca^{2+} 的摄取通过调节细胞质 Ca^{2+} 稳态影响细胞外 Ca^{2+} 进入,因此可能影响肌肉收缩、神经元兴奋性和细胞迁移功能^[44]。线粒体钙单向转运体蛋白(mitochon-

drial calcium uniporter, MCU) 介导的线粒体 Ca^{2+} 摄取,是由 ETC 产生的内膜电位驱动的。添加 SCFA 能够上调牛瘤胃上皮细胞 MCU 基因表达水平,从而促进线粒体 Ca^{2+} 摄取以及 ATP 产生^[45]。研究表明,饲料添加丁酸钠通过调控瞬时受体电位亚家族 V 成员 6 (transient receptor potential channel subfamily V member 6, TRPV6) 通路增加了小鼠肠道 Ca^{2+} 水平^[46]。肠道菌群在催化精氨酸的过程中,可以产生鸟氨酸和一氧化氮 (NO) 等物质^[47]。鸟氨酸循环能够促进线粒体能量代谢,鸟氨酸脱羧酶作为鸟氨酸循环、多胺合成代谢途中的第一个限速酶^[48],增加了多胺、NO 的生成。多胺通过调控细胞 Ca^{2+} 稳态以及 E-钙黏蛋白 (*E-cadherin*) 的基因表达,维持肠上皮屏障功能以及细胞膜通透

性^[49]。当肠道中革兰阴性菌数量增加或肠道屏障受损时,肠道微生物会产生 LPS,胡晓飞等^[50]通过给肉仔鸡腹腔注射 $500 \mu\text{g} \cdot \text{kg}^{-1}$ (BW) LPS,发现其对生长性能并无显著作用,但是对胴体品质和胸肌肉品质产生了不良影响,其原因是 LPS 诱导线粒体功能损伤、线粒体 Ca^{2+} -ATP 酶活性下降,从而影响肌纤维钙离子转移系统功能^[51]。

综上所述,肠道微生物定植后调控机体生长发育,同时,产生大量代谢产物,在维持肠道稳态、辅助动物抵御有害环境和调控线粒体功能方面具有重要作用。肠道微生物及其代谢产物具有很好地调节线粒体状态的作用,通过影响微生物之间的群体响应和调控、线粒体功能相关蛋白表达等途径来发挥作用(图 1)。

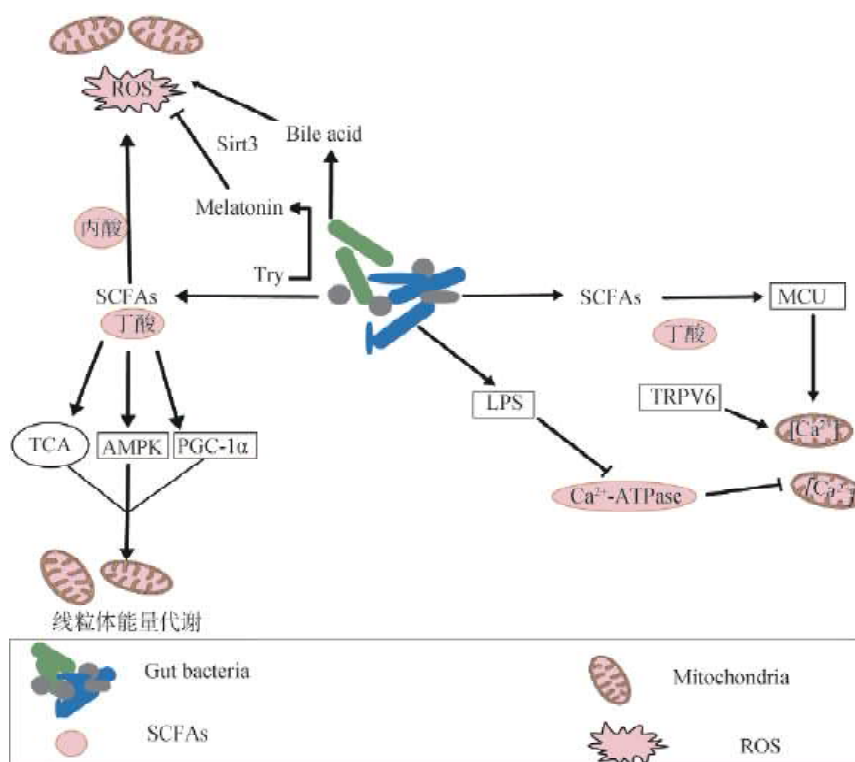


图 1 肠道微生物及其代谢产物对线粒体功能的调节途径

Fig. 1 Regulation of mitochondrial function by intestinal microbes and their metabolites

3 肠道微生物对线粒体功能的影响因素

3.1 饲料营养

饲料种类、水平和结构是影响动物肠道微生物组成和代谢的重要因素。有研究表明,低蛋白质水平(13%)饲料显著提高了育肥猪回肠中的消化链球菌与梭菌科相对丰度,有助于维持肠道微生物稳态和乙酸的产生,这提示肠道微生物对蛋白质营养的

利用与饲料蛋白质水平相关^[52-53];而高蛋白质水平(23%)饲料增加仔猪肠道内 pH,抑制肠杆菌与乳酸杆菌属的生成,促进致病微生物的增殖^[54]。Ijaz 等^[55]研究发现,饲喂高脂饲料显著降低小鼠肠道中的阿克曼氏菌、乳酸杆菌、双歧杆菌属丰度以及 PGC-1 α 蛋白水平,从而抑制线粒体生物合成。Regmi 等^[56]用不同直链淀粉含量(<5%、20%、28%和 63%)饲料饲喂仔猪,发现饲喂 63%直链淀

粉显著提高仔猪粪中的乳酸杆菌、双歧杆菌丰度以及短链脂肪酸与丁酸含量。与 2.5% 粗纤维饲料水平相比, 7.5% 粗纤维饲料水平显著提高了母猪纤维降解菌丰度和 SCFA 浓度^[57]。Zeitl 等^[58]发现, 饲喂发酵木质纤维素粗纤维饲料可降低肉鸡肠道厚壁菌门和乳杆菌属丰度和微生物代谢产物浓度^[59]。添加剂方面, 白藜芦醇、姜黄素等植物提取物具有抗氧化、抗炎等生物功能。给饲喂高脂日粮的大鼠添加白藜芦醇显著提高了肠道丁酸产生菌的比例以及 SCFA 浓度, 从而促进线粒体能量代谢水平^[60]。姜黄素通过丝裂原活化蛋白激酶 (mitogen activated protein kinase, MAPK)、核因子 E2 相关因子 2 (nuclear factor erythroid 2 related factor, Nrf2) 信号通路降低 ROS 的水平, 缓解热应激诱导的线粒体氧化应激损伤^[61]。此外, 姜黄素能够降低仔猪大肠杆菌相对丰度以及 Toll 样受体 4 (Toll-like receptors 4, TLR4) 蛋白表达, 从而抑制 ROS 的产生, 最终缓解线粒体功能障碍^[62]。

3.2 饲料转化效率

饲料转化效率与线粒体功能有着密切关系, 相同的环境和饲料条件下, 与高饲料转化率肉鸡相比, 低饲料转化率肉鸡肝线粒体功能和呼吸链复合物 (I、II、III 和 IV) 的活性下降及 ROS 和蛋白质羰基含量升高^[63-64]。同样, 与低饲料转化率羔羊相比, 高饲料转化率羔羊相关的呼吸链复合物酶活性显著下降^[65]。转录组学分析表明, 低剩余采食量猪肌肉中的超氧化物歧化酶 (superoxide dismutase, SOD)、谷胱甘肽过氧化物酶 3 (glutathione peroxidase 3, GPX3)、过氧化氧化还原蛋白 2 (peroxiredoxins 6, PRDX6) mRNA 水平显著下降, 从而抑制 ROS 的产生^[66]。上述研究表明, 动物饲料转化效率与其线粒体功能密切相关。肠道菌群方面, 不同饲料转化效率肉鸡的回肠、盲肠微生物多样性差异不显著, 但低剩余采食量肉鸡盲肠中 *Oscillibacter*、梭状芽孢杆菌科丰度显著升高^[67]。高饲料转化率猪盲肠乳杆菌属和拟杆菌纲中的普雷沃菌属的相对丰度显著高于低饲料转化率猪^[68-69]。因此, 不同饲料转化效率畜禽肠道微生物的改变是否参与调节机体线粒体功能值得进一步探究。

3.3 饲养阶段

不同生长阶段动物肠道微生物的组成和代谢水平对生长发育具有重要影响。Liu 等^[70]研究表明, 哺乳期间仔猪空肠中较高的乳酸杆菌和拟杆菌属丰

度, 能够促进线粒体生物合成相关基因的表达^[71]。也有研究发现, 仔猪断奶后其结肠中 *Alloprevotella* 和 *Oscillibacter* 相对丰度降低, 且肝线粒体功能相关基因泛素氧化酶 A2 亚基 (ubiquinone oxidoreductase subunit A2, NDUFA2) 和泛素氧化酶 A5 亚基 (ubiquinone oxidoreductase subunit A5, NDUFA5) mRNA 表达水平以及 ATP 含量显著降低, 表明仔猪断奶诱导了线粒体氧化应激和功能障碍^[72-73]。与妊娠后期相比, 哺乳期母猪粪便中的拟杆菌属丰度显著增加, 这意味着不可消化的饲料成分可被发酵并产生 SCFA, 从而促进线粒体功能相关基因的表达水平^[74]。Qi 等^[75]比较了不同生长阶段对猪肠道菌群的影响, 发现 6 月龄猪肠道中瘤胃球菌科以及有益细菌 *Oscillospira* 丰度增加, 可促进机体丁酸盐的产生^[76]。肉鸭方面, 相比生长后期 (6~10 周龄), 2~4 周龄肉鸭十二指肠与空肠中乳酸乳球菌丰度相对较高, 乳酸乳球菌与线粒体转运蛋白表达水平密切相关, 从而为早期生长发育提供能量^[77-78]。总之, 不同生长阶段动物肠道微生物的组成和代谢产物的变化可参与线粒体功能调节, 进而影响宿主生长发育。

3.4 其他因素

动物机体各种应激可介导肠道微生物菌群及其代谢产物变化进而影响线粒体能量代谢水平。在小鼠上, 高温急性热应激和中风应激显著降低肠道乳酸杆菌丰度, 并抑制抗氧化活性, 从而导致 ROS 产量的增加以及线粒体氧化损伤^[79-80]。高脂日粮诱导的小鼠慢性应激可降低其厚壁菌门与拟杆菌门比例及其代谢产物丁酸和 SCFA 浓度, 从而影响线粒体生物合成^[81]。Koncz 等^[82]发现, 紫外线诱导小鼠肾细胞超氧化物的增加, 从而降低线粒体 NADH 以及 Ca^{2+} 水平。日龄方面, 研究发现 5 月龄猪肠道菌群多样性随着月龄增加呈上升趋势, 5 月龄后保持稳定, 但其乳酸杆菌、双歧杆菌的相对丰度呈降低趋势^[83]。Amit-Romach 等^[84]采用 16S rRNA 基因靶向分析 4、14、25 日龄肉鸡肠道微生物多样性, 发现 4 日龄肉鸡十二指肠和盲肠乳酸菌属是主要优势菌属, 且其比例随着日龄增加而增多。已有研究发现, 乳酸杆菌增加可显著提高肠道短链脂肪酸浓度和抗氧化酶活性, 并抑制 ROS 产生, 从而影响宿主线粒体功能^[85]。

4 小结

研究肠道微生物与线粒体功能的关系对动物健

康有重要意义。目前,研究多侧重于肠道微生物及其代谢产物对宿主组织和细胞的影响及其作用机制,需进一步加强对细胞器功能(如线粒体功能)的研究。肠道微生物组成和水平通过何种途径影响线粒体功能,以及线粒体与肠道微生物对话机制如何实现尚不清楚。因此,今后应加强研究肠道微生物及其代谢产物对宿主线粒体的调节作用,为饲养手段靶向线粒体功能调控动物生长发育提供新思路和新方法。

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Effect of oral spray with *Lactobacillus* on growth performance, intestinal development and microflora population of ducklings

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Objective: The aim of this study is to investigate the effect of oral spray with probiotics on the intestinal development and microflora colonization of hatched ducklings.

Methods: In Exp. 1, an one-way factorial design was used to study the antibacterial activity of the probiotics and metabolites on *Escherichia coli* (*E. coli*) without antimicrobial resistance. There were four experimental groups including saline as control and *Lactobacillus*, *Bacillus subtilis*, combined *Lactobacillus* and *Bacillus subtilis* groups. In Exp. 2, 64-day-old ducklings were allotted to 2 treatments with 4 replicated pens. Birds in the control group were fed a basal diet supplemented with *Lactobacillus* fermentation in the feed whereas birds in the oral spray group were fed the basal diet and administrated *Lactobacillus* fermentation by oral spray way during the first week.

Results: In Exp. 1, the antibacterial activities of probiotics and metabolites on *E. coli* were determined by the diameter of inhibition zone in order: *Lactobacillus*>combined *Lactobacillus* and *Bacillus subtilis*>*Bacillus subtilis*. Additionally, compared to *E. coli* without resistance, *E. coli* with resistance showed a smaller diameter of inhibition zones. In Exp. 2, compared to control feeding group, oral spray group increased ($p<0.05$) the final body weight at d 21 and average daily gain for d 1-21 and the absolute weight of the jejunum, ileum and total intestine tract as well as cecum *Lactobacillus* amount at d 21.

Conclusion: *Lactobacillus* exhibited a lower antibacterial activity on *E. coli* with resistance than *E. coli* without resistance. Oral spray with *Lactobacillus* fermentation during the first week of could improve the intestinal development, morphological structure, and microbial balance to promote growth performance of ducklings from hatch to 21 d of age.

Keywords: Ducklings; Intestinal Development; *Lactobacillus*; Oral Spray

INTRODUCTION

The immediate post-hatch period is critical for the development of gastrointestinal tract to minimize the mortality and keep uniformity of young poultry [1]. During first post-hatched days the small intestine undergoes dramatic physiological and morphological changes to increase nutrient digestion and absorption [2,3]. For example, the weight and length of small intestine increases more rapidly than the whole body mass and reaches a maximum between 3 and 7 days [4]. In addition, in newly hatched birds a major change in the source of nutrients occurs by switching from yolk nutrition to enteral nutrition [5]. Early nutrient supply to young poultry is essential for improving the intestine growth and nutrient intake [6] via stimulating digestive enzymes secretion and increasing yolk sac nutrient utilization [7]. Therefore, it is necessary to explore whether the use of neonatal probiotics supplements could promote intestinal health early in life to maximize the nutrient efficiency in later life.

Using probiotics to improve the intestine health of poultry is not a new concept, however, a complete understanding of when and how to use probiotics still has great potential.

The beneficial effects of probiotics served as alternative feed additives to antibiotics on improving the intestinal microbial balance, morphological structure and feed utilization have been proved in poultry production [8]. Therefore, it is important to select probiotics as supplements to aid in the proper development and microflora colonization of the intestinal tract in hatched birds as soon as possible [9]. Due to the dependence on the residual yolk nutrition and the lower feed intake of birds during the first few days post-hatch [10], it is speculated that provision of probiotics supplied by feeding might not be enough to exert some effect on the rapid development of intestine of birds in early life. Therefore, a new method of supplying probiotics using oral spray was adopted to obtain the greater intestinal health and nutrient efficiency in newly hatched birds in the present study. Firstly *in vitro*, the inhibitory effect of *Lactobacillus* and metabolites on *Escherichia coli* (*E. coli*) (antimicrobial resistance vs non-antimicrobial resistance) was investigated (Exp. 1). Secondly, *in vivo*, the effect of *Lactobacillus* fermentation in oral spray way during the first week of on growth performance, intestinal development, and gut flora amount was evaluated in ducklings from hatch to 21 d of age (Exp. 2).

MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee of South China Agricultural University (SCAU-AEC-2010-0416). This study included 2 experiments as follows: the antibacterial activity of probiotics and metabolites on *E. coli in vitro* (Exp. 1) and the effect of *Lactobacillus* fermentation in oral spray way during the first week on performance and intestinal development of ducklings from hatch to 21 d of age *in vivo* (Exp. 2).

Microorganisms and medium

Avian *E. coli* ATCC25922 without antimicrobial resistance was purchased from China General Microbiological Culture Collection Center (Beijing, China), while avian *E. coli* ATCC 25922 with antimicrobial resistance was kindly provided by Professor Sun in College of Veterinary Medicine of South China Agricultural University (Guangzhou, China). *Lactobacillus* and *Bacillus subtilis* were isolated from a commercial product in our lab by conventional microbiological identification methods [11]. *E. coli*, *Lactobacillus* and *Bacillus subtilis* were cultured in mediums (Boyao Biotechnology Company, Shanghai, China) of Eosin-Methyl Blue Agar broth (#BS1041), MRS broth (#BS1138), and Nutrient Agar broth (#BS1002), respectively.

Antimicrobial sensitivity test

Firstly, *Lactobacillus* and *Bacillus subtilis* were fermented in Luria broth (#BS2078) for 24 h in anaerobic and aerobic con-

ditions to prepare the solutions of probiotics fermentation without filtration, respectively. In Exp. 1, an one-way factorial design was used to study the antibacterial activity of the probiotics and metabolites on *E. coli* without antimicrobial resistance. There were four experimental groups including saline as control and *Lactobacillus*, *Bacillus subtilis*, combined *Lactobacillus* and *Bacillus subtilis* groups. The combined mixture was prepared by an equal volume of *Lactobacillus* and *Bacillus subtilis* fermentations. Oxford cups were punched into the plates and were loaded with 2 mL probiotics culture stock solution. After overnight incubation at 37°C, the diameters of the inhibition zones were determined to examine the antibacterial activity of against test *E. coli* [12]. All procedures were performed in three replicates for three times. Then, the *Lactobacillus* fermentation group presenting a greater inhibitory effect on the *E. coli* was screened and selected to evaluate the antibacterial activity between two *E. coli* sources with and without antimicrobial resistance.

Birds, sample collection and analyses

In Exp. 2, 64-day-old Cherry Valley ducklings were weighed individually and allotted to 2 treatments with 4 replicated pens of 8 ducklings per pen based on similar body weight (BW). All ducklings were reared on wire floors in an environmentally controlled room with adjustment of temperature and humidity from 0 to 21 d of age. Birds in the control group were fed a basal diet supplemented with probiotics fermentation in the feed whereas birds in the oral spray group were fed the basal diet and administrated probiotics fermentation by oral spray during the first week. To determine the antibacterial activity of probiotics and metabolites on *E. coli in vitro*, the screened *Lactobacillus* was fermented in 50 g/L brown sugar solution as prepared like in Exp. 1. For the oral spray group, 1 mL *Lactobacillus* fermentation (10^9 colony forming units [CFU]/mL) were taken and supplied for each duckling by oral spraying at 1 d of age. Oral spray produces a spray of balanced tiny droplets of fermentation of *Lactobacillus* and delivers it directly into duckling's mouth twice each day. The dose of liquid fermented *Lactobacillus* was increased step-wise by 1 mL/d for each duckling until 7 d of age. For the control group, ducklings were fed the basal diet supplemented with an equal amount of *Lactobacillus* fermentation to that used in the spray group per pen during the first week. From 8 to 21 d of age, all ducklings in the two groups were just fed the same basal diet without any *Lactobacillus* fermentation supplementation or oral spray. Feed intake was recorded each day per pen. The basal diet was formulated to meet or exceed the nutrient requirements recommended by NRC (1994) for ducklings at the starter period. Compositions and nutrient levels of the basal diet are presented in Table 1. Feed and water were provided *ad libitum* and no mortality of birds were observed throughout the experimental period. At d 7 and

Table 1. Composition and nutrient levels of the basal diet (as-fed basis)

Items	
Ingredient (%)	
Corn	56.23
Soybean meal	25.64
Rapeseed meal	8.78
Wheat middling	2.63
Soybean oil	2.53
Dicalcium phosphate	1.42
Limestone	1.09
L-lysine HCl	0.13
DL-methionine	0.20
Salt	0.25
Choline chloride	0.10
Vitamin and mineral premix ¹⁾	1.00
Total	100.00
Nutrient levels	
	Calculated values
Metabolizable energy (MJ/kg)	12.27
Crude protein (%)	20.01
Calcium (%)	0.92
Total phosphorus (%)	0.65
Available phosphorus (%)	0.39
Lysine (%)	1.10
Methionine (%)	0.51
Methionine+cysteine (%)	0.81

¹⁾ Provided per kilogram of diet: vitamin A, 4,000 IU; vitamin D₃, 2,000 IU; vitamin E, 24 IU; thiamine, 2.0 mg; riboflavin, 12 mg; pyridoxine, 4.0 mg; vitamin B₁₂, 0.02 mg; calcium pantothenate, 10 mg; folate, 0.15 mg; niacin, 50 mg; biotin, 0.15 mg; Choline (Choline chloride), 1,000 mg; Cu (CuSO₄·5H₂O), 8 mg; Fe (FeSO₄·7H₂O), 80 mg; Zn (ZnSO₄·7H₂O), 90 mg; Mn (MnSO₄·H₂O), 70 mg; Se (NaSeO₃), 0.3 mg; I (KI), 0.4 mg.

21, after 12 h feed withdrawal, birds were weighed by each replicate pen. The average daily gain (ADG), average daily feed intake (ADFI), and feed:gain ratio (F:G) were calculated. At d 21, based on the average BW per pen, 2 birds in each pen were euthanized by CO₂ inhalation, and the duodenum, jejunum, and ileum were separated for the measurements of weight and length. The relative weight and length of the duodenum, jejunum, and ileum were calculated based on the BW. Then, segments of about 1.5 cm from the middle of duodenum, jejunum, and ileum were excised and flushed with ice-cold saline and immediately placed in 4% paraformaldehyde for morphometric analysis. The indices of villus height, crypt depth and muscular thickness were measured

using computer-aided light microscope image analysis as described by Uni et al [4]. The chyme in caecum of one duckling was selected for measuring the content of total colonies, *E. coli* and *Lactobacillus* by the plate CFU method [13].

Statistical analyses

In Exp. 1, the data about the diameter of inhibition zones on *E. coli* without antimicrobial resistance were analyzed by one-way analysis of variance using the general linear model procedure of SAS 9.2 (SAS, 2009). Differences among means were tested by the least significant difference method. The data about the diameter of inhibition zones between two *E. coli* sources with and without antimicrobial resistance (Exp. 1) and growth performance, intestine weight and length, morphological structure, and cecal flora number of ducklings between the control and oral spray groups were analyzed by an independent samples t-test. Statistical significance was set at $p < 0.05$.

RESULTS

Diameter of inhibition zone

In Exp. 1, the diameter of inhibition zones from *Lactobacillus* group was greater than that from *Bacillus subtilis* group or combined *Lactobacillus* and *Bacillus subtilis* group ($p < 0.01$), while the diameter of inhibition zones from combined *Lactobacillus* and *Bacillus subtilis* group was greater than that from *Bacillus subtilis* group ($p < 0.01$, Table 2; Figure 1). Under *Lactobacillus* fermentation treatment, *E. coli* with antimicrobial resistance showed a smaller diameter of inhibition zones compared to *E. coli* without antimicrobial resistance ($p < 0.05$, Table 3; Figure 2).

Growth performance

In Exp. 2, oral spray with *Lactobacillus* fermentation during the first week had no effect on the final BW at 21 d and ADG at d 1 to 7 as well as ADFI and F:G at d 1 to 7 and d 1 to 21 ($p > 0.05$; Table 4). Compared to the control group, oral spray group had an increased final BW at 21 d and ADG of birds at d 1 to 21 ($p < 0.05$; Table 4).

Weight and length of intestine

The data of absolute weight and length of duodenum, jejunum,

Table 2. Effect of probiotics on the diameter of inhibition zone of *Escherichia coli* without resistance *in vitro*

Inhibition zone	Probiotics groups				p-value
	Saline	<i>Lactobacillus</i>	<i>Bacillus subtilis</i>	<i>Lactobacillus</i> + <i>Bacillus subtilis</i>	
Diameter (mm)	-	24.4 ± 0.99 ^a	11.1 ± 1.16 ^c	19.2 ± 1.30 ^b	< 0.0001

Data was expressed as mean ± standard deviation (n = 3).

"-": No detected inhibition zone.

^{a-c} Means within the same row lacking a common superscript differ ($p < 0.05$).

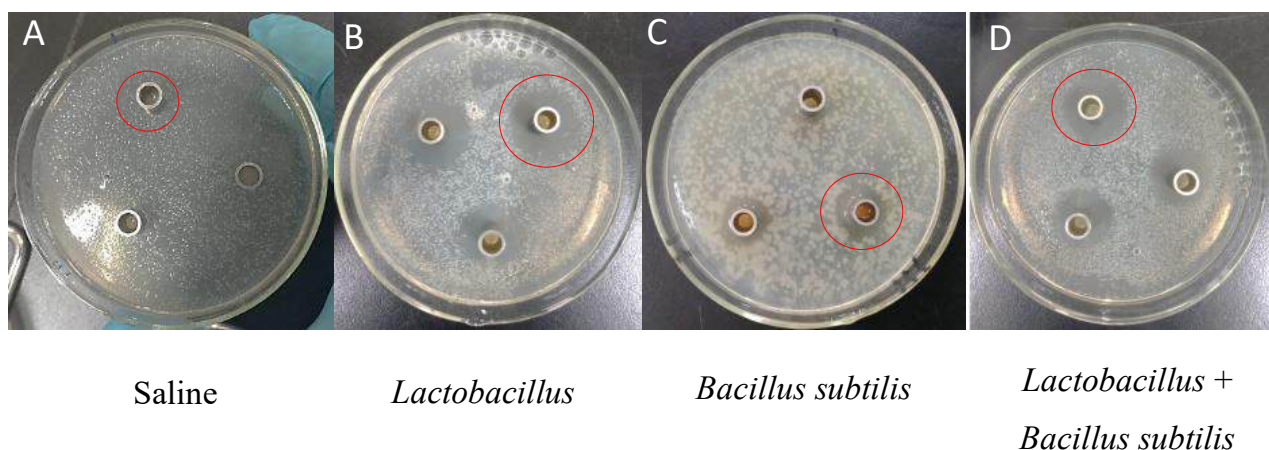


Figure 1. Representative inhibition zone of *Escherichia coli* (*E. coli*) without resistance. The diameters of the inhibition zones were determined to examine the antibacterial activity of probiotics culture stock solution against test *E. coli* (see red circle). The order of antibacterial activity was that *Lactobacillus* (B) > combined *Lactobacillus* and *Bacillus subtilis* (D) > *Bacillus subtilis* (C) > saline (A).

Table 3. Effect of *Lactobacillus* on the diameter of inhibition zone of *Escherichia coli* with or without resistance *in vitro*

Inhibition zone	<i>Escherichia coli</i> sources			p-value
	Saline	Non-resistance	Resistance	
Diameter (mm)	-	24.4 ± 0.50 ^a	23.0 ± 1.23 ^b	0.0076

Data was expressed as mean ± standard deviation (n = 3).

"-", No detected inhibition zone.

^{a,b} Means within the same row lacking a common superscript differ (p < 0.05).

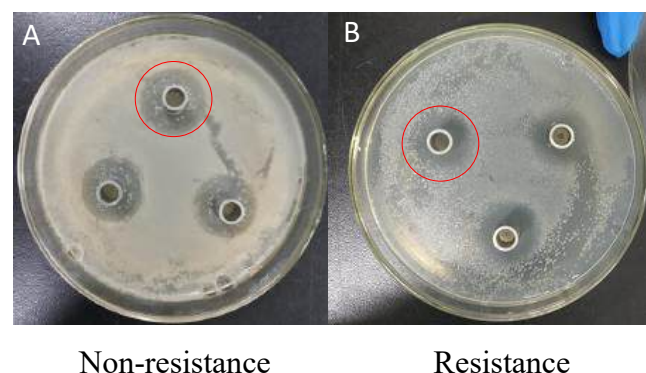


Figure 2. Representative inhibition zone of *Escherichia coli* (*E. coli*) with or without resistance. The *Lactobacillus* fermentation with a greater inhibitory effect on the *E. coli* was selected to evaluate the antibacterial activity between two *E. coli* sources with and without antimicrobial resistance (see red circle). The order of antibacterial activity was that non-resistance (A) > resistance (B).

ileum and total intestine tract at d 21 are presented in Table 5. Oral spray group increased (p < 0.05) the absolute weight of jejunum, ileum, and total intestine tract and did not influence other above-mentioned indices (p > 0.05) of ducklings at d 21 compared to the control group.

Intestinal histomorphology

The data and representative light microscopy of villus height, crypt depth, musculature thicknesses and villus height: crypt depth ratio of duodenum, jejunum and ileum are shown in Table 6, Figure 3, respectively. Oral spray with *Lactobacillus* fermentation during the first week increased (p < 0.05) the villus height and villus height: crypt depth ratio of duodenum and jejunum and musculature thicknesses of jejunum as well as decreased the crypt depth of duodenum compared

Table 4. Effect of oral spray with *Lactobacillus* on growth performance of ducklings from hatch to 21 d of age

Period	Group	Final BW (g/bird)	ADG (g/d/bird)	ADFI (g/d/bird)	F:G (g/g)
D 1-7	Control	159.7 ± 7.7	16.9 ± 1.4	27.6 ± 5.3	1.62 ± 0.20
	Oral spray	165.7 ± 6.0	17.7 ± 1.1	26.5 ± 3.6	1.50 ± 0.18
	p-value	0.26	0.37	0.76	0.39
D 1-21	Control	740.3 ± 20.6 ^b	32.5 ± 0.9 ^b	66.4 ± 4.1	2.05 ± 0.17
	Oral spray	801.1 ± 37.7 ^a	35.3 ± 1.8 ^a	70.7 ± 3.8	2.01 ± 0.15
	p-value	0.03	0.03	0.17	0.72

Data was expressed as mean ± standard deviation (n = 4).

BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; F:G, feed:gain ratio.

^{a,b} Means within the same column lacking a common superscript differ (p < 0.05).

Table 5. Effect of oral spray with *Lactobacillus* on the absolute intestinal weight and length of ducklings at 21 d of age

Items	Group	Duodenum	Jejunum	Ileum	Total tract
Absolute weight (g)	Control	4.45 ± 0.53	10.0 ± 0.89 ^b	9.25 ± 0.75 ^b	23.7 ± 1.7 ^b
	Oral spray	4.84 ± 0.68	11.3 ± 1.20 ^a	10.7 ± 1.38 ^a	26.9 ± 2.7 ^a
	p-value	0.22	0.02	0.02	0.01
Absolute length (cm)	Control	26.3 ± 2.0	62.8 ± 4.6	59.5 ± 4.4	148.6 ± 9.4
	Oral spray	26.8 ± 2.0	64.7 ± 4.0	64.3 ± 7.2	155.8 ± 10
	p-value	0.63	0.39	0.13	0.16

Data was expressed as mean ± standard deviation (n = 8).

^{a,b} Means within the same column lacking a common superscript differ (p < 0.05).

Table 6. Effect of oral spray with *Lactobacillus* on intestinal histomorphology of ducklings at 21 d of age

Segments	Group	Villus height (μm)	Crypt depth (μm)	Musculature thicknesses (μm)	Villus height: crypt depth
Duodenum	Control	365 ± 134 ^b	144 ± 23 ^b	542 ± 136	2.49 ± 0.64 ^b
	Oral spray	436 ± 89 ^a	123 ± 30 ^a	585 ± 74	3.84 ± 1.43 ^a
	p-value	0.02	0.001	0.14	< 0.0001
Jejunum	Control	296 ± 89 ^a	111 ± 21	438 ± 115 ^b	2.76 ± 0.99 ^b
	Oral spray	396 ± 80 ^b	107 ± 34	617 ± 91 ^a	4.08 ± 1.68 ^a
	p-value	< 0.0001	0.59	< 0.0001	< 0.0001
Ileum	Control	325 ± 45	102 ± 16	446 ± 50	3.28 ± 0.74
	Oral spray	343 ± 67	112 ± 23	475 ± 85	3.10 ± 0.38
	p-value	0.24	0.07	0.11	0.24

Data was expressed as mean ± standard deviation (n = 8).

^{a,b} Means within the same column lacking a common superscript differ (p < 0.05).

to the control group, but did not affect the intestinal histomorphology of ileum (p > 0.05).

Cecal microflora population

Compared with the control group, oral spray with *Lactobacillus* fermentation during the first week increased (p < 0.05, Table 7) the cecal *Lactobacillus* amount and had no effect (p > 0.05) on the amounts of *E. coli* and total colonies and *Lactobacillus*:*E. coli* ratio.

DISCUSSION

The use of probiotics has become more common to achieve greater productivity and health benefits in the poultry production [8]. Numerous *in vivo* studies in broilers [14], turkeys [15], and layers [16] have proved that probiotics as feed additive can improve nutrient utilization, gut health, and immune function, resulting in better production performance, such as greater BW gain and resistance to infectious bacteria. However, several other workers reported that no beneficial effects were observed in birds given diets supplemented with or without probiotics [17,18]. Variations in the efficacy of probiotics may depend upon the stability and efficiency of the probiotics and species or strains of microorganisms given to the host [19]. Considering the susceptibility to *E. coli* infections in poultry at the post-hatched period, the inhibitory effect of probiotics

against *E. coli* was investigated *in vitro* in the present study. According to the diameter of inhibition zone, the antibacterial activity of probiotics culture was *Lactobacillus* > combined *Lactobacillus* and *Bacillus subtilis* > *Bacillus subtilis*. As indicated previously [20], the manner by which *Lactobacillus* inhibits the growth and proliferation of pathogenic bacteria is by lowering the pH with the production of primary metabolites such as organic acids and hydrogen peroxide. The use of antibiotics in poultry feed as a growth promoter has been restricted in many countries around the world [21]. Probiotics are considered alternative microbial feed additives to enhance growth and disease prevention for birds by improving the intestinal microbial balance [22]. Therefore, in the present study the screened *Lactobacillus* were selected to evaluate the antibacterial activity between two *E. coli* sources with and without antimicrobial resistance as determined by the diameter of the inhibition zone. Compared to *E. coli* without resistance, *E. coli* with resistance displayed a smaller diameter of inhibition zones under *Lactobacillus* fermentation treatment, implying that *E. coli* with antimicrobial resistance exhibited a greater resistance of *Lactobacillus* fermentation. It is suggested that the possibility of replacing antibiotics in poultry production might not only depend on one alternative feed additive alone, such as probiotics, enzymes, and acidifiers etc, but requires a comprehensive nutrition strategy together with good breeding and management conditions [20].

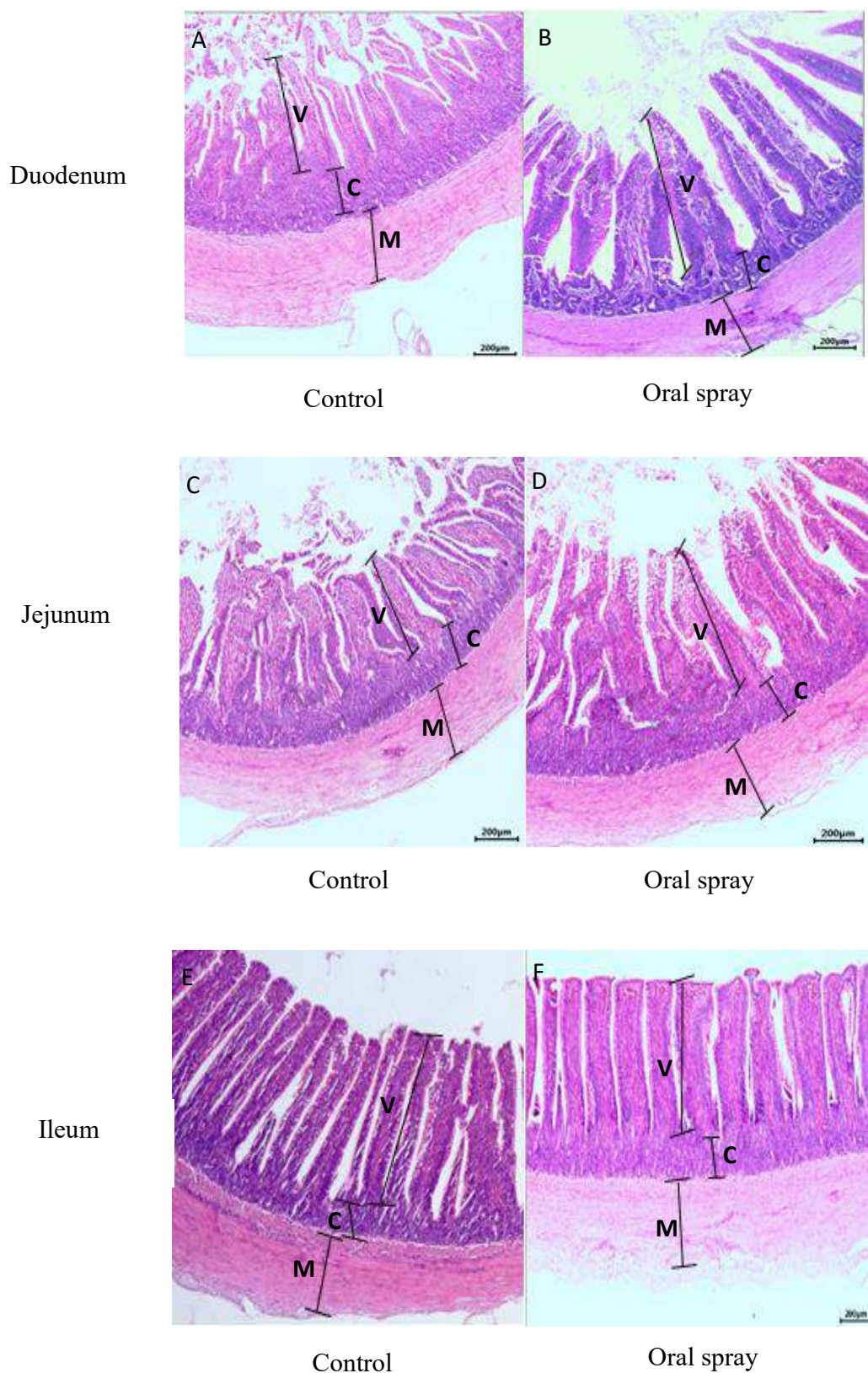


Figure 3. Representative light microscopy (×200) of the histomorphology of duodenum (A, B), jejunum (C, D) and ileum (E, F) of the ducklings at 21 d of age. V, villus; C, crypt; M, muscularis mucosa; control group, the basal diet supplemented with *Lactobacillus* fermentation; oral spray group, ducklings fed the basal diet and administrated *Lactobacillus* fermentation by oral spray way.

Table 7. Effect of oral spray with *Lactobacillus* on cecum flora amount of ducklings at 21 d of age (log CFU/g)

Items	<i>Escherichia coli</i>	<i>Lactobacillus</i>	Total colonies	<i>Lactobacillus:Escherichia coli</i> ratio
Control	8.65 ± 0.83	8.30 ± 0.97 ^b	7.86 ± 0.58	0.97 ± 0.18
Oral spray	8.01 ± 0.46	9.59 ± 0.15 ^a	8.46 ± 0.19	1.20 ± 0.08
p-value	0.22	0.038	0.09	0.06

CFU, colony forming units.

Data was expressed as mean ± standard deviation (n = 4).

^{a,b} Means within the same column lacking a common superscript differ (p < 0.05).

The key point of adding beneficial bacteria to improve the productive performance and intestine health of poultry lines is in understanding completely when and how to use them. Some studies reported that there are positive effects on production efficiency of broiler chickens fed diets containing *Lactobacillus* cultures [14,23,24] or *Lactobacillus* fermentation administered intragastrically [25]. Other reporters found that no significant differences were observed in weight gain of chicken given diets with or without *Lactobacillus* cultures [17,18]. These inconsistent results may be due to the differences in the stability and specificity of the *Lactobacillus* strain to the host, exact dose and supply way of *Lactobacillus* as well as the developmental period and nutritional status of the birds. For example, in the newly hatched chick, the small intestinal development and function appears to be immature and should be further improved to minimize the mortality and keep uniformity of young poultry [4]. Therefore, it is important to select probiotics as supplements to aid in the proper development and microflora colonization of the intestinal tract in the early life of hatched birds as soon as possible [9]. In the current study, the beneficial effects of the *Lactobacillus* fermentation supplements supplied by oral spray were examined. Compared to the feeding control group, oral spray with *Lactobacillus* fermentation during the first week had no effect on growth performance of ducklings during 1 to 7 d of age while positively increased the final BW at 21 d and ADG from 1 to 21 d. Due to the dependence on the residual yolk nutrition and the lower feed intake of birds during the first few days post hatch, it was presumed that provision of probiotics supplied by the traditional feeding way might be insufficient to exert some effect on the rapid development of intestine of birds in early life and then could not maximize the value of the nutrient efficiency compared to oral spraying. In our study, the greater dose of *Lactobacillus* fermentation by oral spray at the critical post-hatch period might be more conducive to the proper development of the intestinal tract to obtain the greater digestion and utilization of nutrients in long run.

The interaction of intestinal growth, digestive functions, and enteral nutrition are critical for hatched poultry during the post-hatch period [4]. In order to increase digestion and absorption of nutrients from the exogenous feed, physiological and morphological changes of the small intestine are dramatic

in birds at the first post-hatched days [2,3]. For example, the weight and length of the small intestine increased more rapidly than the whole body mass and reach a maximum between 3 and 7 days [3,4]. Therefore, early nutrient supply to young poultry is essential for improving the intestine growth and nutrient intake [6]. Access to supplements stimulating digestive enzyme and yolk sac nutrient utilization in early life can promote the intestinal development [7]. In the current study, ducklings given *Lactobacillus* fermentation immediately during the first week exhibited a greater absolute weight of the jejunum, ileum, and total intestine tract. Similar results about promoting intestine development were consistent with those reported in broilers fed probiotics [26]. Therefore, the sooner the gastrointestinal tract achieves functional capacity, the more nutrients can be utilized efficiently, leading to increased weight gain from d 1 to 21. In addition, the immediate post-hatch period is critical for intestinal morphological development in order to enlarge the intestinal absorptive surface and increase nutrient supply [3]. Thus, some of the enhanced growth effects of early nutrition may be explained by changes in intestinal tract development. Moreover, oral spray with *Lactobacillus* and metabolites during the first week increased the villus height and villus height: crypt depth ratio of duodenum and jejunum. The improved intestinal morphology was parallel with simultaneously increased intestinal weight by *Lactobacillus* fermentation administration in the present study, in turn suggesting that oral spray with *Lactobacillus* fermentation could stimulate intestinal development to improve nutrients digestion and absorption for eventually better growth performance in ducklings at 21 d of age.

Newly hatched chickens with immature immune function and unstable intestinal flora were susceptible to bacterial infection [9]. Thus, the colonization of beneficial microorganisms should be encouraged to fight against pathogen infection during post-hatch period as soon as possible. An increase amount of *Lactobacillus* was observed in the cecum of ducklings subjected to oral spray with *Lactobacillus* fermentation in the present study. Similar results were observed in newborn birds by feeding [24] or inoculation with *Lactobacillus* strains [27]. Therefore, the improved intestinal microbial environment resulted from *Lactobacillus* fermentation administration of ducklings in the present study. Since *Lactobacillus* ad-

ministration could increase cecal *Lactobacillus* colonization, *Lactobacillus* could inhibit cecal harmful bacteria colonization by competition for nutrients and adhesion sites on the intestinal epithelium [28]. However, there is no consistent beneficial effects of *Lactobacillus* and metabolites on the antibacterial activity of *E.coli* between Exp. 1 (*in vitro*) and Exp. 2 (*in vivo*). In fact, the degree of *Lactobacillus* effect *in vivo* depends upon the dose or type/blend of *Lactobacillus*, the duration of feeding, bird's age, overall hygiene conditions on farm and environmental factors. It is implied that the survival ability and adhesive capability of *Lactobacillus* as well as the exact dose and duration of *Lactobacillus* to produce the beneficial effects should be evaluated in our future study. Additionally, *E.coli* colonisations in ducklings reared in a comfortable and clean environment might be kept at a stable and lower level and not be affected easily by *Lactobacillus* treatment.

In conclusion, *Lactobacillus* with the better anti-bacteria ability exhibited a lower antibacterial activity on *E. coli* with antimicrobial resistance than *E. coli* without resistance *in vitro*. *In vivo*, oral spray with *Lactobacillus* fermentation during the first week could improve the intestinal development, morphological structure, and microbial balance to promote growth performance of ducklings from hatch to 21 d of age.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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硒营养对种禽繁殖性能的影响研究进展

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摘要: 硒是种禽营养中的必需微量元素。种禽硒营养补充主要来源无机硒(亚硒酸盐或硒酸盐)和有机硒(酵母硒或硒代蛋氨酸), 不同形式硒源的硒生物学利用效率差异较大。首先, 硒作为种公禽繁殖能力的重要微量元素, 有利于维持和改善精液品质。其次, 种蛋硒含量对维持胚胎发育及其抗氧化系统稳定改善胚胎发育发挥着重要作用; 通过母代硒营养途径可提高胚胎抗氧化能力以提高孵化率。此外, 在各种应激条件下提高种禽硒摄入量, 可保证种禽体内有足够的硒储备, 以调节硒蛋白的合成来缓解应激损伤带来的不利影响。本文综述了硒营养对种公禽精液质量、产蛋性能、蛋硒沉积、胚胎硒转移效率及改善后代仔鸡抗氧化性能等繁殖性能的研究进展, 为硒营养改善种禽繁殖性能提供理论依据。

关键词: 种禽; 硒; 抗氧化; 胚胎发育; 精液品质

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硒是家禽必需微量元素, 硒缺乏会导致家禽生产和繁殖性能下降。此外, 家禽规模化生产过程中存在各种应激源, 硒作为各种抗氧化硒蛋白的辅助因子, 协助维持抗氧化防御系统, 防止组织氧化损伤。特别是现代遗传育种技术的提高, 过多重视种禽的产蛋性能和生长速度等指标, 导致其抗病和抗应激能力下降, 对环境各种应激源更为敏感^[1]。因此, 硒营养改善种禽生产性能的关注点, 需要从硒营养缺乏向满足不同生产条件下最佳生长性能的动态硒需求等方向做出重要转变。NRC (1994) 推荐肉禽对硒的需求量为 0.1 mg/kg, 上限为 2 mg/kg, 但缺乏种禽硒营养需要推荐量。一般推荐种禽硒的添加水平为 0.2~0.3 mg/kg^[2]; 而在实际生产中, 由于提高生产性能和抗应激能力的需求, 硒的添加量远远超过 NRC (1994) 推荐的需要量。如通过适当提高饲料硒水平或添加生物学利用率高的有机硒可有效调节硒蛋白的合成来应对家禽生产中各种应激挑战。因此,

本文综述了种禽硒水平和硒源对种公禽精液质量及种蛋硒沉积、胚胎硒转移效率和后代仔鸡抗氧化性能的积极影响。

1 硒与精液质量

硒在维持精子质量方面发挥着重要作用, 是保证种公禽生产性能的重要因素^[2]。硒蛋白参与清除组织和细胞中的氧自由基, 保护精(原)细胞免受损伤, 提高精液质量^[3]。种公禽饲料中添加硒可显著提高肝脏、睾丸、精子和精浆硒谷胱甘肽过氧化物酶(Se-GSH-Px)活性, 且降低精液储存过程中精子对脂质过氧化的敏感性^[1]。种公禽补充硒可提高精液多不饱和脂肪酸(PUFAs)比例, 并降低精子对脂质过氧化的敏感性, 保护精子的完整性^[4]。研究发现, 储存过程中精子 PUFA 浓度显著下降水平与其脂质过氧化正相关; 尤其是精液中 PUFA (22:4n-6) 最易发生脂质过氧化反应^[5]。一般来说, 鸡精液中硒的平均浓度为 47 ng/g, 精液与精子的硒比例约为 8:1; Se-GSH-Px 在鸡精子中占总酶活性的 75% 以上。研究发现, 饲料中添加硒可显著提高精液硒浓度 2 倍以上(101 ng/g), 显著提高精液量(42.11%)、精子密度(38.53%)和精子活力(21.25%); 受精率、孵化率和血液中睾酮、促卵泡素、促黄体素含量均显著升高^[6]。研究表明, 添加 0.3 mg/kg 硒和 100 mg/kg

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维生素 E 可提高人工采精时种鹅完全射精反应的频率 (82.7% vs 73.5%), 显著提高射精量、精子浓度和活精子比例, 并降低未成熟精子的百分比和精液丙二醛 (MDA) 含量^[7]。饲料中添加纳米硒可以提高老龄种公禽的生育能力, 当公禽摄入量达 0.3 mg/kg 纳米硒时效果更佳^[8], 有效缓解精液储存 6 h 引起的精子活力下降 (8.7% vs 3.95%), 并保持精浆中总脂质和磷脂浓度相对稳定^[9]。添加有机硒可提高热应激 (33~36℃) 条件下种鸡精子数量和活性, 降低精子死亡百分比^[10]; 补充有机硒可改善地塞米松诱导的急性氧化应激引起的精液质量下降^[11]。因此, 在种禽饲料中补充硒特别是有机硒可高效维持高精液质量, 并能有效地转移到生殖器官和精液, 并表现出额外的抗应激效果。

2 硒营养与产蛋性能

一般来说, 饲料中应用亚硒酸钠会表现出一定的剂量毒副作用, 且生物学利用效率较低; 而有机硒 (如酵母硒) 安全性高, 有利于改善种禽生产体况。试验表明, 长期饲喂酵母硒更有利于改善蛋鸡健康状况, 提高蛋鸡生产性能, 且在产蛋后期使用效果更佳^[12]。目前, 不同硒源对蛋鸡产蛋性能影响的研究结果并不一致。如相比于无机亚硒酸钠, 饲料中添加 0.6~0.9 mg/kg 酵母硒可显著提高蛋鸡的产蛋率和蛋壳厚度^[13-14]; 不同硒源处理对蛋鸡产蛋性能无显著影响^[15]。研究结果差异可能与基础饲料硒含量和来源、试验周期和鸡只产蛋周龄等因素相关。

3 硒营养与蛋硒沉积

硒几乎平均分布于蛋黄 (58%) 和蛋清 (42%) 之中, 但硒在蛋黄中的沉积效率为 13%~14%, 在蛋清中仅为 8%~9%^[16]。蛋黄和蛋清中硒的主要形式都是硒代蛋氨酸, 其中硒代蛋氨酸占蛋清总硒的 53%~71%, 占蛋黄总硒 12%~19%^[17], 补充性无机硒并不能在卵内有效积聚^[18]。因此, 鸡蛋硒浓度取决于其饲料硒水平和硒源形式^[2]。与无机硒相比, 有机硒源能更有效地转移到鸡蛋中^[22]。饲料中硒含量与蛋硒含量呈线性和二次正相关, 且酵母硒组的蛋鸡蛋硒沉积率较无机硒组更高^[19]。王华付等^[20]发现, 鸭蛋的硒含量随有机硒添加量增加而显著提高, 且在饲料中添加 1.85 mg/kg 有机硒可达到生产富硒鸭蛋标准。王向荣等^[21]研究表明, 饲料中

有机硒添加量为 0.40 mg/kg 和 0.80 mg/kg 时, 可显著提高产蛋高峰期临武鸭的蛋硒含量。一方面, 有机硒 (如 DL- 硒代蛋氨酸类似于氨基酸形式的主动吸收) 较无机硒 (如亚硒酸钠在动物肠道中被动吸收) 更易被吸收进入血液循环并提高种蛋硒含量^[23]。另一方面, 有机硒 (如硒代蛋氨酸和酵母硒) 主动吸收后可直接用于合成蛋白, 而无机硒 (如亚硒酸钠) 则需发生硒代胱氨酸转化后才被吸收。其中, 饲料中有机硒含量与蛋黄硒含量的相关性最高^[2], 因为硒代蛋氨酸能够替代蛋氨酸特异性地结合到鸡蛋蛋清中。不同有机硒源之间的鸡蛋硒转移效率同样存在差异。与有机酵母硒相比, 硒代蛋氨酸转移至鸡蛋的效率更高。与酵母硒相比, 硒代蛋氨酸可更有效地提高组织、种蛋蛋黄和蛋白及 1 日龄雏鸡肾脏和胸肌中硒含量^[24]。也有研究证实, 硒代蛋氨酸羟基类似物的鸡蛋硒转移效率显著高于酵母硒 (76.26% vs 56%)^[22]。因此, 实际生产过程中推荐补充 0.2~0.3 mg/kg 硒代蛋氨酸作为有机硒源解决鸡蛋硒富集的问题。而近期研究表明, 饲料中添加 0.3 mg/kg 纳米硒或酵母硒可显著提高蛋鸡肝脏和肾脏中的硒和蛋硒沉积, 也可考虑通过补充纳米硒和酵母硒用于富硒鸡蛋生产^[25]。

4 硒营养与孵化率

现普遍认为, 孵化过程是一个氧化应激过程, 提高胚胎的抗氧化能力可提高孵化率。鸡胚对脂质过氧化反应很敏感, 因为胚胎组织的脂类中多不饱和脂肪酸含量较高^[26], 需要抗氧化剂来保护。硒从饲料中转移到蛋黄和蛋白中, 并转移到发育的胚胎中^[27]。蛋清硒很可能在胚胎发育的前 2 周转移至胚胎组织, 而蛋黄硒主要在孵化的最后 1 周发生转移^[2]。而无机硒存在许多局限性, 如毒性、与其他矿物质和维生素拮抗作用、转移的效率低等。种母禽饲料添加硒特别是有机硒, 有利于提高种蛋孵化率^[28], 鸡蛋中硒含量增加还可提高哈氏单位和蛋白高度, 有效提供胚胎发育所需营养物质^[29-30], 进而改善受精蛋孵化和健雏率^[31]。鸡胚发育组织中 GSH-Px 活性表现为硒依赖 (Se-GSH-Px) 和非硒依赖 (非 Se-GSH-Px) 2 种形式^[32], 且这些酶的分布具有组织特异性。组织中 Se-GSH-Px 活性显著高于非 Se-GSH-Px, 且组织 Se-GSH-Px 与非 Se-GSH-Px 的活性比例在胚胎发育中后期持续升高, 其中第 11~15 胚龄为快速增长期^[33]。因此, 种禽饲料中添加硒特别是

有机硒有利于胚胎中后期组织抗氧化系统的维稳,以提高孵化率。研究发现,种禽饲料使用氧化鱼油所引起的种蛋胚胎死亡率从3.5%上升至10.6%,显著降低了孵化率和雏鸡初生重;而饲料添加0.4 mg/kg 酵母硒可有效缓解氧化鱼油诱导氧化应激引起的胚胎死亡率^[34]。因此,推测使用有机硒可提高组织硒存量并合成更多GSH-Px,以消除应激产生过多自由基^[35]。

5 母代硒营养与子代性能

随着母代营养与跨代表观遗传理论的发展,现普遍认为母体环境信息转化以类似印记反应的方式传递到后代发育过程中^[36]。新生雏鸡的胚胎发育以及孵化后早期存活率均受母体饲料中不同营养成分的影响^[37]。由此可见,母代营养可对子代发育、代谢产生重大影响。研究发现,饲料中添加0.15 mg/kg 亚硒酸钠+0.15 mg/kg L-硒代蛋氨酸可有效提高种母鸡的繁殖性能和子代雏鸡的抗氧化能力和健康整齐度^[38]。已有报道,种鸡饲料中添加硒可显著提高1~42日龄子代肉仔鸡日增重和采食量^[39]。此外,母体饲料中添加硒代蛋氨酸可显著提高后代雏鸡饲料转化效率,降低死亡率^[40]。一方面,母体营养通过直接影响卵黄营养组成变化,进而影响孵化后雏鸡的生长发育。另一方面,母体激素、免疫因子和营养物质向卵子的转移和沉积,以表观遗传方式影响后代的生长发育、生理和行为^[41]。如鸡胚发育早期卵黄甲状腺激素和卵黄酮是影响后代生理效应的潜在媒介物质^[42]。而母体充足的硒营养可介导甲状腺激素代谢和硒蛋白相关基因表达途径对后代肉仔鸡产生特定的影响^[2,43]。母体的硒营养与子代肉仔鸡组织硒沉积显著正相关。种蛋硒浓度增加可引起雏鸡肝脏、大脑、胸肌和腿肌中硒浓度线性增加,该现象可持续至孵化后2周^[44]。由此可见,母代硒营养可改变其子代的硒代谢水平且该影响在孵化后数周仍持续存在。氧化性能方面,种母鸡饲料硒缺乏可降低种母鸡和子代肉鸭血浆和肝脏中GSH-Px活性,并导致其血液和肝脏中MDA水平升高^[45]。种鸡饲料添加0.30~0.6 mg/kg 酵母硒饲料4周,21日龄后代雏鸡肌肉组织硒含量和硒蛋白均显著增加,肌肉脂质和蛋白质氧化损伤及滴水损失均显著降低^[46]。与低硒饲料组(0.2 mg/kg)相比,高硒组(0.4 mg/kg)子代肉仔鸡组织GSH-Px活性在孵化后2~4周仍显著升高^[47]。不同硒源之间的研究发现,与

亚硒酸钠相比,基础饲料中添加0.15 mg/kg 酵母硒和硒代蛋氨酸均可显著提高1日龄雏鸡肝脏和肾脏硒蛋白含量和基因表达水平^[48]。母体饲料中添加硒代蛋氨酸可改善1日龄雏鸡胸肌的GSH-Px和超氧化物歧化酶(SOD)活性、肾脏GSH浓度,并降低肝脏和胰腺的MDA含量^[49]。也有研究发现,不同硒源(硒代蛋氨酸、酵母硒、亚硒酸钠)均能有效提高肝脏及血浆GSH-Px活性,硒代蛋氨酸效果更佳^[50]。如前所述,硒代蛋氨酸可非特异性地在肌肉蛋白中积累能够建立起硒储备,其可在应激条件下硒需求量增加时被调用,作为硒源合成新的硒蛋白(如GSH-Px、硫氧还蛋白还原酶等)。因此,在种禽生产中应当更加重视母体硒特别是有机硒营养的传递效应。

6 结语及展望

近20年来,有关种禽硒营养方面的研究持续受到关注,种禽饲养中可通过添加各种形式硒(无机或有机硒)来满足其需要量,其中在氧化应激条件下种禽对硒营养的需求更大。不同硒源的生物学利用效率不同,有机硒能更有效地从饲料转移到种蛋,并转移至胚胎和子代组织中,进一步合成多种活性硒蛋白,参与调节机体的各种功能,包括维持氧化还原平衡和抗病力等。特别在应激条件下可额外合成更多硒蛋白以提高机体抵抗力。其中,种禽-胚胎发育-子代生长一体化的硒营养对于种禽繁殖性能、胚胎发育及子代生产性能的影响及其潜在表观遗传机制需进一步加强研究。

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Research Progress on the Effects of Selenium Nutrition on Poultry Breeders

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Abstract: Selenium (Se) is an essential trace element for maintaining the reproductive performance of poultry breeders. The inorganic selenium (such as selenite or selenate) and organic selenium (such as yeast selenium or selenomethionine) are the main Se supplement used in poultry diets. The Se bioavailability varies among different forms of Se sources. Firstly, Se, as an important trace element for reproductive ability of male poultry breeders, is beneficial to maintaining and improving semen quality. Secondly, Se content in eggs plays an important role in maintaining embryo development and stabilizing antioxidant system. Through maternal Se nutrition pathway, the antioxidant capacity of embryos can be improved to improve hatching rate. In addition, increasing the Se intake of poultry breeders under various stress conditions can ensure enough Se reserves in poultry breeders to regulate the synthesis of selenoprotein to alleviate the adverse effects of stress injury. In this paper, the effects of Se nutrition on reproductive performance of poultry breeders were reviewed, and the research progress of different Se levels and sources on semen quality, egg performance, egg Se deposition, embryo Se transfer efficiency and improving antioxidant performance of offspring broilers were summarized, providing theoretical basis for selenium nutrition to improve reproductive performance of poultry breeders.

Keywords: Breeders; Selenium; Antioxidants; Embryonic development; Semen quality

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家禽支链氨基酸营养需要研究进展

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摘要: 支链氨基酸 (branched-chain amino acids, BCAA) 在家禽生长、生产性能、免疫功能和肠道健康等方面发挥着重要作用, 涉及器官发育、免疫反应、肌肉蛋白周转和基因调控等关键过程。然而, 单一 BCAA 的过量、缺乏或比例失衡均会影响家禽蛋白质合成和肠道健康, 因此保持 BCAA 比例的平衡对于家禽的生产性能至关重要。尽管 NRC (1994) 提供了肉鸡、蛋鸡和种鸡的 BCAA 推荐量, 但不同品种、日粮蛋白质水平以及生长阶段的家禽对 BCAA 的需求存在差异。同时, 由于抗生素生长促进剂的禁用及养殖端所面临的多种疾病的挑战, 也需要重新评估家禽对 BCAA 的推荐摄取水平。但 BCAA 在维持肠道完整性和肠道微生物组成和调控机体蛋白周转效率的作用机理研究仍有待进一步探究。本综述在阐述 BCAA 在家禽中的营养生理作用以及其对生产和健康的基础上, 推荐了不同饲养条件下不同肉鸡品种 (肉鸡、蛋鸡和种鸡) BCAA 需要量, 以期为家禽低蛋白饲料 BCAA 推荐量提供理论参考。

关键词: 家禽; 支链氨基酸; 生产性能; 免疫功能; 肠道健康

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Advances in Research on the Nutritional Requirements of Branched-Chain Amino Acids in Poultry

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Abstract: Branched-chain amino acids (BCAA) play important roles in poultry growth, performance, immune function and intestinal health. They are involved in key processes such as organ development, immune response, muscle protein turnover and gene regulation. However, an excess, deficiency or imbalance of a single BCAA can affect poultry protein synthesis and intestinal health. Therefore, maintaining a balanced ratio of BCAA is critical for poultry performance. Although the NRC (1994) provides recommended amounts of BCAA for broilers, laying hens, and breeders, there are variations in BCAA requirements among poultry breeds, dietary protein levels, and growth stages. Additionally, the ban on antibiotic growth promoters and the challenges of multiple diseases in poultry farming necessitate a re-evaluation of the recommended levels of BCAA intake. Furthermore, further research is needed to explore the mechanisms of action of BCAA in maintaining intestinal integrity and regulating gut microbial composition and enhancing protein turnover efficiency in the body. This review aims to describe the nutritional and physiological roles of BCAA in poultry, as well as their effects on production and health. It also provides BCAA requirements for different broiler breeds (broilers, laying hens and breeders) under various feeding conditions. These recommendations aim to provide theoretical references

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for the recommended levels of BCAA in low-protein diets for poultry.

Keywords: poultry; branched-chain amino acids; productive performance; immune function; intestinal health

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蛋白质是促进家禽生长发育、提高生产性能的关键营养物质。近年来,蛋白质资源的短缺以及家禽摄入过量蛋白质对环境造成的负面影响已成为制约家禽养殖业发展的重要因素^[1]。因此,开发低蛋白(crude protein, CP)饲料至关重要。目前低 CP 饲料的开发主要针对赖氨酸(lysine, Lys)、蛋氨酸(methionine, Met)和苏氨酸(threonine, Thr)等限制性必需氨基酸,而关于支链氨基酸(branched-chain amino acids, BCAA)的研究相对较少。一方面, Lys 和 Met 等合成氨基酸的添加容易导致 BCAA 的缺乏和比例失衡;另一方面,由于三种 BCAA 结构相似,容易引起代谢竞争和对酶降解的干扰。在亮氨酸含量通常较高的低 CP 饲料中,这种情况进一步加剧了 BCAA 比例失衡,从而对家禽的生长产生负面影响^[2]。因此,在研究低 CP 饲料时,应特别关注 BCAA 在家禽生长中发挥的作用及适宜的添加比例。

基于此,本文综述了 BCAA 的营养生理作用及其对家禽生产性能、产蛋性能、免疫功能及肠道健康的影响,并根据 NRC (1994) 提供的家禽 BCAA 推荐量探究其在肉鸡、蛋鸡和种鸡中的不同需求。基于现有 BCAA 需要量的相关数据,本文还指出了目前存在的研究空白,为重新评估疾病挑战下和不同饲养条件下的 BCAA 需要量提供了研究方向。

1.1 BCAA 的营养生理作用

家禽的生长、生产、免疫、酶反应和组织周转等多种活动都需要蛋白质的参与。BCAA 是指分子结构中侧链具有分支结构的三种必需氨基酸,分别为亮氨酸(leucine, lLeu)、异亮氨酸(isoleucine, lIle)和缬氨酸(valine, lVal);其中, Leu 为 2-氨基-4-甲基戊酸, Ile 为 2-氨基-3-甲基戊酸, Val 为 2-氨基-3-甲基丁酸。三种 BCAA 结构相似,与支链脂肪酸一样携带疏水侧链。BCAA 在支链氨基酸转氨酶(branched-chain aminotransferase, lBCAT)的作用下初步降解,后经支链 α -酮酸脱氢酶(branched-chain α -keto acid dehydrogenase complex, lBCKD)作用完全降解, lBCKD 同时可导致辅酶 A 复合物的不可逆分解。该酶促反应往往由其中一种 BCAA 触发(通常是 Leu),最终导致其他 BCAA 被降解^[3]。

尽管家禽高蛋白饲料含有更多限制性氨基酸可满足其生长早期的营养需求,但 BCAA 的生物学利用率尚不清楚。此外,家禽饲料中任一 BCAA 水平过高或过低均会影响其他两种的吸收与利用。例如,饲料中 Leu 过量和 Ile 不足均会阻碍雏鸡的生长,同时导致 BCAA 比例失衡,不利于其他 BCAA 的利用;特别是在低 CP 饲料中添加 Leu 会增加其与 Ile 和 Val 的拮抗作用,并加剧 Ile 和 Val 的降解,阻碍家禽生长^[4]。然而,在满足饲料中 Ile 和 Val 最低需求时,过量 Leu 不会表现出不利影响^[5]。在玉米-豆粕型饲料中,除 Lys、Met 和 Thr 三种限制性氨基酸外,BCAA 也可能成为限制性因素,但目前仍无法确定将哪一种氨基酸作为家禽饲料的第四限制性氨基酸^[6]。相较于 Ile, Val 在 Leu 参与的反应中更容易出现拮抗反应和酶降解,目前可确定 Val 是玉米-豆粕型饲料的限制性氨基酸,因此低 CP 饲料通常会额外添加 Val 以满足家禽所需;而 Val 水平过高(0.82%)并不会对 Leu 与 Ile 的利用产生较大影响^[7]。

尽管 BCAA 在调节免疫功能和肠道健康上的重要性已得到普遍认可,但目前有关家禽靶向免疫、微生物群和肠道健康方面的研究应用十分有限。Konashi 等^[8]研究表明,低 BCAA 饲料显著降低了 10~24 日龄雄性科宝肉鸡胸腺和法氏囊的重量,说明 BCAA 在淋巴器官发育和免疫机能中起着关键作用。有研究发现 BCAA 可以通过调节靶组织基因表达和蛋白酶信号转导影响家禽的免疫功能及肠道健康^[9]。而相较于其他 BCAA, Leu 能够激活 mTOR 通路、上调蛋白酶以增强家禽的免疫功能^[10]。此外,禽肠道菌群也会对 BCAA 的消化吸收产生影响。因此,在确定饲料 BCAA 的最佳比例之前,有必要评估家禽肠道菌群多样性对 BCAA 需要量的影响。

2.1.1 BCAA 对肉禽生产性能的影响

NRC (1994) 提供的 BCAA 推荐需要量仅适用于 2~6 周龄的肉鸡, 且该推荐量是在最低 CP 水平饲料下得出的。而随着新品系的培育和饲养模式的变化, 家禽的营养需求也在不断发生变化^[11-12]。BCAA 比例失衡会导致多余的部分被用于能量而非蛋白质的合成, 而目前家禽 BCAA 的最佳比例尚未明确。

任何一种 BCAA 的过量与缺乏均会导致 BCAA 比例失衡, 进而对肉鸡的生产性能产生不利影响。Leu 促进蛋白质合成的能力最强, 但 Leu 过量会降低血浆中 Ile 和 Val 的浓度。Chen 等^[13]研究表明, 将饲料 Leu 水平由 1.88% 提高到 2.73% (控制 Ile 和 Val 比例分别为 Leu 的 59% 和 69%), 可显著提高 1~21 日龄肉仔鸡末重、采食量、饲料利用率和胸肌率。Erwan 等^[14]研究表明, 饲料中添加 0.5% Leu 可显著提高 3~6 周龄科宝肉鸡胴体重并减少脂肪沉积, 同时发现口服包被型 Leu ($6\text{mmol}/10\text{mL kg}^{-1}\text{BW}$) 能够显著降低 7 日龄肉仔鸡血浆 Ile 和 Val 水平。一些研究者认为, 饲料配方中 Leu 的最佳水平应根据饲料原料类型来确定^[15-16]。在 Ile 水平方面, 蒋守群等^[17]研究发现, 随着饲料 Ile 从 0.55% 增加到 0.66%, 22~42 日龄黄羽肉鸡平均日增重和饲料利用率显著提高。Kidd 等^[18]发现低 Ile (饲料 Ile 水平为 $4.2\text{g} \cdot \text{kg}^{-1}$) 会显著降低雄性罗斯肉鸡胸肌率; 而在 0.51% Ile 的玉米-豆粕型饲料中继续添加额外的 50% Ile 可提高 3~8 周龄雄性肉鸡生产性能。Park 等^[19]通过分段回归分析确定 Ile 在肉仔鸡饲料中的添加量应为 0.63%~0.65% (占 CP 水平的 3.28%~3.38%), 且添加其他氨基酸 (Lys、Arg 除外) 会加剧 Ile 的失衡。因此, 在满足主要限制性氨基酸需要量的基础上, 也要适当考虑 Ile 的需要量及 BCAA 的比例。在 Val 水平方面, Maynard 等^[20]研究发现, 在高 Leu 和 Ile 饲料中添加 Val 可显著降低 29~42 日龄科宝肉鸡羽毛的异常率。因此补充 Leu 和 Ile 之前, 应先满足 Val 的需要。从这一角度可认为 Val 是家禽玉米-豆粕型饲料的第四限制性氨基酸。Amirdahri 等^[21]发现, 缺乏 Val 会导致 8~21 日龄雌性科宝肉鸡的生产性能下降、羽毛生长异常和腿部畸形。饲料中 Ile 和 Val 比例的改变, 同样会使肉鸡的生产性能下降。Corrent 等^[22]研究发现, 7~21 日龄罗斯肉鸡饲料 Ile:Lys 应为 0.7~0.8, Val:Lys 应为 0.8 左右, Leu:Lys 是可变的, 但需保持在 1.30 左右。Lee 等^[23]发现, 在花生粕-动物蛋白混合饲料中添加 Ile/Lys (67%~69%) 和 Val/Lys (75%~78%) 显著降低了 0~48 日龄雄性罗斯肉鸡氮排放并增加胸肉质量。同时有研究发现, 在饲料中添加 $1\text{g} \cdot \text{kg}^{-1}$ 的 Ile 可显著提高 28~42 日龄雄性罗斯肉鸡胸肌率; 添加 $1\text{g} \cdot \text{kg}^{-1}$ 的 Val 可显著提高 28~42 日龄肉鸡平均日增重; 而 Ile 和 Val 联合补充则会降低 0~21 日龄肉鸡料重比及腹脂率, 并提高 42 日龄肉鸡末重^[24-25]。Miranda 等^[26]研究发现, 在不限饲料 CP 水平的情况下, Val:Lys 和 Ile:Lys 分别为 0.77 和 0.67 时 1~6 周龄雄性科宝肉鸡的生产性能显著提升, 脂肪沉积减少。Lima 等^[27]采用折线模型估算 30 周龄科宝肉鸡的 Val 和 Ile 需要量分别为 803 和 $708\text{mg} \cdot \text{d}^{-1}$ 。在其他 BCAA 比例组合方面, Ospina-Rojas 等^[28]研究发现, 饲料 1.19% Leu+0.86% Val 水平可显著提高 21~42 日龄雄性科宝肉鸡的采食量和平均日增重; 此外, 饲料 Leu 水平为 1.13% 时胸肌率最高, Val 水平为 0.71% 时腿肌率最高, 因此添加 Val 的同时应考虑 Leu 水平, 以提高肉鸡的生产性能。Maynard 等^[23]研究发现, 在饲料低 CP (19%) 水平下, 低 Ile (0.78%) 和 Leu (1.57%) 组 15~35 日龄雌性科宝肉鸡的翅重和采食量明显高于低 Val (0.87%) 组; 而低 Ile 组 15~35 日龄雄性科宝肉鸡的料重比则明显低于低 Val 和 Leu 组。

关于 3 种 BCAA 的组合效果, Pastor 等^[29]采用非线性回归模型确定了雄性科宝肉鸡在 10~20 日龄和 25~35 日龄 Lys、Leu、Ile 和 Val 的理想比例分别为 100:94:55:65 和 100:106:56:72。Sakomura 等^[30]采用线性回归模型估算出在体重代谢基础上 23 周龄科宝肉鸡维持所需的 Ile、Leu 及 Val 分别为每日 94、52 和 $155\text{mg} \cdot \text{kg}^{-0.75}$, 在蛋白代谢基础上分别为每日 329、172 和 $546\text{mg} \cdot \text{kg}^{-0.75}$ 。现有的文献和推荐标准指出, 家禽生长所需的 BCAA 较少, 但随着日龄增长, 生长后期 BCAA 与 Lys 的比值应有所提升^[11-12]。但 Kop-Bozbay 等^[31]研究发现, BCAA 水平并不会对生长后期科宝肉鸡和 16~42 日龄罗斯肉鸡的生产性能造成显著影响, 这可能是饲料中 BCAA 的来源不同造成的。其他特殊家禽方面, Martinez 等

[32]和 Hanafy 等[33]在对日本鹌鹑的研究中发现, 饲料 Val 水平为 $6.661\text{g} \cdot \text{kg}^{-1}$ 或 0.2%可显著提升其采食量和体重。Kop-Bozbay 等[34]研究 1~7 日龄雏火鸡发现, 饲料 Leu : Ile : Val 为 3:1:2 时, 平均日增重和胸肌率显著提高。

3.1.1 BCAA 对蛋禽产蛋性能的影响

饲料 BCAA 能够在家禽肝组织中通过脂肪酸代谢产生卵黄和蛋白[35], 在蛋鸡产蛋过程中发挥重要作用。饲料 Ile 在不同条件下对蛋鸡日产蛋量和蛋品质产生不同影响。Miller 等[36]发现, 饲料 0.53%Ile 水平就可满足以血粉为主要蛋白来源的单冠白莱航鸡的维持和产蛋需要。Ullah 等[37]研究发现, 在饲料 Ile 含量从 0.66%提升至 0.72%时可显著提高 20~46 周龄海兰蛋鸡的日产蛋量和产蛋重。Peganova 等[38]研究发现, 饲料 Ile 水平高于 1.0%时产蛋期罗曼蛋鸡的蛋品质降低; 在低 Val+Leu (0.63%+0.72%) 组合中, Ile 水平从 0.57%提升至 1.15%时蛋鸡的日产蛋量降低。也有研究[39]显示, 提高 Ile 水平并未使蛋鸡产蛋性能表现出显著差异。上述研究结果不一致的原因推测可能是不同饲料 CP 水平或不同家禽品种对 Ile 的消化率不同引起的。在 Val 水平方面, Toprak 等[40]研究表明, 在饲料 Val 含量从 0.81%提升至 0.87%可显著提高罗曼蛋鸡的产蛋率和蛋品质。对于其他特殊家禽, Hanafy 等[33]研究发现, 在饲料 18%CP 水平下, 0.2%Val 可显著提高 36~96 日龄日本鹌鹑的产蛋率。而 Jian 等[41]研究发现 33~41 周龄凤达蛋鸡的产蛋率随着饲料 Val 水平升高而升高, 而哈氏单位、蛋壳厚度、蛋黄颜色出现下降。相对肉禽, 蛋禽以及主要饲料成分中不同的 BCAA 含量及其利用率仍有待完善。

4.1.1 BCAA 对种禽胚胎发育的影响

充分的 BCAA 营养供给对初生幼禽至关重要。为改善家禽胚胎的生长发育, 除了在孵化后提供特定营养外, 还可以通过卵内注射为发育中后期家禽胚胎提供足够的营养和生物活性物质。Kita 等[42]研究发现, 卵内注射 1%的 Leu 和 Ile (453 mg Leu 和 271 mg Ile) 可以加速鸡胚的生长, 缩短雏鸡的孵化时间。Chowdhury 等[43]研究发现, 卵内注射 500 μL 含 34.5 μmol Ile 的无菌水显著提高了出雏后肉鸡初生重及热应激期间的热耐受性。Han 等[44]研究发现, 与注射 Ile 或 Val 相比, 在第 7 天向胚胎卵黄囊注射 500 μL 含 35 μmol Ile 的无菌水显著降低了出雏时雏鸡的体温并提高其 5 日龄的体重; 而注射 500 μL 含 70 μmol Leu 的无菌水显著提高了鸡胚血浆甲状腺素水平并显著降低出雏 10 日龄时发生热应激的雄性雏鸡直肠温度。崔洋洋等[45]在卵内注射 9.06 mg Leu, 显著改善了慢性热应激雌性罗斯肉鸡的十二指肠绒毛高度和回肠隐窝深度。上述研究均表明, 卵内注射 Leu 可以提高雏鸡的耐热性。Bhanja 等[46]研究发现, 卵内注射 4~7 mg BCAA 可显著提高鸡胚第 1 周的胚重。Kop-Bozbay 等[47]研究发现, 将 0.2%的 Leu、Ile 和 Val 以 3 : 2 : 1 的比例混合注入鸡胚羊膜腔, 鸡胚孵化率显著降低, 但鸡胚的健雏率与初生重显著提高。

5.1.1 BCAA 对家禽免疫功能的影响

家禽肝组织中的 BCAA 主要由 Val 刺激粒状淋巴细胞和无粒白细胞产生, 参与自然杀伤细胞的增殖[48]。若 BCAA 添加充足, 家禽黏膜会加速分泌免疫球蛋白 A, 减少固有层的致病性增殖。而作为白细胞、IL-12 等促炎细胞因子和树突状细胞功能的动力来源, BCAA 还在促进先天性和适应性免疫反应中发挥重要作用[49]。Sartori 等[50]研究发现, 添加 BCAA 可促进小鼠间充质细胞增殖, 显著下调 p-NF- κ B/NF- κ B 并上调 p-STAT-3/STAT-3 mRNA 表达, 同时减少 IL-6 和 TNF- α 并增加抗炎介质水平, 证明 BCAA 具有合成代谢和免疫调节的作用。董小英等[51]研究表明, 在饲料低 CP 水平 (17%) 下, 添加 BCAA 会显著降低仔猪血浆尿素和上皮内淋巴细胞数量并增加十二指肠绒毛长度和免疫球蛋白 A 数量。Prates 等[52]研究发现, 在 $25\text{mg} \cdot \text{kg}^{-1}$ 低脂多糖 (lipopolysaccharide, LPS) 水平下, 额外补充 0.3%的 BCAA、Arg 和 Cys, 可部分逆转断奶仔猪因 LPS 应激而增加的炎症反应及激素变化产生的应激生物标志物。在肉鸡方

面, Liu 等^[53]研究发现, 在 19 胚龄鸡胚肠细胞中添加 Leu 可观察到 NF- κ B 磷酸化并下调炎症反应。Hale 等^[54]研究发现, 饲料低 Ile 水平 (0.42%) 显著降低了 30~42 日龄雌性罗斯肉鸡的胸腺重量和 CD⁸⁺T 细胞数量。Konashi 等^[8]研究发现, 饲料低 BCAA 水平 (13.5lg · kg⁻¹ Ileu, 8lg · kg⁻¹ Ile 和 8.2lg · kg⁻¹ IVal) 显著降低了 10~24 日龄雄性科宝肉鸡胸腺和法氏囊的重量, 说明 BCAA 在淋巴器官发育和免疫功能中起着关键作用。

6.1.1 BCAA 对家禽肠道发育和菌群组成的影响

为了更好地吸收养分, 家禽需要增加肠绒毛高度以产生更大的吸收表面积。有研究表明, 饲料 1.37%~2.17%Leu 水平可显著提高 AA 肉鸡空肠和回肠中的绒毛高度及十二指肠、空肠和回肠中的绒毛隐窝比^[55]。Liu 等^[56]研究发现, 在饲料中添加 400mg · kg⁻¹ Ile 可显著增加初生 AA 肉鸡空肠和回肠绒毛高度。Allameh 等^[57]在低 CP 饲料中补充可消化 Val (9.9lg · kg⁻¹), 罗斯肉鸡空肠和回肠的绒毛高度及杯状细胞的数量显著增加。BCAA 可在转氨过程中为 Glu 和 Asp 等氨基酸的合成提供氨基, 而上述两种氨基酸被认为是小肠黏膜细胞内蛋白质周转和营养物质运输的主要原料^[58]。有研究表明, 若 BCAA 比例不平衡, 补充 Ile 不会对仔猪肠细胞的生长和增殖产生促进作用^[59]。常银莲^[60]发现, 上调饲料 BCAA 水平可激活 mTOR 信号通路以促进初生 AA 肉鸡肠道蛋白质的合成, 从而促进肠道上皮细胞的增殖, 增加肠绒毛高度。

目前, BCAA 对肠道菌群的具体影响仍不明确, 有待进一步研究。Van der Wielen 人^[61]研究发现, 从 31 日龄科宝肉鸡盲肠中分离出的乳酸发酵菌 (菌株 G17^T)、丙酸梭菌 (93<5%) 及新丙酸梭菌 (93<5%) 相似, 且当底物中存在 BCAA 时生长平缓。Jian 等^[41]发现随着饲料 Val 水平的提高, 33 周龄罗曼蛋鸡盲肠 *Fusobacterium*、*Aeriscardovia*、*Anaerobiospirillum*、*Aerococcus*、*Corynebacterium* 和 *Campylobacter* 的相对丰度显著降低, *Oribacterium* 和 *Frisingicoccus* 的相对丰度显著增加。Liu 等^[56]发现, 饲喂 800mg · kg⁻¹ Ile 饲料时, 初生 AA 肉鸡盲肠在门水平上的 Firmicutes 和 Bacteroidota 的相对丰度显著降低, Proteobacteria 和 Cyanobacteria 的相对丰度显著增加。上述结果显示, 添加 BCAA 可能对家禽肠道菌群普遍存在有益影响, 从而促进家禽的生长。

7 小 结

BCAA 作为蛋白质合成的关键调控因子之一, 对于促进肌肉生长发育和提高免疫机能起着至关重要的作用。近年来, 更多研究表明 BCAA 在维持家禽肠道完整性、营养转运蛋白和肠道微生物上具有重要作用, 但对其具体作用机制仍有待明确, 特别在家禽低蛋白饲料配方中, 应根据基础饲料 CP 含量、饲料原料种类及其 BCAA 含量和利用率, 精准预测 BCAA 适宜需要量及其比例。在现有禁抗条件下, 未来的研究应根据家禽采食量变化、肠道健康、应激水平和免疫状态来精准调整适宜的可消化 BCAA 的需要量, 以实现家禽饲料节本增效。

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四、科研成果

证书号第 5650541 号



发明专利证书

发明名称：一种提高家禽产蛋性能和孵化率的饲料及其应用

发明人：王文策；冯艳；杨琳；朱勇文；叶慧；夏戴阳；黎宇；黄靓
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专利号：ZL 2020 1 0429333.1

专利申请日：2020 年 05 月 20 日

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其他事项参见续页

证书号第6224578号



发明专利证书

发明名称：一株植物乳杆菌、分离方法、用途和药品、食品

发明人：王文策;杨琳;付阳;朱勇文;叶慧;朱姗姗;莫潜渊
罗浩桐;马渭青;李金泽;陈昱君;邓小兼;杨硕;宋国荣

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证书号第15150018号



实用新型专利证书

实用新型名称：一种带清洁度检测的种蛋清洗装置

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证书号第 4597038 号



发明专利证书

发明名称：一种利用酶水解能法评价鹅饲料代谢能的方法

发明人：王文策;杨琳;杨静;朱勇文;叶慧;黎宇;汪珩;夏戴阳
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证书号第20904215号



实用新型专利证书

实用新型名称：便于收集排泄物的家禽通用代谢笼

发 明 人：朱勇文;徐伟汉;陈弘睿;林渤;谢诗胜

专 利 号：ZL 2023 2 2664057.9

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中华人民共和国国家标准

GB/T 45103—2024

肉鸭营养需要量

Nutrient requirements of meat-type duck

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国家市场监督管理总局
国家标准化管理委员会 发布



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前 言

本文件按照 GB/T 1.1—2020《标准化工作导则 第 1 部分：标准化文件的结构和起草规则》的规定起草。

请注意文件的某些内容可能涉及专利。本文件的发布机构不承担识别专利的责任。

本文件由中华人民共和国农业农村部提出。

本文件由全国畜牧业标准化技术委员会(SAC/TC 274)归口。

本文件起草单位：中国农业科学院北京畜牧兽医研究所、中国农业科学院饲料研究所、全国畜牧总站。

本文件主要起草人：唐静、谢明、郭占宝、冯宇隆、朱勇文、吴永保、张云生、汪超、庄蕾、魏杰、张博、江勇、赵健楠、汪忠艳、赵小丽、黄苇、周正奎、侯水生。



肉鸭营养需要量

1 范围

本文件规定了北京鸭及其配套系、番鸭和兼用型鸭的营养需要量。
本文件适用于饲料生产企业和各种类型养殖场(户)肉鸭饲料配制,半番鸭参照番鸭执行。

2 规范性引用文件

下列文件中的内容通过文中的规范性引用而构成本文件必不可少的条款。其中,注日期的引用文件,仅该日期对应的版本适用于本文件;不注日期的引用文件,其最新版本(包括所有的修改单)适用于本文件。

GB/T 10647 饲料工业术语
NY/T 823 家禽生产性能名词术语和度量计算方法

3 术语和定义

GB/T 10647 和 NY/T 823 界定的以及下列术语和定义适用于本文件。

3.1

营养需要量 **nutrient requirements**
动物在维持正常生理活动、机体健康和达到特定生产性能时对营养素需要的最低数量。

3.2

肉鸭 **meat-type duck**
用于提供肉产品的鸭品种(配套系)。
注:包括北京鸭及其配套系(简称北京鸭)、兼用型鸭及番鸭与半番鸭。

3.3

鸭酶解能 **duck enzymatic hydrolysis gross energy; EHGE**
基于鸭消化道酶谱配制仿生消化液,饲料原料在人工仿鸭消化道内环境下消化后,饲料总能减去未消化残渣总能后的能值。

4 北京鸭营养需要量



- 4.1 商品代北京鸭营养需要量应符合表 1、表 2 的要求,以达到表 3 的生产性能。
- 4.2 北京鸭种鸭营养需要量应符合表 4~表 7 的要求,以达到表 8 和表 9 的生产性能。

5 番鸭营养需要量

- 5.1 商品代番鸭营养需要量应符合表 10、表 11 的要求,以达到表 12 的生产性能。
- 5.2 番鸭种鸭营养需要量应符合表 13、表 14 的要求,以达到表 15、表 16 的生产性能。

6 兼用型鸭营养需要量

- 6.1 商品代兼用型鸭营养需要量应符合表 17、表 18 的要求,以达到表 19 的生产性能。
6.2 兼用型种鸭营养需要量应符合表 20~表 23 的要求,以达到表 24、表 25 的生产性能。

7 饲料原料成分及营养价值表

肉鸭常用饲料原料成分及营养价值表参见附录 A。

表 1 商品代北京鸭饲粮表观代谢能、酶解能、粗蛋白质和氨基酸需要量
(自由采食,以 88%干物质为计算基础)

项目	周龄(Week)			
	育雏期 0 周龄~2 周龄	生长期 3 周龄~5 周龄	育肥期 6 周龄	
			自由采食	填饲
鸭表观代谢能(AME)/[MJ/kg(kcal/kg)]	11.93(2 850)	12.14(2 900)	12.35(2 950)	12.56(3 000)
鸭酶解能(EHGE)/[MJ/kg(kcal/kg)]	12.35(2 950)	12.56(3 000)	12.77(3 050)	12.98(3 100)
粗蛋白质(CP)/%	19.5	17.5	16.0	13.0
粗蛋白质/表观代谢能(CP/AME)/[g/MJ(g/Mcal)]	16.3(68.4)	14.4(60.3)	13.0(54.2)	10.4(43.3)
赖氨酸/表观代谢能(Lys/AME)/[g/MJ(g/Mcal)]	0.92(3.86)	0.70(2.93)	0.57(2.37)	0.48(2.00)
总氨基酸(Total amino acids)				
赖氨酸(Lys)/%	1.10	0.85	0.70	0.60
蛋氨酸(Met)/%	0.45	0.40	0.35	0.30
蛋氨酸+胱氨酸(Met+Cys)/%	0.82	0.72	0.65	0.55
苏氨酸(Thr)/%	0.72	0.60	0.55	0.47
色氨酸(Trp)/%	0.20	0.18	0.16	0.14
异亮氨酸(Ile)/%	0.72	0.57	0.45	0.40
精氨酸(Arg)/%	1.00	0.85	0.70	0.60
真可利用氨基酸(True available amino acids)				
真可利用赖氨酸(TA-Lys)/%	0.98	0.76	0.60	0.53
真可利用蛋氨酸(TA-Met)/%	0.42	0.37	0.32	0.28
真可利用蛋氨酸+胱氨酸(TA-Met+Cys)/%	0.72	0.64	0.58	0.49
真可利用苏氨酸(TA-Thr)/%	0.62	0.52	0.48	0.40
真可利用色氨酸(TA-Trp)/%	0.19	0.17	0.15	0.13
真可利用异亮氨酸(TA-Ile)/%	0.65	0.50	0.39	0.34
真可利用精氨酸(TA-Arg)/%	0.95	0.80	0.66	0.57

表 2 商品代北京鸭饲料矿物质和维生素需要量
(自由采食,以 88%干物质为计算基础)

项目	周龄(Week)			
	育雏期 0 周龄~2 周龄	生长期 3 周龄~5 周龄	育肥期 6 周龄	
			自由采食	填饲
矿物质 ^a (Minerals)				
钙(Ca)/%	0.90	0.85	0.80	0.80
总磷(Total P)/%	0.65	0.60	0.55	0.55
非植酸磷 ^b (NPP)/%	0.42	0.40	0.35	0.30
钠(Na)/%	0.15	0.15	0.15	0.15
氯(Cl)/%	0.12	0.12	0.12	0.12
铁(Fe)/(mg/kg)	60	60	60	60
铜(Cu)/(mg/kg)	8.0	8.0	8.0	8.0
锰(Mn)/(mg/kg)	100	80	80	80
锌(Zn)/(mg/kg)	60	60	60	60
碘(I)/(mg/kg)	0.30	0.30	0.30	0.30
硒(Se)/(mg/kg)	0.30	0.30	0.30	0.20
维生素 ^c (Vitamins)				
维生素 A(Vitamin A)/(IU/kg)	4 000	3 000	2 500	2 500
维生素 D ₃ (Vitamin D ₃)/(IU/kg)	2 000	2 000	2 000	2 000
维生素 E(Vitamin E)/(IU/kg)	20	10	10	10
维生素 K(Vitamin K)/(mg/kg)	2.0	2.0	2.0	2.0
硫胺素(Thiamin)/(mg/kg)	2.0	1.5	1.5	1.5
核黄素(Riboflavin)/(mg/kg)	10	10	10	10
烟酸(Niacin)/(mg/kg)	50	50	50	50
泛酸(Pantothenic acid)/(mg/kg)	11	11	11	11
吡哆醇(Pyridoxine)/(mg/kg)	4.0	3.0	3.0	3.0
生物素(Biotin)/(mg/kg)	0.20	0.15	0.15	0.15
叶酸(Folic acid)/(mg/kg)	1.0	1.0	1.0	1.0
维生素 B ₁₂ (Vitamin B ₁₂)/(mg/kg)	0.02	0.02	0.02	0.02
胆碱(Choline)/(mg/kg)	1 000	1 000	1 000	1 000
^a 矿物质需要量包括饲料原料中提供的矿物质量。 ^b 非植酸磷需要量为未添加植酸酶时的需要量。 ^c 维生素(除胆碱外)需要量不包括饲料原料中提供的维生素量。				

广东省地方标准

DB44/T 2566—2024

乌鬃鹅商品代饲养标准

Feed standard of commercial meat-type wuzong goose

地方标准信息服务平台

2024 - 11 - 11 发布

2025 - 02 - 11 实施

广东省市场监督管理局 发布

地方标准信息服务平台

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地方标准信息服务平台

前 言

本文件按照GB/T 1.1—2020《标准化工作导则 第1部分：标准化文件的结构和起草规则》的规定起草。

请注意本文件的某些内容可能涉及专利。本文件的发布机构不承担识别这些专利的责任。

本文件由广东省农业农村厅提出并组织实施。

本文件由广东省畜牧业标准化技术委员会（GD/TC 9）归口。

本文件起草单位：华南农业大学。

本文件主要起草人：杨琳、王文策、杨静、朱勇文、叶慧、董泽敏、张秀芬、汪珩、申佳佳、黎宇、黄靓、马渭青、夏戴阳、陈建颖、罗浩桐、张艺、刘书峰、尹亚红、高威。

地方标准信息服务平台

乌鬃鹅商品代饲养标准

1 范围

本文件规定了乌鬃鹅商品代生长期划分、营养需要量、体重和耗料量、常用饲料成分及营养价值。本文件适用于集约化养殖模式条件下乌鬃鹅商品代的养殖和配合饲料厂的饲粮配制。

2 规范性引用文件

下列文件中的内容通过文中的规范性引用而构成本文件必不可少的条款。其中，注日期的引用文件，仅该日期对应的版本适用于本文件；不注日期的引用文件，其最新版本（包括所有的修改单）适用于本文件。

GB/T 10647 饲料工业术语
NY/T 823 家禽生产性能名词术语和度量统计方法

3 术语和定义

GB/T 10647、NY/T 823 界定的以及下列术语和定义适用于本文件。

3.1

乌鬃鹅 **wuzong goose**

乌鬃鹅又称清远乌鬃鹅，属于小型肉用鹅种。成年公鹅体重可达3.0 kg~3.5 kg，母鹅体重可达2.5 kg~3.0 kg。

3.2

商品代 **commercial meat-type geese**

通过品种选择、纯系培育等生产的、主要用于生产供人类消费肉产品的商品肉鹅。

4 乌鬃鹅商品代生长期划分及营养需要量

4.1 生长期划分

根据乌鬃鹅的生长发育和生产性能特点，将其生长期划分为育雏期（1~28 日龄）、生长期（29~56 日龄）、育肥期（57~91 日龄）。

4.2 营养需要量

乌鬃鹅商品代各阶段营养需要量见表1。

表1 乌鬃鹅商品代各阶段营养需要量

营养指标	育雏期（1~28日龄）	生长期（29~56日龄）	育肥期（57~91日龄）
代谢能/（MJ/kg）	11.29~12.1	11.71~12.4	11.91~12.37
代谢能/（Mcal/kg）	2.7~2.89	2.80~2.96	2.85~2.96
粗蛋白质/%	18	14	10
蛋能比/（g/MJ）	16.5	11.9	11.9
蛋能比/（g/Mcal）	69.1	49.9	49.9
粗纤维/%	4	6	6
赖氨酸/%	1.05	0.85	0.75
蛋氨酸/%	0.45	0.4	0.3
蛋氨酸+胱氨酸/%	0.78	0.68	0.52
苏氨酸/%	0.8	0.55	0.33
色氨酸/%	0.19	0.15	0.15
精氨酸/%	1.15	0.6	0.54
钙/%	0.8	0.7	0.5
总磷/%	0.7	0.6	0.4
非植酸磷/%	0.4	0.3	0.25
钠/%	0.15	0.15	0.15
氯/%	0.22	0.22	0.22
铁/（mg/kg）	20	20	20
铜/（mg/kg）	5	5	5
锌/（mg/kg）	55	55	55
锰/（mg/kg）	7	70	70
碘/（mg/kg）	0.4	0.3	0.3
硒/（mg/kg）	0.1	0.1	0.1
亚油酸/%	1	0.8	1
维生素A/（IU/kg）	5700	3600	3000
维生素D /（IU/kg）	500	500	500
维生素E /（IU/kg）	40	20	10
维生素K /（mg/kg）	0.5	0.5	0.5
硫胺素/（mg/kg）	6	5	5
核黄素/（mg/kg）	2	2	2
泛酸/（mg/kg）	10	10	10
烟酸/（mg/kg）	85	65	65
生物素/（mg/kg）	0.15	0.1	0.15
叶酸/（mg/kg）	2.5	2	2.5
维生素B12 /（μg/kg）	25	25	25
胆碱/（mg/kg）	2000	2000	1200
注：公母混养，以88 %干物质为基础，自由采食。			

5 体重和耗料量

乌鬃鹅商品代体重预测值见表2。
乌鬃鹅商品代耗料量预测值见表3。

表2 乌鬃鹅商品代体重预测值

单位为g

日龄	体重
1	95
7	220
14	480
21	880
28	1100
35	1330
42	1560
49	1790
56	2020
63	2250
70	2500
77	2850
84	3100
91	3200
注1：公母混养，以88 %干物质为基础，自由采食。	
注2：以上数字均为平均值。	

表3 乌鬃鹅商品代耗料量预测值

单位为g/只

日龄	每周耗料量	累计耗料量
0~7	0	0
8~14	210	210
15~21	550	760
22~28	950	1710
29~35	1050	2760
36~42	1500	4260
43~49	1600	5860
50~56	1760	7620
57~63	1650	9270
64~70	1500	10770
71~77	1500	12270
78~84	1350	13620
85~91	1400	15020
注：公母混养，以88 %干物质为基础，自由采食。		

6 常用饲料成分及营养价值

常用饲料描述及营养成分、氨基酸含量，矿物质及维生素含量见附录A。

7 常用矿物质饲料中矿物质元素的含量

常用矿物质饲料中矿物质元素的含量见附录B。

地方标准信息服务平台

五、其他业绩



第五届全国大学生动物科学专业技能大赛

荣誉证书

朱勇文 老师：

在第五届全国大学生动物科学专业技能大赛中带领学生团队
荣获：

团体特等奖

特发此证，以兹表彰。

教育部高等学校动物生产类专业教学指导委员会





第一届广东省本科高校动物生产类
大学生创新与设计大赛

特等奖

(生态畜牧场规划设计)

广东省本科高校动物生产类专业教学指导委员会
二〇二〇年一月

第一届广东省本科高校动物生产类
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二〇二〇年一月

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特等奖

(动物生产类大学生创新项目)

广东省本科高校动物生产类专业教学指导委员会
二〇二〇年一月

第一届广东省本科高校动物生产类
大学生创新与设计大赛

特等奖

(动物生产类大学生创新项目)

广东省本科高校动物生产类专业教学指导委员会
二〇二〇年一月

第一届广东省本科高校动物生产类
大学生创新与设计大赛

特等奖

(动物生产类大学生创新项目)

广东省本科高校动物生产类专业教学指导委员会
二〇二〇年一月

荣誉证书

CERTIFICATE OF HONOR

朱勇文：

在广州农村科技特派员大赛中荣获

优秀农村科技特派员

广州产业投资控股集团
有限公司

广州市科技进步
基金会

广州生产力促进中心
有限公司

2024年1月25日

荣誉证书

CERTIFICATE OF HONOR

朱勇文：

在广州农村科技特派员大赛中荣获

二等奖

广州产业投资控股集团
有限公司

广州市科技进步
基金会

广州生产力促进中心
有限公司

2024年1月25日

广东省农业农村厅

粤农农函〔2021〕951号

关于提供首批粤黔东西部协作 定向实用技术的函

贵州省科学技术厅：

为贯彻落实李希书记在陪同贵州省党政代表团考察我省农业科技创新工作时的指示要求，进一步做好做实粤黔农业科技协作，我厅针对贵州十二大农业主导产业（茶叶、食用菌、蔬菜、牛羊、特色林业<竹、油茶、花椒、皂角等>、水果、中药材、刺梨、生态渔业、辣椒、生态家禽），面向全省定向征集粤黔实用转化技术，截至目前已收集首批成熟可推广技术93项，其中来自科研院所55项、高校27项、企业11项。

由于技术资料较多，现将粤黔东西部协作定向实用技术统计表（见附件1）提供贵厅，如有进一步合作意向，请径联系广州国家现代农业产业科技创新中心。

- 附件：1. 粤黔东西协作定向实用技术统计表
2. 广州国家现代农业产业科技创新中心简介



（联系人：潘广，联系电话：13418560528，邮箱：
g zg jnykc zx@gd. gov. cn）

附件1

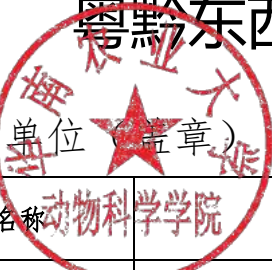
粤黔东西协作定向实用技术统计表(93项)		
序号	技术名称	技术提供单位
1	农业食品产业数字化的操作系统——组学区块链	中国农业科学院深圳农业基因组研究所
2	结合超低覆盖度全基因组测序和深度学习的基因组选择平台	中国农业科学院深圳农业基因组研究所
3	杨梅产业化种植关键技术的示范推广	广东省农业科学院果树研究所
4	桃李无公害栽培技术示范与推广	广东省农业科学院果树研究所
5	泥炭藓（海花草）人工栽培技术	广东省农业科学院环境园艺研究所
6	赤灵芝代料栽培及孢子粉收集技术	广东省农业科学院蔬菜研究所
7	辣椒轻简化高效栽培关键技术	广东省农业科学院蔬菜研究所
8	蔬菜生产全过程生物农药防治病虫害技术体系	广东省农业科学院植物保护研究所
9	广东蔬菜重要病虫害鉴定及防控关键技术集成与应用	广东省农业科学院植物保护研究所
10	重大植物病害烟草病毒病绿色防控关键技术	广东省农业科学院植物保护研究所
11	设施蔬菜病虫害生态控制技术	广东省农业科学院植物保护研究所
12	菠萝内外品质流水线式无损检测与分级技术	广东省农业科学院农业质量标准与监测技术研究所
13	农产品质量安全风险评估与预警技术	广东省农业科学院农业质量标准与监测技术研究所
14	农产品品质鉴定评价与品牌提升技术服务	广东省农业科学院农业质量标准与监测技术研究所
15	樱桃番茄新品种及配套设施无土栽培技术	广东省农业科学院设施农业研究所
16	优质肉鸡效率育种关键技术	广东省农业科学院动物科学研究所
17	从源头减少氮排放、降低环境污染的技术研究与示范推广	广东省农业科学院动物科学研究所
18	优质鸡安全高效综合饲料配置及健康养殖技术	广东省农业科学院动物科学研究所
19	家禽无抗养殖技术	广东省农业科学院动物科学研究所
20	黄羽肉鸡精准营养与安全低排放饲料配制技术	广东省农业科学院动物科学研究所
21	黄羽肉鸡肉质品质营养调控技术	广东省农业科学院动物科学研究所
22	黄羽肉鸡鸡高效繁殖营养调控关键技术	广东省农业科学院动物科学研究所
23	商品猪优质高效绿色无抗饲料配制技术及不同体重阶段商品猪精准营养技术	广东省农业科学院动物科学研究所
24	农业废弃物生物高效处理及资源化再生利用成套技术	广东省农业科学院农业资源与环境研究所
25	狂犬病检测和综合防控技术	广东省农业科学院动物卫生研究所
26	养殖废气生物安全与环保处理及回收再利用技术	广东省农业科学院动物卫生研究所
27	猪口蹄疫精准免疫技术临床综合应用	广东省农业科学院动物卫生研究所
28	猪重要病原菌的现场快速检测技术	广东省农业科学院动物卫生研究所
29	家禽疫病的快速诊断及综合防控	广东省农业科学院动物卫生研究所
30	猪流行性腹泻综合防控关键技术	广东省农业科学院动物卫生研究所
31	猪繁殖与呼吸综合征（猪蓝耳病）综合防控技术示范与推广	广东省农业科学院动物卫生研究所
32	鸭传染性浆膜炎综合防控技术	广东省农业科学院动物卫生研究所
33	家禽重要肠道细菌病绿色防控关键技术	广东省农业科学院动物卫生研究所
34	畜禽水产生态健康养殖微生物资源合理利用关键技术	广东省农业科学院农业生物基因研究中心
35	广东生态茶园建设技术	广东省农业科学院茶叶研究所
36	大叶种红茶引进与生态智慧管控及产业化应用	广东省农业科学院茶叶研究所
37	白茶高效陈化技术	广东省农业科学院茶叶研究所
38	高韵味生普加工技术	广东省农业科学院茶叶研究所
39	甜韵红茶加工技术	广东省农业科学院茶叶研究所
40	柑橘茶加工技术	广东省农业科学院茶叶研究所
41	茶全产业链智慧管控关键技术集成与推广	广东省农业科学院茶叶研究所
42	高香型茶树品种引进	广东省农业科学院茶叶研究所
43	优质抗逆鲜食玉米示范应用	广州市农业科学研究院
44	油绿703菜心的推广应用	广州市农业科学研究院
45	玉田3号菜心的推广应用	广州市农业科学研究院
46	新优高产多抗小果型中国南瓜系列品种示范推广	广州市农业科学研究院
47	热带亚热带特色植物种苗快繁技术研究及产业化示范	广州市农业科学研究院
48	应用环保安全、无抗药性的农用植物油防控果、蔬、茶病虫害	广东省科学院动物研究所
49	辊轴式对虾剥壳装备	广东省现代农业装备研究所
50	缩短芒果产期的方法	广东省科学院南繁种业研究所
51	特色藤本果树高产栽培技术	广东省科学院南繁种业研究所
52	茶园废弃生物质基材料关键制备技术及其在土壤修复中的应用	广东省科学院生态环境与土壤研究所
53	基于果蔬原料的复合多菌种协同定向生物转化技术	华南协同创新研究院
54	狮头鹅饲养管理技术	汕头市白沙禽畜原种研究所
55	水肥一体化灌溉技术在大田蔬菜上应用	江门市农业科学研究所
56	一种基于填料和折叠板的废水生物膜反应器	华南理工大学
57	大宗食物资源生产富含肽呈味基料及调味品共性关键技术	华南理工大学

58	香蕉系列精深加工食品	华南理工大学
59	一种超低分子铁皮石斛多糖的酶工程制备技术	华南理工大学
60	刺梨精深加工关键技术研发及产业化	华南理工大学
61	利用专利技术开展农产品的健康种养殖与高附加值深加工产品开发	中山大学
62	水果、刺梨的果酒发酵技术研究和发酵产品开发	暨南大学
63	富集天然叶酸的芽菜生产技术	暨南大学
64	新型高效萃取分离油茶籽中茶油、茶皂素及其综合利用新技术推广与示范	华南农业大学
65	气吸式蔬菜精量直播机	华南农业大学
66	茶青内含物无损快速检测装置	华南农业大学
67	移动式果蔬产地快速喷淋预冷装置	华南农业大学
68	肉鸭高效低排饲料配制技术应用与示范	华南农业大学
69	重要经济作物生态全营养栽培管理技术	华南农业大学
70	蓄冷气调运输箱	华南农业大学
71	一种公牛附睾注药绝育药物	佛山科学技术学院
72	优质麻鸡早熟自别雌雄配套系的选育	佛山科学技术学院
73	地方鸡特色种质性状挖掘与产业化开发	佛山科学技术学院
74	无患子（圆皂角）的综合开发利用	佛山科学技术学院
75	基于黑斑蛙的山区多元高效稻渔生态养殖	佛山科学技术学院
76	小花瓣·大梦想——蝴蝶兰“植物工厂”，助推产业兴旺	韶关学院
77	优质肉羊（湖羊）快速繁殖及健康养殖技术	韶关学院
78	保健型高花青素紫叶芥菜部分品种成果转化	韶关学院
79	药用石斛生态种苗繁育和种植技术与产业化	韶关学院
80	智能化食用菌工厂化生产技术和设备	深圳技术大学
81	特种珍禽生态养殖技术推广	汕尾职业技术学院
82	道地药材信前胡种植技术规范	深圳职业技术学院
83	区域性土壤与环境综合恢复治理	深圳市正合食品有限公司
84	移动一体式闭环除湿热泵干燥机	广东威而信实业有限公司
85	促进红薯叶产业化的生态种养结合方法推广	梵叶农业（罗定）有限公司
86	有机碳肥生产技术	广东丽福来商贸有限公司
87	金稻超声波种子处理技术	广州市金稻农业科技有限公司
88	茶多酚深加工技术	深圳市博礼潮商投资股份有限公司
89	功能性农业（富硒农业及农业产品深加工）	一辈子（广东）健康产业有限公司
90	一种防腐防蛀防霉防开裂竹材技术与制备	广东建中新竹材科技有限公司
91	装配式净化猪舍	广东信宇环保设备有限公司
92	农产品深加工--固态有氧生物发酵技术开发应用	广东碧辉生物技术有限公司
93	智能化食用菌工厂化生产技术和设备	大连富森智能科技有限公司

附件

粵黔东西协作定向实用技术登记表

推荐单位（盖章）



成果名称	肉鸭高效低排饲料配制技术应用与示范
成果 所有权人信息	成果单位（或所有权人）： 华南农业大学
	成果权属： <input type="checkbox"/> 独占 <input checked="" type="checkbox"/> 共有（共有权人：朱勇文，杨琳，王文策）
	联系人：朱勇文 联系电话：18818912892 电子邮箱：zhuyw0724@scau.edu.cn
所属产业领域	<input type="checkbox"/> 茶叶 <input type="checkbox"/> 食用菌 <input type="checkbox"/> 刺梨 <input type="checkbox"/> 生猪 <input type="checkbox"/> 牛羊 <input type="checkbox"/> 特色林业 <input type="checkbox"/> 中药材 <input type="checkbox"/> 水果 <input type="checkbox"/> 蔬菜 <input type="checkbox"/> 辣椒 <input checked="" type="checkbox"/> 生态家禽 <input type="checkbox"/> 生态渔业 <input type="checkbox"/> 其他，请注明_____（可多选）
成果属性	<input type="checkbox"/> 原始创新 <input checked="" type="checkbox"/> 集成创新 <input type="checkbox"/> 引进消化创新
成果成熟度	<input type="checkbox"/> 实验室阶段 <input type="checkbox"/> 小试阶段 <input checked="" type="checkbox"/> 中试阶段 <input type="checkbox"/> 市场化阶段 <input type="checkbox"/> 其他，请注明_____
成果应用情况	<input type="checkbox"/> 未转让 <input type="checkbox"/> 技术转让 <input checked="" type="checkbox"/> 实际应用 <input type="checkbox"/> 规模化生产 <input type="checkbox"/> 其他，请注明_____
成果转化方式	<input type="checkbox"/> 技术转让 <input checked="" type="checkbox"/> 技术许可 <input type="checkbox"/> 技术入股 <input type="checkbox"/> 创业融资 <input type="checkbox"/> 其他，请注明_____
成果投资方式	<input type="checkbox"/> 成果方提供资金支持 <input checked="" type="checkbox"/> 转化方提供资金支持
成果简介 (限 1500 字左右)	<p>（包括<u>产业概况及存在问题</u>、<u>成果简介</u>、<u>成果创新点</u>等方面内容）</p> <p>本成果从饲料资源高效利用、低碳、低氮、低磷和微量元素减量等方面建立肉鸭高效低排饲料配制技术体系，在肉鸭主产区集成、应用与示范，最终实现提高肉鸭饲料利用率、节约饲料资源、减少肉鸭营养过剩、降低排放的目标。</p> <p>一、成果简介</p> <p>1. 系统测定了不同饲料原料和混合日粮的鸭酶水解能和代谢能，分析两者之间的相关性，建立以单一因子酶水解能估测饲料代谢能的数学回归模型，研究获得了生长前期肉鸭酶水解能需要量，创建了以仿生酶法为基础的鸭饲料酶水解能评价技术体</p>

	<p>系 1 套，为肉鸭饲料有效能的精准和快速测定提供数据支持。</p> <p>2. 确定并优化了非淀粉多糖酶体外评价参数（温度、pH、酶谱等），体外评价了非淀粉多糖酶对饲料（饲粮类型、酶协作等）能量利用率和仿生能值的影响，并探讨了优化的非淀粉多糖酶在肉鸭饲料中应用，验证了非淀粉多糖酶能够降低肉鸭采食量、料重比和蛋白摄入量，提高了饲料能量和蛋白消化率。</p> <p>3. 构建了肉鸭常用饲料原料磷的数据库，比较研究了肉鸭饲料加酶与不加酶磷的真消化率，构建了饲料磷释放量随植酸酶活性的回归数学方程，提出了饲料添加植酸酶替代无机磷的当量值；研究了多层立体笼养结构下肉鸭低蛋白日粮配制技术及对肉鸭饲料氮排放的影响，提出三层立体笼养模式下肉鸭蛋白需要量。</p> <p>4. 研究了不同来源与配比的微量元素组合对肉鸭生长性能和鸭粪矿物元素排泄量的影响，提出了肉鸭微量元素高效利用技术参数 1 套。</p> <p>二、成果创新点</p> <p>组装集成肉鸭高效低排饲料配制与使用技术规范 1 种，提出了一种用于测定鸭饲料养分利用率的体外仿生消化酶法，申请国家发明专利 1 项（202010011510.4），利用仿生消化酶法研究评价了肉鸭 20 多种常用饲料原料样品的酶消化能，编制了肉鸭饲料原料酶水解能测定技术规程，形成企业标准 1 项（Q/AHQY 002-2019），获得以仿生酶法为基础的鸭饲料酶水解能数据库 1 套。</p>
<p>行业与市场分析</p> <p>（限 500 字左右）</p>	<p>（包括行业前景、预期经济效益、社会效益、生态效益、市场容量等内容）</p> <p>我国是世界最大的鸭肉生产和消费国，2020 年肉鸭出栏量 44.6 亿只，产值达 1132.8 亿元，肉鸭产业创造了巨大的社会与经济效益。目前，我国鸭饲料配方技术由于饲料营养数据库不完善，导致营养物质利用率低，排泄物中氮、磷、铜、锌、锰含量较高。因此，期望通过肉鸭高效低排饲料配制技术应用与示范，提高肉鸭饲</p>

	料转化率、避免营养过剩、节约饲料资源，实现肉鸭饲料碳排放降低 3%以上，氮、磷、微量元素排放降低 5%以上，从源头上有效控制和缓解粪便造成的环境污染问题。
备注	请提供 10-20 张技术照片、1-2 个短视频技术简介及成果如文章、标准、专利等、等扫描件作为附件。