

申 报	系列：教学科研并重
	专业：动物营养与饲料科学
	职称：副高级

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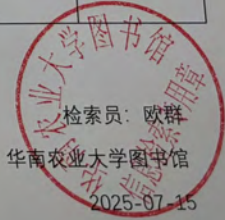
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“美育+”视域下高校“红绿交融”人才培养策略研究



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“互联网+教育”背景下高等学校畜牧学学科人才培养路径研究

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摘要: 在全球信息化浪潮的推动下,“互联网+教育”已成为高等教育改革的重要趋势。在这一背景下,探索“互联网+教育”背景下高等学校畜牧学学科人才培养的路径,对于推动我国畜牧业现代化和可持续发展具有重要意义。研究探讨“互联网+教育”背景下培养畜牧学学科人才的培养路径,旨在通过实证分析的方式构建畜牧学学科人才培养新体系。研究共包含三部分,第一部分分析了“互联网+”概念及其在教育领域的应用,第二部分探讨了“互联网+教育”背景下畜牧学人才培养面临的挑战,第三部分提出了“互联网+教育”背景下畜牧学学科人才的培养路径,以为高等学校教育改革提供参考。

关键词: “互联网+”; 高等学校; 畜牧学; 人才培养

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在全球数字化浪潮的席卷之下,“互联网+”已从一种概念演变为推动各行业变革的强劲动力,教育领域自然也不例外。尤其在高等教育中,“互联网+教育”正以前所未有的速度重塑着教学模式。畜牧学这一历史悠久且实践性极强的学科,在面临数字化转型时,展现出了前所未有的活力。然而,机遇总是伴随着挑战。畜牧学是一门高度依赖实地观察与操作的学科,如何在数字化学习环境中确保其实操性不被削弱,避免理论与实践脱节,成了教育工作者所面临的重大挑战。

一、“互联网+”概念及其在教育领域的应用

“互联网+”概念的提出旨在通过互联网技术与传统行业的深度融合,推动产业转型升级,提升经济效率和服务质量。这一理念的核心是利用互联网的开放性、共享性和高效性,打破行业壁垒,实现资源的优化配置和价值的最大化。“互联网+”在教育领域的应用,即“互联网+教育”,是指将互联网技术应用于教育全过程,包括教学、管理、服务等环节,以提高教育质量和效率,促进教育公平,实现个性化学习。^[1]

在高等教育中,特别是在畜牧学这样实践性较强的学科教学中,“互联网+教育”的应用展现出巨大潜力。它不仅能够提供丰富的在线学习资源,如视频课程、虚拟实验室等,还能够通过大数据分析,精准识别学生的学习需求和进度,为学生提供个性化的学习路径和反馈。此外,数字化平台的发展打破了传统教育的空间局限,让学生有机会与全球各地的行业专家和科研人员进行实时交流与合作,这种跨文化的沟通不仅拓展了学生的国

际视野,还大大增强了他们解决实际问题的能力。

二、“互联网+教育”背景下畜牧学人才培养面临的挑战

(一)技术与资源的整合难题

尽管“互联网+教育”为畜牧学人才培养带来了前所未有的机遇,但如何有效地整合技术与教育资源却是一大挑战。一方面,虽然网络上充斥着海量的畜牧学相关资料,但资料的质量参差不齐,筛选出高质量的教学资源并将其融入课程体系,需要教师具备较高的信息素养和专业判断力。另一方面,虚拟实验、智能教学系统等新兴技术的引入,要求学校在硬件设施、软件平台以及师资培训等方面进行相应的投入和升级,这无疑增加了教育成本。^[2]此外,技术与教学的融合并非一蹴而就,需要时间去摸索和优化,其间可能会出现技术故障、操作不当等问题,影响教学效果。

(二)实践与理论的平衡挑战

畜牧学是一门实践性极强的学科,传统的教学模式往往强调理论知识的传授,而忽视了实践能力的培养。“互联网+教育”的引入,虽然丰富了教学手段,但在实践教学方面仍然存在短板。虚拟实验虽然可以模拟部分真实场景,但无法完全替代实地操作。因此,如何在充分利用网络资源加强理论教学的同时,保证学生有足够的实践机会,实现理论与实践的有机结合,是当前亟待解决的问题。

(三)评价体系的转型难题

传统的评价方法通常聚焦于检验学生对理论知识的

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吸收和理解程度,而忽略了对其实践技能和创新能力的考查。在“互联网+教育”背景下,学生的学习方式和成果呈现形式发生了变化,传统的评价体系已无法充分反映和适应这些新变化。如何科学、公正地评价线上讨论、虚拟实验报告等形式的学习成果,一度成为教育工作者遇到的难题。此外,虽然大数据分析能提供学生学习过程的量化数据,但如何将这些数据转化为有效的评价依据,促进学生全面发展,仍是一个复杂的技术和伦理问题。评价体系的转型,需要教育者从理念到实践进行全面革新,以适应新时代人才培养的需求。

三、“互联网+教育”背景下畜牧学人才培养路径

(一) 课程体系改革

1. 优化课程结构,融入“互联网+”元素

在“互联网+教育”的大背景下,优化畜牧学课程结构,融入“互联网+”元素,是实现人才培养目标的关键步骤。在实践过程中,高等学校应重新审视现有课程体系,找出与“互联网+教育”相契合的课程模块,如数据分析、远程监控、智能养殖等,将其作为核心课程纳入教学计划。与此同时,学校还需利用网络资源开发线上课程,如中国大学MOOC课程,让学生能够自主选择感兴趣的内容进行深度学习,以满足不同层次学生的学习需求。此外,高等学校还可以利用虚拟实验室技术,改善实体实验室资源不足的问题,使学生能够在安全、便捷的环境中进行实验操作,增强实践能力。

2. 加强信息技术相关课程建设,提升信息素养

在“互联网+”时代,信息素养已成为人才必备的核心竞争力之一。因此,在畜牧学课程体系中,应加强信息技术相关课程的设置,如数据科学基础、编程语言、云计算与物联网等。这些课程不仅可以提升学生的信息化处理能力,还能激发他们对新兴技术的兴趣,培养其运用信息技术解决实际问题的能力。此外,学校还可以组织学生参加各类科技竞赛和实践活动,如数据分析大赛、智能养殖项目,实现以赛促学,帮助学生进一步提升信息素养。

3. 增设跨学科课程,拓宽学生视野

为了适应畜牧业发展的多元化需求,培养具有跨界思维的复合型人才,增设跨学科课程是必要的。例如,学校可以开设生物信息学、环境科学、经济学等相关课程,让学生了解畜牧学与其他学科的交叉点,培养其跨领域解决问题的能力。此外,通过开展跨学科项目,如“智能牧场管理系统设计”,鼓励学生将畜牧学知识与信息技术、管理学原理相结合,不仅能够拓宽学生的知识面,还能培养其团队合作和项目管理能力。^[3]

(二) 培养学生自主学习能力

1. 构建个性化学习资源库

在“互联网+”时代,海量的信息资源为学生提供了更丰富的学习材料,但也带来了筛选和整合资源的挑战。因此,高校应构建一个包含视频教程、在线课程、

虚拟实验室、专业文献数据库等在内的综合学习资源库。这一资源库应根据学生过往的学习记录、个人兴趣和设定的学习目标,精准分析并自动为学生推送最为匹配的学习资料。例如,通过分析学生在畜牧疾病诊断模拟实验中的表现,系统可以智能推荐相关的病理学课程或病例研究,帮助学生查漏补缺。这种个性化学习方式,不仅能够激发学生的学习兴趣,还能有效提升其自主探索和解决问题的能力。

2. 强化在线互动与合作学习

传统的畜牧学教育往往侧重于教师讲授和实地实习,但在“互联网+教育”模式下,学生间的互动和合作变得更为重要。高校应当利用在线平台促进学生之间的讨论、协作和项目合作。例如,设立线上小组讨论区,鼓励学生围绕特定的畜牧学案例进行分析和讨论,或开展跨地域的虚拟养殖项目,让学生在实践学习中学习团队沟通、资源管理及问题解决技巧。此外,教师可以通过在线平台定期开展直播答疑,即时解答学生的疑惑,增强师生间的互动。这种基于互联网的互动与合作学习模式,能够有效培养学生的批判性思维能力和团队协作精神。

(三) 培养学生创新创业能力

1. 构建跨学科创新平台

在“互联网+教育”背景下,畜牧学的教育不应局限于单一学科的知识传授,而应搭建一个跨学科的创新平台,鼓励学生从多角度思考问题,促进知识的交叉融合。例如,高校可以开设“智慧畜牧”系列课程,课程需涵盖物联网技术、大数据分析、生物信息学、市场营销等多个领域的前沿知识。由此,学生不仅能够深入了解畜牧学的核心概念,还能学习如何利用现代科技改进养殖方式,提高生产效率。此外,学校还可以邀请行业专家和成功创业者举办讲座和研讨会,分享最新的行业动态和创业经验,激发学生的创新灵感,引导他们思考如何将理论知识应用于实际的创业项目。

2. 设立创新创业孵化基地

为了推动学生将创新思维转化为实际行动,高校应设立专门的创新创业孵化基地,为学生提供一个从想法到实践的完整支持体系。这个基地需配备先进的科研设施和创业导师团队,为学生团队提供项目指导、资金支持、市场调研、产品开发等一系列服务。例如,在孵化基地内,学生可以着手研究如何运用物联网技术来实现对动物健康的连续监控,或设计基于区块链技术的畜产品供应链追溯系统。^[4]同时,孵化基地还应举办创业大赛、路演和投资人见面会等活动,帮助学生团队获得外部资源和市场认可,促进创新成果的商业化。

3. 强化校企合作与实践实训

推动理论与实践相结合是提升学生创新创业能力的关键。高校应加强与企业的合作,建立稳定的校企合作关系,为学生提供实习实训和就业创业的平台。企业可



以为学生提供真实的工作环境和项目,让学生在实践学习中如何将理论知识应用于解决实际问题。例如,学生可以参与到企业的新产品研发中,从市场需求分析到产品设计,再到市场推广,亲身体验创业的全过程。

(四) 加强师资队伍建设

1. 加强教师信息技术培训,提升教学水平

在“互联网+教育”背景下,教师的角色正在发生深刻转变,教师不只是知识的传授者,更是学生学习的引导者和促进者。为了提升教师的专业技能并优化教学效果,定期开展信息技术工作坊、在线学习平台使用培训以及虚拟实验操作示范等活动至关重要。这些活动旨在让教师熟练掌握一系列前沿技术与工具,包括在线课程的设计与制作、数据分析软件的应用以及虚拟实验室系统的操作。更重要的是,信息技术的应用使教师能够有效监控和评估学生的学习进展,实现精准教学,最终显著提升教育质量和学习效果。^[5]

2. 引进具有“互联网+”背景的专业人才

为了充实师资队伍、提升教师的教学与科研水平,高等学校应积极引进具有“互联网+”背景的专业人才。这类人才通常具备深厚的学科知识和信息技术能力,能够将互联网技术与畜牧学教育深度融合,推动教学模式的创新;能够设计和开发高质量的在线课程,利用大数据分析学生的学习行为,优化教学内容和方法,提高教学效果;还能引领科研方向,开展基于互联网的畜牧学研究,如智能养殖系统、动物健康监测平台等,为学生提供前沿的科研项目和实践机会。此外,这类人才还能够搭建校企合作桥梁,引入行业资源和技术,为学生提供更多实习就业机会,提升毕业生的市场竞争力。

3. 建立跨学科教学团队,促进知识融合

在畜牧学教育中,建立跨学科教学团队是培养复合型人才的关键。通过聚焦不同学科背景的教师,如信息技术、生物学、环境科学、管理学等,可以有效促进知识的交叉融合。例如,信息技术教师可以教授学生运用大数据分析动物生长数据,生物学教师可以讲解动物生理机制,环境科学教师可以带领学生探讨畜牧业对生态环境的影响,管理学教师可以讲授畜牧业的经济管理策略。这种跨学科的教学模式不仅能够拓宽学生的知识视野,还能培养他们解决复杂问题的综合能力。更重要的是,跨学科教学团队还能共同指导学生开展跨领域研究项目,如智能畜牧管理系统的设计与实施,让学生在实践中学会整合和应用不同学科的知识,为未来的学术研究和职业发展奠定坚实基础。^[6]

(五) 完善评价体系

1. 构建多元化的评价体系

传统的教育评价往往侧重考查学生的理论知识掌握程度,而忽视了对其实践能力和创新思维的评价。在“互联网+教育”的框架下,高校应当构建多元化的评价体系,既包括对基础知识的考核,也涵盖对实验技能、

科研能力、团队合作精神以及实际问题解决能力的评估。例如,高校可以引入项目制学习,让学生参与实际的畜牧生产项目,并通过在线平台提交报告,教师和行业专家共同进行线上评审,这样既能检验学生的专业知识应用能力,也能促进其综合素养的提升。

2. 强化过程性评价与反馈

在传统的教育模式下,期末考试往往是衡量学生学习成效的主要方式,但这种方式容易导致学生在学习过程中缺乏持续的动力。为了克服这一弊端,“互联网+教育”提供了实时跟踪学习进度的技术手段,学校应充分利用这些工具,建立过程性评价机制。例如,使用学习管理系统(LMS)来记录学生的学习活动,如在线课程的参与度、作业提交情况、小组讨论的活跃度等,这些数据可以作为评价依据。同时,构建即时的反馈机制也至关重要,教师可以通过在线平台及时给予学生指导和建议,帮助他们调整学习策略,提高学习效率。

四、结语

“互联网+教育”为高等学校的畜牧学学科人才培养带来了前所未有的机遇与挑战。通过研究探讨与实践,文章提出要培养出适应时代发展、具有创新能力和实践技能的高素质人才,就必须打破传统教育模式,充分利用互联网技术,创新教学模式,优化课程体系,加强校企合作,提升教师信息化教学能力,营造开放、互动、共享的学习环境。未来,期待更多学者关注这一领域,共同探索“互联网+教育”背景下畜牧学学科人才培养的新路径,助力我国高等教育事业的繁荣发展。

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中国农业科学院北京畜牧兽医研究所简介

中国农业科学院北京畜牧兽医研究所成立于 1957 年，以解决我国畜牧业发展中战略性、基础性、全局性、关键性重大科技问题为责任，开展动物遗传资源与育种、动物生物技术与繁殖、动物营养与饲料、草业科学、动物医学和畜产品质量与安全等六大学科的基础研究、应用基础研究和成果转化推广，为畜牧业高质量发展提供科技支撑。

研究所有职工 225 人，聘用人员 196 人，博士后 46 人，研究生 607 人。其中，中国工程院院士 2 人、国家自然科学基金委“杰青”2 人、国家重大项目首席科学家 13 人、现代农业产业技术体系首席 2 人、农业先进工作者 3 人、国务院津贴专家 9 人、建国 70 周年纪念章获得者 21 人。建有畜禽营养与饲养全国重点实验室、畜禽生物育种全国重点实验室、国家畜禽种质资源库等国家级科研平台 5 个，农业农村部动物遗传育种与繁殖重点实验室等省部级平台 12 个。

建所以来，承担国家重点研发计划等项目千余个，以第一完成单位获国家奖 24 项。育成了中国西门塔尔牛、华西牛、中畜草原白羽肉鸭、白羽肉鸡、京星系列黄羽肉鸡等畜禽新品种（系）15 个，突破了畜禽健康养殖及智能作业装备创制等系列关键技术。获授权专利 286 项，发表论文 4600 多篇。在畜禽重要性状遗传机制解析、大动物基因修饰、饲料营养价值评定、饲用酶工程等方面达到国际领先水平。



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班主任在本科生心理健康和专业素质培养中的作用——以动物科学专业为例

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摘要: 学生群体的心理健康影响着高校本科教育的质量和效果。在教学和生活中, 班主任是大学生与课程和专业之间的联系桥梁。本文以动物科学专业班主任为例, 阐述了班主任在不同的教育教学活动中对本科生心理健及专业素质培养起到的关键作用。班主任工作对引导本科生形成正确的价值观和积极向上的心态, 增加学生的专业认同感, 以及帮助学生成长为心理健康的高素质专业人才起到至关重要的作用。

关键词: 班主任; 本科生; 心理健康; 专业素质; 动物科学

建设教育强国是中华民族伟大复兴的基础工程, 培养品学兼优的专业人才是高等教育的历史使命。本科生是高素质专业人才培养的最大群体, 本科教育是提高高等教育质量最重要的基础。本科阶段是学生世界观、人生观、价值观形成的关键期, 本科生的心理健康问题不利于高等教育的良性发展。一系列内外在因素(生理和心理的异常与冲突、时代背景的影响、校园的生态环境、家庭环境等)的综合作用影响着本科生的心理健康^[1], 从而影响本科生专业素质的培养质量。作为我国高校教育教学教师队伍中的重要一员, 班主任在本科生心理健康和专业素质培养中直接和间接地发挥着至关重要的作用。

1 本科生心理健康预警和干预

作为“校-院-班-寝”四级心理危机防范体系中的“院”级重要构成元素, 班主任应及时密切地关注本班学生的心理情绪。动物科学一直属于冷门专业, 报考人数常少于招生计划人数, 学生大部分对专业实际情况不了解, 或为调剂生。除了新生常遇到的因环境适应、人际交往等原因出现的情绪问题外, 在大学前一两年以通识课为主的课程学习中, 动物科学专业的学生容易产生沮丧感, 或者出现盲目调换专业的情况。班主任应该在减少本科生迷茫情绪、引导学生对专业形成正确认识和培养大学生专业兴趣中发挥关键作用。

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1.1 关注学生的情绪状况

通过建立班级开放交流日来鼓励引导学生发言和互相交流, 以及定期与班委座谈了解其工作情况, 班主任能加强与学生的联系, 更好地掌握班级的整体情况以及每位学生的近况。同时, 对于存在心理问题倾向的学生, 班主任可以与其进行一对一的谈心和疏导, 邀请家长、辅导员参与沟通访谈, 以便带来更好的疏导效果, 同时与院系、咨询中心建立起联系, 以方便实时通报学生情况和在必要时让学生接受更专业的心理帮助。在访谈过程中, 班主任应多引导学生发现自身优点和长处, 鼓励学生多参与社团和校园文化活动(如华南农业大学紫荆文化节、宠物嘉年华)等, 让学生逐渐形成乐观、积极的生活态度。面对学生提出的问题和困惑, 班主任可以以自身学习和工作过程中遭遇的困难与挫折为例勉励学生, 激励学生建立起面对和解决问题的信心与决心^[2]。不同学生的性格特点、家庭情况和教育背景等存在差异, 因此班主任需要提前了解学生的背景信息, 因材施教地设定谈话方向和内容, 并且在交谈中要善于观察学生 and 了解其思想动态, 才能起到更好的监督和引导作用。

1.2 关注学生的学习动力

学生的学习情况能反映学生自主学习的动力和对大学生活的态度。班主任在学生从高中到大学的顺利过渡中扮演着十分重要的引导角色^[3]。学生在大学期间相比高中更为自由, 学习和生活上都不再受到家长和老师们的时刻监督。因此, 对学生进行一定的约束和强化学习纪律是必要的。班主任在班会中应该对



课堂出勤率进行强调,同时配合实际奖惩制度的实施,减少甚至杜绝旷课、迟到等问题。同时,要关注学生的学习效果,重点包括期末考试分数、挂科和重修科目情况、发表论文和参加学科竞赛等。对学习效果不佳尤其是挂科重修率高,参与活动不积极的同学,班主任应该给予额外关注,找出背后原因并积极采取措施进行干预和引导。班主任可以通过让积极性高和学习效果好的同学,带动自主学习动力不足的学生一起参加自习、学术讲座和学术竞赛等,提高整个班集体的学习活跃度,促进班级营造良好的学习氛围。同时,班主任作为专业课老师要创新教学模式,以“学生为中心”,推动课堂教学改革,激发学生的学习兴趣。鼓励学生尽早联系导师,走入实验室和养殖场,“多听、多看、多了解”,对动物科学专业有一个更具体和科学的认识,激发学生的专业兴趣。

2 主题教育结合实际,课程思政融入课堂教学

传统教学模式常常过分注重理论学习而忽略学生综合素质的培养。班主任通常为专业课教师,有立德树人的职责。班主任应该针对动物科学专业的学科特色,将“课程思政”作为心理素质教育的重要部分浸润在专业课教学的全程中,达成塑造学生健康个性和专业高素养的目标。动物科学专业包含动物生理生化、营养与饲料、遗传与育种、行为与福利等多方面课程知识的学习,班主任作为专业课教师应用心提炼思政元素,帮助学生在课堂中领悟本专业的思政要点,激发学生产生专业兴趣。

2.1 建立正确的社会观和价值观

动物科学是生命科学的一个分支。对人和动物共性专业知识的学习能让学生了解到生命体精细复杂的运作原理,个体生长发育、生存和适应环境的艰难过程可以让学生领悟生命的宝贵,不易产生轻生的念头。另一方面,动物科学专业涉及到动物的研究和处理,因此在课程中可以加强对动物保护、动物福利和动物伦理的教育,引导学生尊重生命、关爱动物和大自然。对于一些道德败坏的思想和行为(如虐待动物、制作和传播相关录像等),应该让学生有明确的

认识,并引导和帮助有问题的学生及时寻求心理治疗。此外,畜牧业在国民经济和生产中一直占据着举足轻重的地位。动物科学专业主要关注农场动物和伴侣动物的遗传基础、品种培育、生理生化、营养代谢、饲料配制、安全产品、加工调制等内容,以期获得“优良的品种”“优质的饲料”“安全的产品”。授课过程中,班主任要让学生认识到动物科学专业及人才在社会生产中的重要地位,培养他们的专业荣誉感、职业道德和社会责任感。

2.2 强化科学实践精神和创新意识

动物科学作为一门科学性很强的专业,在动物科学相关课程中应强调科学精神的培养,鼓励学生进行独立思考和创新实践。班主任可以通过引导学生参与科研项目、实验室实践等活动,激发他们的科研兴趣 and 创新能力。动物科学也是应用性较强的专业,人才的培养应当响应习近平总书记的教导,即“教育引导广大科技工作者传承老一辈科学家以身许国、心系人民的光荣传统,把论文写在祖国的大地上”。通过在专业课教学中加入模范科学家和优秀科技工作者的先进事迹,积极引导学生形成学以致用,脚踏实地投身一线科技工作的实践精神。面对近年来新的国际机遇和挑战,动物科学专业的科研和产业人才在乡村振兴的大舞台上,承担着畜禽种业振兴、建设现代畜牧业等重要使命。班主任应该教导学生产生对农业的热爱,提高他们对农业的兴趣,为党和国家培养出一批有创造力、有知识、有能力的新型农牧业人才。

2.3 培养团队合作和沟通能力

动物科学专业的研究和实践活动往往需要进行团队合作,因此在课程中可以设计一些团队协作的项目和任务,培养学生的团队合作和沟通能力。如在生理学的实验课中将学生进行随机分组,明确学生的分工(负责动物保定、麻醉、采样、善后处理和记录等),过程中学生需要做好自己分内之事,同时相互之间进行有效地沟通以及积极地配合,才能保证实验顺利地完成。再比如在《家畜行为学》的翻转课堂中,安排学生分组介绍各种动物行为,不同的学生要分别负责文字内容查找、图片和视频搜集、幻灯片制作和演讲等工作,最终课堂演讲的精彩呈现离不开成员各司其

职以及相互之间的完美配合。因此,通过团队合作的实践,学生可以了解和体验团队协作的重要性,培养他们的团队精神和合作意识,这对于学生将来步入社会,适应与他人社交和工作至关重要。

2.4 引导学生关注国内外社会热点问题

班主任在课堂中通过引导学生关注社会热点问题,能帮助他们扩大视野和思考范围,培养其社会责任感和公民意识。可以通过课堂讨论、社会实践等方式引导学生思考和解决与动物科学相关的社会问题,提高他们的专业兴趣、社会参与能力和责任感。

例如,2022年10月,我国一家在湖北鄂州建造的26层养猪大楼正式投产,年出栏生猪可达120万头。这一新闻引起了全国和国际养猪行业的广泛关注。楼房猪场这一生产系统顺应我国国情和发展形势,在节约人力成本和用地面积、环保、疫病防控中,较其他生产模式有较强优势。现代化智能设备系统的应用,可实现自动化饲喂、环境控制和疫病监测,同时粪污可实现统一收集再处理,生产过程绿色高效。这类行业新闻在课堂上的分享,能让学生了解我国畜牧行业的发展现状和前沿趋势,给学生的学习、研究和就业方向提供新思路。

2023年10月,四川成都出现的犬咬人事件和衍生出的大肆捕杀无辜流浪动物和投毒等恶性事件,引起了广泛的社会关注。本校动物科学学院老师在《养犬养猫学》的课堂上就提出了这一热点问题,让学生对文明养宠和流浪动物管理等相关问题进行思考,从而引导学生形成科学的专业意识和从业观念。

3 结语

建设高等教育强国需要多方力量的共同努力,而本科生作为高等教育的主体对象,他们的心理健康是高等教育取得成效的基础保障。班主任作为学生专业学习和健康成长的引路人,联系学生和学院、教学和心理咨询中心的纽带,在教育和教学活动的多个环节都发挥着引导、预警、干预和维护学生心理健康的作用。班主任日常工作尤其需要关注那些常被忽视的因素,比如对专业认识不正确和对就业前景迷茫等会深刻影响学生的自我认同感、价值观和心理健康。总之,班主任在引导高校本科生健康成长成才方面担负着重要职责,需要全面关照学生的学习和生活,要结合自身优势和专业认知,为学生心理健康建设和高效优质的专业人才教育做出重要贡献。

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投稿邮箱: xumuyehj@126.com

聘 书

张玲娜 老师：

在动物科学学院 2023 年青年教师教学能力比赛中
荣获“一等奖”

特发此证，以资鼓励



2023 年 12 月 22 日

表9 科研课题情况
张玲娜 主持的课题

序号	项目名称	评审等级	项目来源	合同经费/ 实到经费	立项时间	结题时间	课题组 总人数	本人排 名	是否结 题	备注
1	催产素对猫社交行为的影响	C	广州市科技局	5.0	2023-05-30	2025-03-31	1	1	否	
2	多层（楼房）养猪关键技术与综合性能评估	A	科技部	50.0	2023-12-01	2028-11-01	1	1	否	
3	华农益普思宠物营养产品研发项目	C	横向	60.0	2024-04-25	2025-05-08	4	1	否	
4	人宠互动方式对猫依恋关系和社会行为认知的影响及机制研究	B	广东省基础与应用基础研究基金委员会	15.0	2024-03-08	2026-12-31	1	1	否	

科技处审核人及盖章：

年 月 日

“多层（楼房）养猪智能化舒适环境控制关键技术研发” 课题组织实施协议

课题牵头单位（甲方）：广西扬翔股份有限公司

课题参加单位（乙方）：华南农业大学

甲乙双方就共同实施“十四五”国家重点研发计划“畜禽新品种培育与现代牧场科技创新”重点专项 2023 年度项目“多层（楼房）养猪关键技术与综合性能评估”下的课题“多层（楼房）养猪智能化舒适环境控制关键技术研发”。为完成课题研究目标，本着优势互补、协同配合、共同发展的原则，甲乙双方经友好协商，达成如下协议：

1、乙方承诺在课题执行期内，任务分工为：楼房生长育肥猪环境因子时空变化规律和舒适参数研究。

2、乙方应按时完成任务书规定的研究任务，并达到以下考核指标：

（1）发表 SCI 论文 1-2 篇；

（2）申请国家发明专利 1 件。

3、乙方分配科技经费 50 万元，配套自筹经费 0 万元。在课题实施过程中，乙方执行《财政部 科技部关于印发〈国家重点研发计划资金管理办法〉的通知》（财科教〔2021〕178 号）等科研经费管理规定，严格按照预算支出经费，独立核算并专款专用。

4、课题执行期内，乙方根据课题任务书要求定期向甲方汇报进展和研究数据，提交课题执行报告和科技报告。

5、课题执行过程中，甲、乙双方各自产出的知识成果原则上归各自所有，但甲方有权因非商业目的（如：以政府性会议、报告、文件、统计资料等）使用乙方课题信息，在课题执行期间进行知识产权共享。共同产出的知识成果归共同所有，可根据双方的实际贡献进行具体分配。专利、论文、著作、新品种（系）的登记申请和推广应用情况等，均须标注课题的资助编号。

6、本协议一式六份，甲方持四份，乙方持二份。未尽事宜由双方协商解决。

甲方（单位盖章）：广西扬翔股份有限公司

法定代表人（签章）：

课题负责人（签字）：

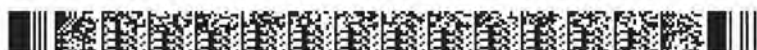
2023 年 12 月 5 日

乙方（单位盖章）：华南农业大学

法定代表人（签章）：

任务负责人（签字）：

2023 年 12 月 5 日



受理编号: c24140500001582

项目编号: 2024A1515012606

文件编号: 粤基金字(2024)7号

广东省基础与应用基础研究基金项目 任务书

项目名称: 人宠互动方式对猫依恋关系和社会行为认知的影响及机制研究

项目类别: 广东省自然科学基金-面上项目

项目起止时间: 2024-01-01 至 2026-12-31

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: 张玲娜

联系电话: 19825610615



(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扫描上传到广东省科技业务管理阳光政务平台。

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。

五、根据《广东省科学技术厅广东省财政厅关于深入推进省基础与应用基础研究基金项目经费使用“负面清单+包干制”改革试点工作的通知》（粤科规范字〔2022〕2号），2022年度及以后立项资助的全部省基金项目（包括省自然科学基金、省市联合基金、省企联合基金项目等）均适用“负面清单+包干制”，项目提交申请书和任务书时无需编制费用明细科目预算。

一、主要研究内容和要达到的目标

猫的社会行为问题影响宠主关系，间接加重弃养、公共卫生和生态健康问题。猫有较强的社会认知能力，主人和猫的互动方式会影响猫的社会行为健康，但机理尚不清楚。猫能和主人形成典型的依恋关系，人宠互动方式决定宠物的依恋类型。不安全依恋类型在人和狗上与个体的行为问题的发生显著相关。而本课题组的近期数据显示不安全的依恋类型会妨碍猫在目标选择测试和无法解决问题测试中的表现，说明不安全依恋会降低猫的社会认知能力。此外，不同人-猫互动方式对猫催产素的释放存在差异化的影响，而催产素在狗的社会行为表达以及狗和主人的感情连结中起了关键介导作用。基于猫和狗上的研究基础，我们提出假设：主人和猫的互动方式影响了猫内源OT 的释放和依恋类型的形成，这会影响猫的行为认知能力，从而长远地影响猫的社会行为健康。为验证这一假设，本研究拟解决的关键问题包括：人和猫的互动方式如何影响猫OT 的分泌和依恋类型？互动刺激的OT 对猫行为认知能力的影响？猫的不健康依恋类型和内源OT 水平能否被长期宠主互动方式的矫正和/或应用外源OT 改变，从而长远地改善猫的社会行为问题？申请人拟在前期工作的基础上，对以上基本问题进行研究，主要通过以下几个方面展开工作：1）在宠物猫上研究宠主互动方式、猫依恋类型和OT 之间的关联，另外设置不同互动方式组（自愿的肢体接触互动、强迫接触互动和无互动等），在实验猫上对比不同互动方式对猫OT 分泌情况的影响，找出能最大程度刺激OT 释放的“有效互动”方式；2）通过目标选择测试和无法解决问题测试，探究“有效互动”和鼻腔外用OT 对猫社会行为认知的影响，以及OTR 拮抗剂是否会消除这种影响，以此证明OT 在介导人宠互动方式对猫行为认知的影响中的作用；3）研究为期6 个月的对人宠互动方式的指导和结合外用OT 能否改善宠物猫的不安全依恋类型和内源OT 的释放，进一步验证不同互动方式刺激OT 释放的差异影响了猫依恋类型的形成。本研究有望揭示人和猫的互动方式影响猫行为健康的内分泌机制，为开发OT 作为改善猫行为问题和人宠关系的天然外用激素提供理论和实践基础。

二、项目预期获得的研究成果及形式

论文及专著情况	国家统计局刊物以上刊物 发表论文（篇）		2		科技报告（篇）		1	
	其中被SCI/EI/ISTP收录 论文数（篇）		2		培养人才（人）			
	专著（册）				引进人才（人）			
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

三、项目进度和阶段目标

(一) 项目起止时间： 2024-01-01 至 2026-12-31		
(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
2024-01-01	2024-12-31	1) 招募志愿者，进行猫依恋类型的评定和互动方式的记录观察，并完成行依恋类型、互动方式和OT 水平相关性分析。 2) 在实验猫上验证互动方式对OT 释放的影响，找出能最大程度刺激OT 释放的“有效互动”方式。
2025-01-01	2025-12-31	1) 完成评价OT 对猫行为认知能力的影响的实验。 2) 进行阶段性总结，并撰写1 篇高质量学术论文。
2026-01-01	2026-12-31	1) 完成外源OT 应用和互动方式指导影响宠物猫依恋类型的实验。 2) 进行项目总结，撰写项目结题报告，以及1 篇高质量学术论文。

四、项目总经费及省基金委经费预算

1. 省基金委经费下达总额：（大写）壹拾伍万圆整；（小写）15万元；					
2. 省基金委经费年度下达计划：					
年度	2024 年	年	年	年	年
经费(万元)	15.00				

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
张玲娜	321282199004051043	34	女	讲师	博士研究生	项目负责人	华南农业大学	张玲娜

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
简仕燕	522127199410296087	30	女	未取得	硕士研究生	动物实验，数据整理和分析	华南农业大学	简仕燕
卞兆威	342225199908165710	25	男	未取得	本科	动物实验	华南农业大学	卞兆威

六、工作分工及财政经费分配

承担/参与单位名称 (盖章)	工作分工	省级财政科技资金分配 (万元)
华南农业大学	经费将用于项目的开展，包括试剂耗材，检测费用，项目参与人员劳务费，出版费等。	15.00
	合计	15.00

七、任务书条款

第一条 甲方与乙方根据《中华人民共和国民法典》及国家有关法规和规定，按照《广东省科学技术厅关于广东省基础与应用基础研究基金（省自然科学基金、联合基金等）项目管理的实施细则（试行）》《省级科技计划项目任务书管理细则》《广东省省级科技计划项目验收结题工作规程（试行）》等规定，为顺利完成（2024）年人宠互动方式对猫依恋关系和社会行为认知的影响及机制研究专项项目（项目编号：2024A1515012606）经协商一致，特订立本任务书，作为甲乙双方在项目实施管理过程中共同遵守的依据。

第二条 甲方的权利义务：

1. 按任务书规定进行经费核拨的有关工作协调。
2. 根据甲方需要，在不影响乙方工作的前提下，定期或不定期对乙方项目的实施情况和经费使用情况进行检查或抽查。
3. 根据《广东省科研诚信管理办法（试行）》等规定对乙方进行科技计划信用管理。

第三条 乙方的权利义务：

1. 确保落实自筹经费及有关保障条件。
2. 按任务书规定，对甲方核拨的经费实行专款专用，单独列账，并随时配合甲方进行监督检查。
3. 经费使用按照广东省级财政科研项目经费使用等有关规定进行管理。
4. 项目依托单位应制定经费使用“负面清单+包干制”内部管理制度并报甲方案案。
5. 使用财政资金采购设备、原材料等，按照《广东省实施〈中华人民共和国招标投标法〉办法》有关规定，符合招标条件的须进行招标。
6. 项目任务书任务完成后，或任务书规定的任务、指标及经费投入等提前完成的，乙方可提出验收结题申请，并按甲方要求做好项目验收结题工作。
7. 若项目发生需要终止结题的情况，乙方须提出终止结题申请，并按甲方要求做好项目终止结题工作。
8. 在每年规定时间内向甲方如实提交上年度工作情况报告，报告内容包含上年度项目进展情况、经费决算和取得的成果等。
9. 按照国家和省有关规定，提交科技报告及其他材料。
10. 利用甲方的经费获得的研究成果，项目负责人和参与者应当注明获得“广东省基础与应用基础研究基金（英文：Guangdong Basic and Applied Basic Research Foundation）（项目编号）”资助或作有关说明。
11. 乙方要恪守科学道德准则，遵守科研活动规范，践行科研诚信要求，不得抄袭、剽窃他人科研成果或者伪造、篡改研究数据、研究结论；不得购买、代写、代投论文，虚构同行评议专家及评议意见；不得违反论文署名规范，擅自标注或虚假标注获得科技计划（专项、基金等）等资助；不得弄虚作假，骗取科技计划（专项、基金等）项目、科研经费以及奖励、荣誉等；不得有其他违背科研诚信要求的行为。
12. 确保本项目开展的研究工作符合我国科技伦理管理相关规定。

第四条 在履行本任务书的过程中，如出现广东省相关法律法规重大改变等不可抗力情况，甲方有权对所核拨经费的数量和时间进行相应调整。

第五条 在履行本任务书的过程中，当事人一方发现可能导致项目整体或部分失败的情形时，应及时通知另一方，并采取适当措施减少损失，没有及时通知并采取适当措施，致使损失扩大的，应当就扩大的损失承担责任。

第六条 本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享，除双方另有约定外，按国家和广东省有关法规执行。

第七条 根据项目具体情况，经双方另行协商订立的附加条款，作为本任务书正式内容的一部分，与本任务书具有同等效力。

第八条 本任务书一式三份，各份具有同等效力。甲、乙方及项目负责人各执一份，三方签字、盖章后即生效，有效期至项目结题后一年内。各方均应负任务书的法律责任，不应受机构、人事变动的影响。

第九条 乙方必须接受甲方聘请的本项目任务书监理单位的监督和管理。监理单位按照甲方赋予的权利对本项目任务书的履行进行审核、进度调查，对项目任务书变更、经费使用情况进行监督管理及组织项目验收。

说明：1. 本任务书中，凡是当事人约定无需填写的内容，应在空白处划（/）。

2. 委托代理人签订本任务书的，应出具合法、有效的委托书。

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）

2024 年 05 月 22 日

依托单位（乙方）： 华南农业大学

法定代表人（或法人代理）： 薛红卫

联系人（项目主管）姓名： 倪慧群

Email: kjcgxk@scau.edu.cn

电话： 020-85283435 / 15920301530

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

2024 年 06 月 03 日

联系人（项目负责人）姓名： 张玲娜

（签名）

Email: 1016043808@qq.com

电话： 19825610615

2024 年 05 月 27 日

任务书编号：2023A04J0117

广州市科技计划项目 任务书

项目名称：	催产素对猫社交行为的影响
承担单位：	华南农业大学
项目负责人：	张玲娜
计划类别：	基础研究计划
专题名称：	2023年度基础与应用基础研究专题
支持方向：	一般项目（博士青年科技人员类）
组织单位：	华南农业大学
起止时间：	2023-04-01 至 2025-03-31
主管处室：	基础研究处

广州市科学技术局制

二〇二三年

填写说明

1. 任务书甲方为广州市科学技术局；乙方为项目承担单位；丙方为项目组织单位。

2. 任务书基于项目申报书转换而成，请按照“广州科技大脑”提示在线填写核实，若存在不填写内容的栏目，请用“无”表示；任务书中的单位名称应为规范全称，并与单位公章一致。

3. 乙方与合作单位的合作协议自动从项目申报书中读取，如需变化调整，须待任务书签订后，按要求及时办理重大变更。

4. 乙方完成项目任务书在线填写，依次提交丙方和甲方审核确认后，按要求登录“穗好办”APP完成电子签章。不具备电子签章条件的单位，经与业务主管处室沟通对接后，可下载电子版项目任务书用A4纸双面打印装订签章；一式六份报甲方和丙方签章，其中甲方两份丙方两份，项目承担单位和项目负责人各一份。

5. 涉密项目请在“广州科技大脑”下载项目任务书模板，按保密要求离线填写报送。

6. 项目申报书是项目任务书填报的重要依据，未经甲方许可，乙方不得修改考核指标，调整主要研究内容。项目任务书将作为项目实施管理、验收结题和监督评估的重要依据。

7. 项目任务书中的“备注”，包括重要的必须补充的内容。

8. “广州科技大脑”是项目管理过程中重要通知和文书的电子送达平台。为确保电子送达渠道畅通，乙方和项目负责人应及时更新维护“广州科技大脑”的单位和个人信息。

一、项目基本信息

项目 基本信息	项目名称	催产素对猫社交行为的影响		
	申请金额 (万元)	5	研究期限 (年)	2
项目摘要	<p>主人和猫的互动方式影响猫的行为健康，但机理尚不清楚。人宠互动决定狗对主人的依恋关系，且不安全依恋类型和狗行为问题的发生显著相关，而催产素（OT）在其中起关键作用。因此，推测OT和依恋类型介导了人宠互动对猫行为健康的影响。本项目将探究猫依恋类型、OT水平和人宠互动方式之间的关联，并通过实验验证OT对猫社交行为健康和依恋类型的影响，为开发OT作为天然外源激素在改善猫行为问题上的应用提供研究基础。</p>			

二、项目单位情况

项目 承担单位	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	15920301530	
		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、项目组成员信息

项目负责人	姓名	张玲娜	证件类型	身份证
	证件号码	321282199004051043	性别	女
	出生年月	1990-04-05	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	讲师	职称	无
	所学专业	动物科学	手机号码	19825610615
	办公电话	020-12345678	电子邮箱	lingna.zhang@scau.edu.cn

四、项目经费信息

本项目总投入：¥（5）万元，其中，市财政科技经费：¥（5）万元，

资金来源	小计	市财政科技经费
2023	5	5
总计	5	5
合 计	5	5

（单位：万元）

本专题纳入“包干制”试点，市财政科技经费按市科技计划项目经费“包干制”相关规定执行。

五、预期代表性成果

项目负责人须在项目实施期内以该项目作为资助项目（须标注该项目编号）至少完成以下情形之一。

1. 项目实施期内，以第一作者/通讯作者发表论文1篇或以上；
2. 项目实施期内，以第一完成人申请或授权专利、软件著作权1项或以上；
3. 项目实施期内，获省级以上科技计划项目或人才项目支持1项或以上；
4. 项目实施期内，获省级以上科技奖励（含列入获奖团队成员名单）1项或以上；
5. 项目实施期内，获得职称晋升。

注：项目申报指南发布日后所形成的与项目直接相关的科研成果，可列为该项目科研成果。项目完成以上情形之一的，由组织单位审核后，验收通过。如无以上情形，各组织单位应根据项目实际完成情况组织验收。

六、备注

专题补充约定条款：

甲方对未履行勤勉尽责义务的相关责任主体，自作出处理结论之日起，依照法律法规规定或任务书约定实施惩戒5年，取消相关责任主体申报市科技计划项目、申领市科技计划项目经费的资格。

项目承担单位（乙方）及项目负责人承诺书

承诺书

本单位/本人作为广州市科技计划项目承担单位/项目负责人，将严格遵守广州市科技计划管理相关规定，严格履行自身责任，加强对项目组人员及合作单位的管理，在此郑重承诺：

（一）确保与本项目有关的全部材料真实、合法、有效，未侵犯其他方知识产权等权利，不存在多头申报、重复申报行为；

（二）严格遵守《广州市科技创新条例》《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》等相关规定，实施项目和经费管理；

（三）严格遵守国家、省、市关于科研诚信和科技伦理的有关法律、法规，相关政策以及各项规定，加强项目实施过程中的科研诚信及科技伦理管理，恪守科研道德准则。

如有违反，本单位/本人愿意接受相关部门做出的各项处理决定，包括但不限于终止项目、停拨经费、核减经费、追回经费，取消一定期限广州市科技计划项目申报资格，记入科研失信行为数据库，将不良行为向社会公开等。

项目承担单位：华南农业大学

日期：2023年03月20日

项目负责人：张玲娜

日期：2023年02月24日

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：李磊 联系电话：83124052
责任处室负责人：麦胜文

2023年03月21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：张玲娜
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03月20日

组织单位（丙方）：华南农业大学
项目经办人：倪慧群

2023年03月20日

广州市科技计划项目验收结果通知书

根据《广州市科技计划项目管理办法》规定及项目验收情况，《催产素对猫社交行为的影响》的验收结论为：验收通过。

计划类别	基础研究计划
专题名称	2023年度基础与应用基础研究专题
起止时间	2023-04-01至2025-03-31
任务书编号	2023A04J0117
市财政科技经费 总额（万元）	5
组织单位	华南农业大学--124400004554165634
承担单位	华南农业大学--124400004554165634
项目负责人	张玲娜--321282199004051043



技术服务合同

甲方：杭州益普思生物科技有限公司

联系地址：杭州市上城区创智绿谷发展中心 1 幢 708 室

联系方式：王东林 18505812777

乙方：华南农业大学

联系地址：广东省广州市天河区五山路 483 号

联系方式：张玲娜 19825610615

杭州益普思生物科技有限公司（下称“甲方”）甲方是一家致力于科学研究与观察来创造优质的宠物营养解决方案，提供科学营养专业配方的宠物食品企业，和 华南农业大学（下称“乙方”），秉承协作开放、优势互补、联合发展为双方合作的基本纲领，就宠物食品、保健品开发项目，经协商一致，达成以下合作协议：

一、合作内容：

1. 乙方有偿为甲方提供功能性宠物食品、保健品研发服务，研究方向包括但不限于缓解宠物应激及 胃肠道健康、老年宠物健康 等方面，具体包含以下内容：

（1）由乙方主导进行研究方向的实验验证，至本协议期限届满前应实现 实验设计目标；实验测试过程中，乙方向甲方公布实验过程中的预算表（包括材料、人员费用，测试费用，学校管理费，联合中心费用等费用），根据实验初步结果，实验流程可进行优化，甲乙双方努力完成实验设计目标。

（2）测试甲方产品效果并提供技术整改意见，至本协议期限届满前积极配合甲方产品实现 宠物食品产品上市 标准；

（3）乙方向甲方提供产品配方和实验报告；



(4) 乙方为甲方及其人员在广州提供线下公司内部专业知识培训或授课（一年 2 次，具体培训时间双方另行协商确定）。乙方协助提供产品答疑解惑手册和产品相关专业知识素材等资料；

(5) 应甲方需要，乙方派遣相关研发人员参与甲方举办的学术研讨会和专家讲座等活动（一年 1~2 次，具体活动时间、地点、人员安排双方另行协商确定）。

2. 双方一致同意根据合作情况，适时共同成立宠物营养研究中心，双方均应积极促成研究中心成立，其中乙方为研究中心提供技术支持；

3. 乙方利用其在宠物营养领域的科研成果及相关联盟、协会平台资源，帮助甲方拓展业务，推动甲方公司在相关领域更快发展；

4. 双方充分发挥各自优势，联合申报各级政府、部门的科学研究、技术开发成果转化及产业化项目；

5. 双方共同申请按照学校的管理和审批流程共建“华南农业大学动物科学学院教学实践基地”；

二. 合作经费及付款方式：

甲方向乙方提供宠物预混合饲料样品（具体种类、数量、方式双方另行签订协议确定），配合乙方完成实验。本项目合作总经费为人民币 60 万元（大写：人民币陆拾万元整），甲方应在 合同生效后 10 个工作日，向乙方全额支付技术服务费 30 万元人民币，并在 2024 年 11 月 8 日 前支付尾款 30 万元人民币。

乙方账户信息如下：

银行账户：中国工商银行广州五山支行

开户名称：华南农业大学

账号：3602002609000310520

乙方收到甲方款项 10 个工作日内开具增值税专用发票给甲方。

甲方开票信息如下：

发票抬头：杭州益普思生物科技有限公司

税号：91330102MADEP4EL14

地址：浙江省杭州市上城区创智绿谷发展中心 1 幢 708 室

开户行：杭州银行钱塘智慧城支行

账号：3301041060001088727

三. 合同履行的期限和方式：

1. 本合同期限自 2024 年 5 月 8 日 至 2025 年 5 月 8 日；
2. 若合同任意一方单方面终止此合同，须提前 1 个月通知对方，由于终止合同造成的损失由提出终止合同方承担（包括但不限于直接损失、可得利益损失、诉讼费用、律师费用等维权费用以及因此而支付的其他合理费用）。
3. 因乙方原因无故提前终止合同的，应向甲方退还已收取的合作经费，因甲方原因无故提前终止合同的，乙方已收取的合作经费不予退还。
4. 本实验只保障实验数据的真实性，但不保障实验数据与实验课题设计的一致性，但乙方应尽最大注意义务，按照正常标准确保实验数据与实验课题设计一致。



四. 知识产权

1. 双方合作过程中产生的配方和产品的知识产权归甲方所有，本项目专利权（包括发明专利和实用新型专利）归甲方所有，是否申请专利以及何时申请专利由甲方决定（甲方申请的，乙方应提供必要配合与支持），甲乙双方可共同发表中文\英文论文，论文以乙方为主，甲方可作为共同作者署名。
2. 双方一致同意，研发成果不会侵犯第三方的所有权和知识产权，且不会给对方造成任何经济或声誉上的损害。



五. 技术保密：

乙方承诺对于与甲方合同研究的项目的技术数据保密：若由于乙方的问题泄露相关信息给第三方，而对甲方造成的损失，由乙方承担。

六•解决合同纠纷的方法:

- 1) 执行本合同发生争议, 由双方协商解决, 也可请求调解,
- 2) 若双方当事人和解或调解不成, 由广州仲裁委员会仲裁解决

七. 本协议书一式 4 份, 其中甲方一份、乙方一份、乙方所在学院一份、乙方项目负责人一份, 自双方签章之日起生效。

以下无正文

甲方 (签章)

法定代表人 (签字)

项目负责人 (签字)

日期: 年 月 日

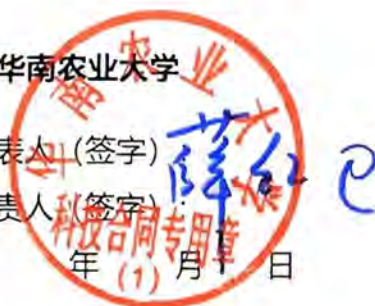


乙方: 华南农业大学

法定代表人 (签字)

项目负责人 (签字)

日期: 年 (1) 月 日



科研空间

打印

近__年参加科研项目情况表

参加科研项目实际可支配经费：__万元。

序号	项目名称	项目级别	项目来源	主持人	合同经费(万元)	实到经费(万元)	结余经费(万元)	立项时间	结题时间	本人排名	课题组总人数
1	二氢杨梅素通过PANoptosis信号通路缓解断奶仔猪腹泻的作用机制研究	A	国家自然科学基金委员会	邓百川	50.0	28.75	28.75	2024-12-01	2028-12-31	2	2
项目__主持人意见：		本人承诺 张玲娜 可在本课题结余经费中支配使用__万元。 承诺人签名： 日期:									
报表人：张玲娜 报表日期： 年 月 日											

国家自然科学基金资助项目批准通知

(预算制项目)

邓百川 先生/女士:

根据《国家自然科学基金条例》、相关项目管理办法规定和专家评审意见,国家自然科学基金委员会(以下简称自然科学基金委)决定资助您申请的项目。项目批准号: 32472927, 项目名称: 二氢杨梅素通过PANoptosis信号通路缓解断奶仔猪腹泻的作用机制研究, 直接费用: 50.00万元, 项目起止年月: 2025年01月至 2028年12月, 有关项目的评审意见及修改意见附后。

请您尽快登录科学基金网络信息系统(<https://grants.nsfc.gov.cn>), **认真阅读《国家自然科学基金资助项目计划书填报说明》并按要求填写《国家自然科学基金资助项目计划书》(以下简称计划书)**。对于有修改意见的项目,请您按修改意见及时调整计划书相关内容;如您对修改意见有异议,须在电子版计划书报送截止日期前向相关科学处提出。

请您将电子版计划书通过科学基金网络信息系统(<https://grants.nsfc.gov.cn>)提交,由依托单位审核后提交至自然科学基金委。自然科学基金委审核未通过者,将退回的电子版计划书修改后再行提交;审核通过者,打印纸质版计划书(一式两份,双面打印)并在项目负责人承诺栏签字,由依托单位科研、财务管理等部门审核、签章并在承诺栏加盖依托单位公章,且将申请书纸质签字盖章页订在其中一份计划书之后,一并报送至自然科学基金委项目材料接收工作组。纸质版计划书应当保证与审核通过的电子版计划书内容一致。**自然科学基金委将对申请书纸质签字盖章页进行审核,对存在问题的,允许依托单位进行一次修改或补齐。**

向自然科学基金委提交电子版计划书、报送纸质版计划书并补交申请书纸质签字盖章页截止时间节点如下:

1. **2024年9月9日16点:** 提交电子版计划书的截止时间;
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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	Semiochemicals from Domestic Cat Urine and Feces Reduce Use of Scratching Surfaces	ANIMALS 出版年: 2024 出版日期: FEB 卷期: 14 3 页码: - 文献号: 520 文献类型: Article		A类	华南农业大学	SCI	IF2-year=2.7 IF5-year=3.2 (2024)	农林科学 2区 Top 期刊: 否 (2025)
2	Dealing With Stress in Cats: What Is New About the Olfactory Strategy?	FRONTIERS IN VETERINARY SCIENCE 出版年: 2022 出版日期: JUL 15 卷期: 9 页码: - 文献号: 928943 文献类型: Review	1	A类	华南农业大学	SCI	IF2-year=3.2 IF5-year=3.5 (2022)	农林科学 2区 Top 期刊: 否 (2022)
3	Effects of species-relevant auditory stimuli on stress in cats exposed to novel environment	JOURNAL OF APPLIED ANIMAL WELFARE SCIENCE 出版年: 2025 出版日期: APR 3 卷期: 28 2 页码: 318-327	通讯作者	B类	华南农业大学	SCI	IF2-year=1.1 IF5-year=1.5 (2024)	农林科学 3区 Top 期刊: 否 (2025)

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4	Abrupt Dietary Change and Gradual Dietary Transition Impact Diarrheal Symptoms, Fecal Fermentation Characteristics, Microbiota, and Metabolic Profile in Healthy Puppies	ANIMALS 出版年: 2023 出版日期: APR 卷期: 13 8 页码: - 文献号: 1300 文献类型: Article	共同通讯作者 (倒数第二)	A类	华南农业大学	SCI	IF2-year=2.7 IF5-year=3.0 (2023)	农林科学 2区 Top 期刊: 否 (2023)
5	Dietary Strategies for Relieving Stress in Pet Dogs and Cats	ANTIOXIDANTS 出版年: 2023 出版日期: MAR 卷期: 12 3 页码: - 文献号: 545 文献类型: Review	共同通讯作者 (倒数第一)	A类	华南农业大学	SCI	IF2-year=6.0 IF5-year=6.7 (2023)	医学 2区 Top 期刊: 否 (2023)
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Article

Semiochemicals from Domestic Cat Urine and Feces Reduce Use of Scratching Surfaces

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Simple Summary: This study aimed to identify the major volatile compounds from cat urine and feces that differed between intact males and females and evaluate these molecules on cat scratching behavior. Results indicated that males had more 3-Mercapto-3-Methyl Butanol (MMB) in the urine and butanoic acid in the feces than females. And the mixture of MMB and butanoic acid had the potential to deter inappropriate scratching behavior in cats, which provided a new strategy for modifying feline destructive behavior in the home.

Abstract: Scratching is a natural behavior in cats but can cause damage to household furnishings. In this work, we sought to identify potential semiochemicals in the urine and feces of domestic cats that may modify cat scratching behavior. Sex differences among adult, intact cats were examined for volatile molecules in their urine ($n = 7$ females, 7 males) and feces ($n = 8$ females, 10 males) using gas chromatography-mass spectrometry (GC-MS). Males had seven times more 3-Mercapto-3-Methyl Butanol (MMB, $p < 0.001$) in the urine and 98% more butanoic acid ($p = 0.02$) in the feces than females. One mL of mineral oil without (i.e., control) or with MMB (0.1 $\mu\text{g/mL}$) and butanoic acid (100 $\mu\text{g/mL}$; i.e., treatment), which corresponds to the estimated biological amount in a single elimination from a male cat, were evaluated for their effectiveness in modifying the use of scratching devices by cats. Two identical cardboard standing scratchers, treated with either the control or the solution containing both semiochemicals delivered through a hanging cotton sock were placed side by side in a home/shelter environment. The preference test consisted of exposing individual cats ($n = 28$) to both scratchers for 20 min and recording the duration and frequency they interacted or scratched each scratcher. The semiochemical solution significantly decreased scratching time (21.19 ± 3.8 vs. 6.08 ± 3.8 s; $p < 0.001$) and interaction time (31.54 ± 5.9 vs. 12.90 ± 5.9 s; $p = 0.0001$) and tended to reduce scratching frequency (1.49 ± 0.3 vs. 0.82 ± 0.3 times; $p = 0.07$) compared with the control solution. The male-representative solution of MMB and butanoic acid was aversive to cats and might have future applications in protecting furniture from the destructive scratching or in modifying behavior of domestic cats.

Keywords: cat; urine; feces; semiochemical; scratching



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1. Introduction

Cats rely heavily on olfactory and chemical communications for individual identification, territory/route marking, and reproductive recognition and as an alarm mechanism [1–3]. Semiochemicals include any chemical signal given off by one individual that provides a message, altering the physiology and/or behavior of another individual, while pheromones are a type of semiochemicals for communication within the same species [4].

In the current study, we will also use semiochemical to describe those chemical communicative compounds, the exact biological functions of which have not yet been completely understood. Candidate pheromones have been reported in cats, with facial pheromones, mammary appeasing pheromone, and pedal interdigital semiochemicals being applied to solve behavioral problems, such as aggression in multi-cat households, urine marking, and anxiety in a novel environment [5,6]. Less work has been carried out on the identification and application of potential pheromones/semiochemicals from cat urine and feces, even though odor signals from cat eliminations may convey individual and/or sex information to conspecifics. For example, intact male cats and estrous female cats exhibit urine marking on vertical surfaces [7]. Feces were found unburied at the peripheral but not the core areas of their home range [8,9]. Feline and its volatile derivatives, including 3-mercapto-3-methyl butanol (MMB) and 3-mercapto-3-methylbutyl formate, can be detected in the urine of male cats and some small felids like bobcats and leopards [10]. With a characteristic sulfur odor, MMB is believed to be a male cat sex pheromone because a higher concentration of MMB is found in the urine of mature intact male cats compared to the urine of females or castrated males [10]. A recent study reported that feces from intact male adult cats had higher levels of propanoic acid, 4-methyl-pentanoic acid, and MMB than feces from non-estrus intact females [11]. The same volatile fatty acids were also detected at the perianal area and were suggested to serve the purpose of individual rather than sex identification in cats [11]. Few studies have evaluated cat urinary and fecal components as behavioral modifiers. Organic extracts from the urine of intact male cats induced sniffing and flehmen response in both laboratory male cats and bobcats of both sexes housed in outdoor enclosure [12]. A patent reported that the use of L-feline in cat litter attracted cats to eliminate in the litter box (U.S. Patent 20160309676A1), but MMB, the major feline metabolite, did not increase the use of litter box in cats [13].

Scratching, as a natural behavior for cats, is speculated to serve functions of nail polishing, extension of hind limbs, and providing visual and chemical signals for conspecific communications [14,15]. The ability to engage in scratching behavior is not only natural, but vital to the welfare of cats because one way to improve animal welfare is providing opportunities for that animal to engage in natural, species-typical behaviors. However, when cat scratching behavior is exhibited indoors on the furniture, it is often considered problematic by cat owners and referred as inappropriate scratching [16]. A common solution to this problem is to redirect this behavior to scratching devices. Successful scratching redirection depends on multiple factors, such as the scratcher type and its location in the household [17–19], individual cat preferences [20,21], and the attractiveness of the scratchers [6]. The use of herbal cat attractants such as catnip and/or silver vine to attract cats and increase playful behavior has been well documented [22]. Catnip and its extracted oil have been reported to induce scratching when placed on scratching devices in adult cats [6] but not in kittens less than 8 weeks old, possibly due to the immature behavioral development in young cats [20]. Application of pheromone/semiochemicals that can attract or deter cats to the provided scratchers might assist in reducing inappropriate scratching. The aim of the current study was to identify and quantify the major volatile compounds from cat urine and feces that differed between intact males and females, and to investigate the effect of these molecules on the use of preferred scratchers in cats.

2. Methods and Materials

2.1. Animals

All research was approved by the Texas Tech University Institutional Animal Care and Use Committee (Protocol 17010-02) prior to the beginning of the work. Healthy adult cats (≥ 1 year of age) were recruited from owners and the local shelter (Lubbock Animal Shelter and Adoption Center, Lubbock, TX, USA). Detailed information of the cats is shown in Appendix A Table A1. Cat owners were blind to the treatments. Ten intact male (M) and eight anestrus female (F) cats from the shelter were included for fecal collection. Seven intact males and seven intact females from the shelter were included for urine collection.

Cats in the shelter were housed individually in kennels (90 cm length \times 65 cm width \times 75 cm height) with segregated areas for defecation, feeding, and resting. They were fed the same diet (Hill's, Science Diet Optimal CareTM, premium natural cat food, Chicken Recipe, Hill's Pet Nutrition, Inc., Topeka, KS, USA) for at least three weeks before sample collection. A total of 28 cats, including 7 M and 7 F from the shelter and 7 neutered male (NM) and 7 spayed female (SF) household cats were included in the behavioral assay. Cats from households were freely kept inside the house and had access all the rooms. Cats from the shelter were not familiar with each other and so were the owned cats except for cats living in the same household.

2.2. Sample Collection

Samples were collected in December 2018 and none of the female cats showed signs of being in estrus (e.g., increased frequency of calling, lordosis, and other sexual behaviors). Unscented nonabsorbent litter (Petconfirm, Nancy Ridge Technology Center, San Diego, CA, USA) was used for urine collection. Fecal samples were collected either from the litter box or with the free-catching method. The researcher checked the litterbox every 30 min during the day (from 8:00 a.m. to 6:30 p.m.) for urine and feces. Contaminated samples were discarded. Urine was collected with centrifuge tubes (Conical Centrifuge Tubes, Falcon[®], Newport, TN, USA) and fecal samples with whirl-pack bags (Sigma-Aldrich, St Louis, MO, USA). Samples were placed on ice immediately after collection, transferred to the lab, and stored at -80°C until extraction.

2.3. Urine Extraction

Urine samples were thawed at room temperature for about 10 min. After thawing, NaCl was added until saturation to precipitate urinary proteins. The urine was then vortexed for 2 min and centrifuged at 3000 rpm, 15°C for 10 min. The supernatant was filtered using a $0.2\ \mu\text{m}$ cellulose Acetate (CA) syringe filter (Whatman, GE Healthcare, Hatfield, UK) and 1 mL of 200 ppm 4-ethyl phenol (97%; Sigma-Aldrich, USA) in ddH₂O as an internal standard solution was added to 9 mL of the filtered urine. The 10 mL mix was then vortexed and filtered through a reversed phase HyperSep C18 SPE cartridge (2 g bed weight; $40\text{--}60\ \mu\text{m}$ particle size; $60\ \text{\AA}$ pore size; 15 mL column capacity; Thermo Fisher Scientific, Bellefonte, PA, USA) at a steady rate of 0.25 mL/sec using a 12-valve vacuum manifold (Thermo Fisher Scientific, Bellefonte, PA, USA). The cartridge was previously conditioned with 10 mL of acetonitrile and 10 mL of ddH₂O. After filtration, the cartridge was washed with 10 mL ddH₂O, and centrifuged at 3000 rpm, at 15°C for 3 min to remove remaining water from the column. Molecules were subsequently eluted with 2 mL acetonitrile. To the eluted sample, 0.1 g NaCl and 0.1 g MgSO₄ were added to separate the aqueous layer and organic layer [23]. After 15 min, the upper layer was carefully transferred to a 2 mL screw-capped Gas Chromatography (GC)-vial for chemical analysis (Thermo Fisher Scientific, Bellefonte, PA, USA).

2.4. Feces Extraction

Two grams of thawed (previously frozen) feces was placed in a centrifuge tube with 0.5 μL of heptanoic acid ($>99\%$, Sigma-Aldrich, USA) as the internal standard and 5 mL of acetonitrile, and vortexed for one minute. Samples were centrifuged at 3000 rpm, 15°C for 10 min. Once centrifuged, the supernatant was filtered with a polytetrafluoroethylene (PTFE) $0.2\ \mu\text{m}$ syringe filter (VWR North America, Denver, CO, USA). Each of 0.1 g NaCl and MgSO₄ was added to the filtered solution to separate the aqueous layer from the organic layer [23]. After 15 min, the upper layer was carefully transferred to a GC-vial for chemical analysis. The litter used for sample collection was also extracted with acetonitrile and analyzed in the same way as feces to confirm that volatiles found in feces were not litter residues.

2.5. Gas Chromatography-Mass Spectrometry (GC-MS)

The fecal and urinary extracts were analyzed using GC-MS (Thermo Scientific Trace GC-MS Ultra connected to single quadrupole ISQ MS, Thermo Fisher Scientific Inc., San Jose, CA, USA) that was equipped with an SPB-PUFA capillary column (30 m length \times 0.25 mm i.d. \times 0.20 μ m; Sigma-Aldrich, USA) containing poly alkylene glycol-bonded stationary phase. One microliter of the sample was injected by an auto sampler in splitless mode into the injection port that was pre-heated to 250 °C. Helium was the carrier gas and flowed at 1.2 mL/min. The temperature program of the oven was as follows: 100 °C for 2 min, then increased by 7 °C/min to 220 °C, and then held at 220 °C for 15 min. The temperature of the mass spectrometer ion source was 225 °C during analysis. Mass spectra were recorded in electron-impact (EI) mode at 70 eV with a mass range from 40 to 450 amu. Compounds of interest were initially identified by matching the obtained mass spectra with a reference library in the instrument control software. The identities of peaks of interest were further confirmed by comparing the mass spectra and retention time with analytical standards, including MMB ($\geq 98\%$, Sigma-Aldrich, USA) and butanoic acid ($\geq 99\%$, Sigma-Aldrich, USA).

2.6. Behavioral Assay

Two socks rinsed either with the control or the volatile-containing solution, were hung on two identical cardboard standing scratchers. Both scratchers were made of a square wood board (44 cm \times 44 cm \times 1.5 cm) with a cardboard column (13 cm \times 77 cm) attached to it (Figure 1). For shelter cats, the experimental room was the feline interaction center at the shelter measured 4.39 m \times 3.63 m. The room had a standing bookcase, a table, a cat tree and some toys. The scratchers were placed next to the bookcase and the other items were removed from the nearby testing area. For household cats, the test occurred in one room at owner's place. The two scratchers were placed side by side in the room and sides were changed between trials. A camera (LINNSE, Camcorder Full HD, Amazon, Seattle, WA, USA) was placed in front of the scratchers to videotape the trials. Cats were introduced to the experimental room one at a time and they were given 20 min to interact with the scratchers. A habituation period was not specified; therefore, the 20 min testing period included the time for the cat to habituate to the room and explore both scratchers. The 20 min test was conducted only once for each cat. The testing area was relatively novel for shelter cats, which is the feline interaction center at the shelter with space for potential adopters to interact with candidate cats. As for owned cats, the test occurred in one of the rooms in the house, therefore, was familiar for the cats. A single trained and validated person watched the videos and recorded how long (duration) and how often (frequency) scratching and interactions exhibited to the scratchers occurred over the 20 min experimental period. Behavioral coding by the same person was validated in a previous study where use of scratchers in cats was studied in detail and high intra-observer agreements were found for these behavioral measurements (Spearman's rank-order correlation $\rho > 0.98$) [21]. The videos were watched using fast-forwarding at $\times 5$ times to find video segments of interest. Each short video was then watched in detail with continuous sampling in real time. The definition of scratching and interactions are presented in Table 1. The interactions mentioned in the current study referred to the total interactions, which included scratching and other non-scratching interactions.



Figure 1. An example of two cardboard standing scratchers with hanging socks treated control or volatile-containing solution, respectively. The scratchers had a square wood base that measured 44 cm × 44 cm × 1.5 cm and an attached round standing column (77 cm in height × 13 cm in diameter) that contained a center square wood stick (5 cm × 5 cm) covered with round stacked cardboard. One centimeter height of the cardboard column contained four pieces of the stacked cardboard.

Table 1. Behavioral measures exhibited to scratchers by cats [24].

Behavior	Definition
Scratching	With front claws extended, cat grips the material, and its claws withdraw and extend alternately.
Interaction	Other active behaviors exhibited by cats on the scratcher and the sock, including climbing on, pawing, kicking or rubbing against the scratcher; sniffing, licking, pawing, or rubbing against the sock.
-not scratching	Sum of scratching and non-scratching interactions.
Total interaction	
Preference Index (PI)	PI of specific measurement was calculated as the measurement of one scratcher divided by the measurement summed for both control and treated scratchers.

2.7. Statistical Analysis

All statistical analyses were conducted using SAS 9.4 (SAS Inst., Inc., Cary, NC, USA). Peak areas of volatiles from the chemical analysis were calculated using Qual Browser within Xcalibur (Thermo Fisher Scientific Inc., Waltham, MA, USA). To be included in the final analysis, the molecule was required to be present in all individuals of the same sex and not be presented in the litter used for sample collection. An area ratio for individual peaks was calculated as the peak area of the interested molecule divided by the peak area of the internal standard. Data of peak area ratio were examined for parametric analysis using the Shapiro–Wilks and Levene’s test. Data that met the assumption for normality were analyzed using the student t-test and the degree of freedom was adjusted with Satterthwaite approximation if the assumption of equal variance was not met. Wilcoxon rank sum test was applied to analyze the data when assumption of normality was not met. The sex effect was considered significant at $p \leq 0.05$. Calibration curves were made by running analytical standard at different concentrations using the same GC-MS protocol [25]. The equation of the curve was further used to estimate the fecal or urinary concentrations of the analytes of interest.

For the behavioral data, assumptions of parametrical analyses were not met based on the Shapiro–Wilks test and Levene’s test and were analyzed with the Wilcoxon signed rank test. Preference index (PI) data, calculated as described in Table 1, were transformed using the arcsine square root transformation and analyzed as repeated measures using GLIMMIX. The model included cat sex and treatment (TRT) and the interaction between sex and treatment (sex*TRT) with cat as random effect. Significant differences were considered at $p \leq 0.05$ and a tendency at $0.05 < p \leq 0.10$.

3. Results

3.1. Urine Volatiles

Six volatiles were identified in the urine of both intact male and female cats (Figure 2, Table 2). When the peak area ratio of these volatiles was compared between sex, male cats had higher 3-mercapto-3-methyl butanol (MMB, $p = 0.02$) and 4-heptanol, 2, 6-dimethyl ($p = 0.05$) in their urine than females (Table 3).

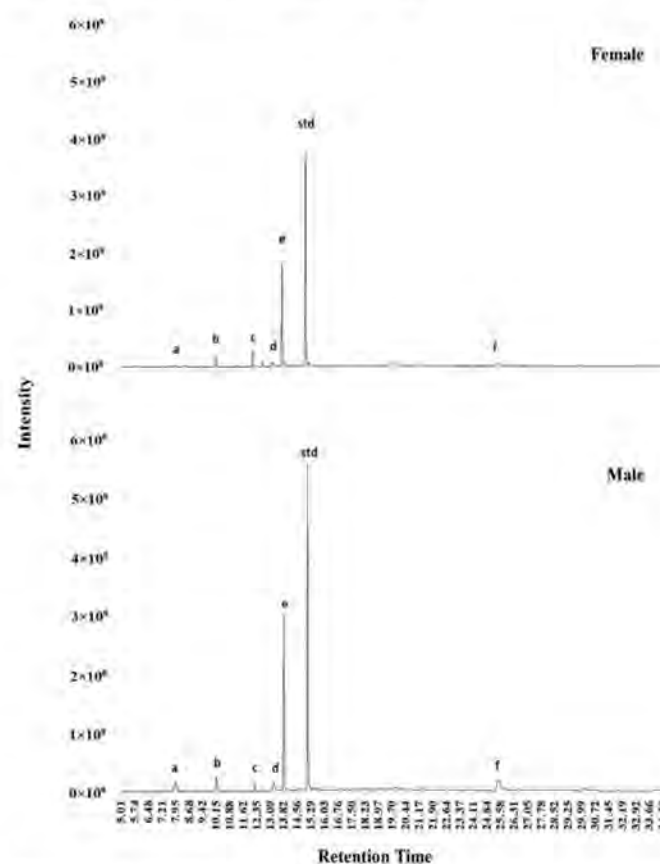


Figure 2. Representative chromatogram of volatiles in the urine of male and female cats. Letters a–f correspond to the candidate molecules in Table 2. Std: standard.

Table 2. Major volatiles in the urine of cats.

Peak ^a	RT	Candidate	Formula	<i>m/z</i> ^b	Matching ^c
a	7.92	3-Mercapto-3-Methyl Butanol *	C ₅ H ₁₂ OS	69	92.15%
b	10.17	2-4-Dimethyl-Benzaldehyde	C ₉ H ₁₀ O	133	23.48%
c	12.24	Cis-Jasmone	C ₁₁ H ₁₆ O	79	43.04%
d	13.33	2,3-Dethydropiperidin-6-one	C ₅ H ₇ NO	97	49.14%
e	13.82	P-Cresol/4-Methyl-Phenol *	C ₇ H ₈ O	107	31.30%
f	25.64	4-Heptanol, 2, 6-Dimethyl	C ₉ H ₂₀ O	69	35.85%

^a Letter match with peaks in Figure 2. RT: retention time. ^b Based on peak of molecular mass spectrum. ^c Matching rate between obtained mass spectrum and NIST library database. * Volatile confirmed with analytical standard.

Table 3. Peak area ratio of volatiles in the urine of intact male and female cats.

Candidate Molecule	Female	Male	SE ^a	Df ^b	Statistics ^c	p-Value ^d
3-Mercapto-3-Methyl Butanol	0.02	0.14	0.02	6	−3.04	0.02
2-4-Dimethyl-Benzaldehyde	0.11	0.09	0.01	12	0.92	0.38
Cis-Jasmone	0.09	0.04	0.02	7	1.42	0.19
2,3-Dethydropiperidin-6-one	0.06	0.08	0.02	12	−0.48	0.64
P-Cresol/4-Methyl-Phenol	0.59	0.65	0.15	8	−0.27	0.79
4-Heptanol, 2, 6-Dimethyl	0.06	0.24	0.04	7	−2.43	0.05

^a SE, standard error of least square means. ^b Df, degree of freedom (Satterthwaite approximation was applied when data were not homoscedastic). ^{c,d} Test statistics and the significant level of sex effect based on the student *t*-test (*n* = 7 females, *n* = 7 males).

3.2. Feces Volatiles

Twenty major volatiles were identified in the feces of both intact male and female cats (Figure 3, Table 4). When the peak area ratio of these volatiles was compared between sexes, male cats had higher butanoic acid (*p* = 0.04) and lower 9-octadecenoic acid (*Z*)-ethyl ester (*p* = 0.03) in their feces compared to female cats (Table 5).

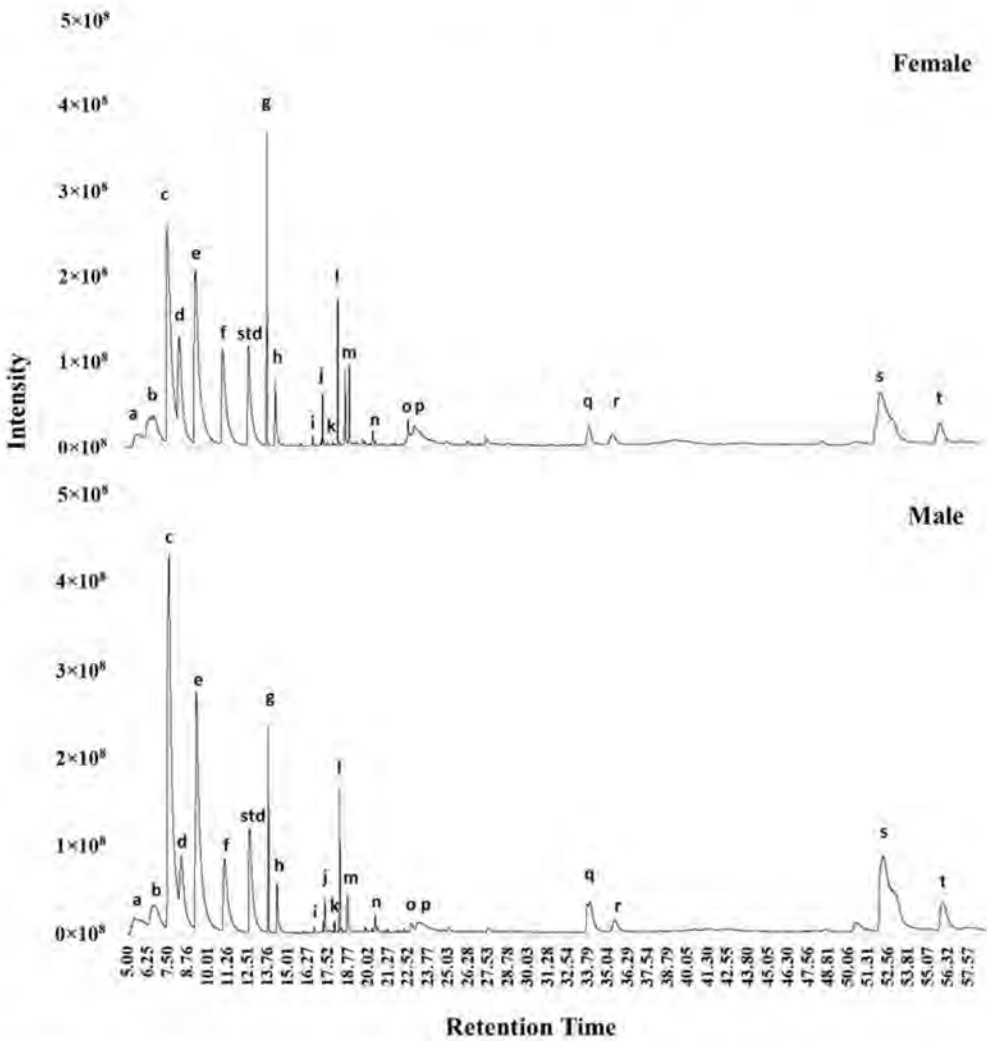


Figure 3. Representative chromatogram of volatiles in the feces of female and male cats. Letters a–t correspond to the candidate molecules in Table 4. Std: standard.

Table 4. Major volatiles in the feces of cats.

Peak ^a	RT	Candidate Molecules	Formula	m/z ^b	Matching ^c
a	5.50	Acetic acid *	C ₂ H ₄ O ₂	60	57.89%
b	6.50	Propanoic acid	C ₃ H ₆ O ₂	74	34.47%
c	7.59	Butanoic acid/butyric acid *	C ₄ H ₈ O ₂	60	78.24%
d	8.35	Isovaleric acid/3-methyl-butanoic acid *	C ₅ H ₁₀ O ₂	60	69.56%
e	9.32	Pentanoic acid	C ₅ H ₁₀ O ₂	60	46.37%
f	11.13	Hexanoic acid	C ₆ H ₁₂ O ₂	60	80.65%
g	13.82	P-cresol/4-methyl-phenol *	C ₇ H ₈ O	107	30.73%
h	14.39	2-Piperidinone	C ₅ H ₉ NO	99	76.51%
i	16.71	4-Methyl-5-thiazoleethanol	C ₆ H ₉ NOS	112	67.16%
j	17.27	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	88	22.48%
k	17.98	Q-Docecalactone	C ₁₂ H ₂₂ O	85	40.66%
l	18.30	1-H indole	C ₈ H ₇ N	117	31.36%
m	18.77	Hexadecen-1-ol, trans-9-	C ₁₆ H ₃₂ O	55	14.35%
n	20.56	9-Octadecenoic acid (Z)- ethyl ester	C ₂₀ H ₃₈ O ₂	55	14.97%
o	22.73	Carbonic acid, ethyl octadecyl ester	C ₂₁ H ₄₂ O ₃	91	17.64%
p	23.11	Propanedioic acid, phenol	C ₉ H ₈ O ₄	91	35.60%
q	34.11	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	55	14.35%
r	35.62	Oleic acid/8-Octadecenoc acid	C ₁₈ H ₃₄ O ₂	55	17.54%
s	52.39	cis-Vaccenic acid	C ₁₈ H ₃₄ O ₂	55	16.37%
t	56.24	Lenoelaidic acid	C ₁₈ H ₃₂ O ₂	67	10.89%

^a Letter match peaks in Figure 3. RT = retention time. ^b Based on peak of molecular mass spectrum. ^c Matching between obtained mass spectrum and NIST library database. * Volatile confirmed with analytical standard.

Table 5. Peak area ratio of volatiles of feces in intact male and female cats.

Candidate Molecule	Female	Male	SE ^a	Statistics ^b	p-Value ^c
Acetic acid *	0.40	0.52	0.07	−1.07	0.14
Propanoic acid	0.66	0.56	0.10	0.70	0.49
Butanoic acid/butyric acid	2.78	4.85	0.63	−2.28	0.04
Isovaleric acid/3-methyl-butanoic acid	0.93	0.68	0.10	1.76	0.10
Pentanoic acid	2.40	2.81	0.43	−0.61	0.55
Hexanoic acid	1.22	0.93	0.21	0.98	0.34
P-cresol/4-methyl-phenol *	0.53	0.33	0.12	1.69	0.09
2-Piperidinone *	0.25	0.20	0.05	0.62	0.53
4-Methyl-5-thiazoleethanol	0.02	0.02	0.00	0.86	0.40
Hexadecanoic acid, ethyl ester *	0.09	0.08	0.03	0.36	0.72
Q-Docecalactone *	0.01	0.02	0.00	−1.95	0.05
1-H indole *	0.24	0.22	0.07	0.27	0.79
Hexadecen-1-ol, trans-9- *	0.16	0.12	0.04	0.89	0.37
9-Octadecenoic acid (Z)-athyl ester *	0.13	0.04	0.03	2.22	0.03
Carbonic acid, ethyl octadecyl ester *	0.31	0.29	0.01	0.20	0.84
Propanedioic acid, phenol *	0.23	0.44	0.11	−0.71	0.48
Hexadecanoic acid	0.15	0.15	0.03	0.02	0.99
Oleic acid/8-Octadecenoc acid *	1.76	2.41	0.43	−1.16	0.25
cis-Vaccenic acid *	0.40	0.50	0.14	−0.62	0.53
Lenoelaidic acid *	0.40	0.52	0.07	−1.07	0.14

^a SE, standard error of least square means. ^{b,c} Statistics and significance level of sex difference ($n = 8$ females, $n = 10$ males) based on the student *t*-test or Wilcoxon rank sum test. * Data do not meet the assumption for normality and the Wilcoxon rank sum test was used.

Of the four volatiles differed in the urine and feces between male and female cats, MMB (the recognized male sex marking pheromone) and butanoic acid (abundant in the feces) were selected for further investigation. The other two molecules were not selected because their identities were not able to be confirmed using analytical standards. Specifically, standard MMB was dissolved in acetonitrile to make an original standard solution in 500 ppm, then diluted to 3.9 ppm with twofold serial dilution method. The standard solution of butanoic acid ranged from 5000 ppm to 62.5 ppm in acetonitrile, also using twofold serial dilutions. The calibration curve for MMB and butanoic acid were built with the concentrations of different standard solution and their corresponding peak areas ($R^2 \geq 0.99$). Content of MMB and butanoic acid (corrected for the fecal dry matter content) in the urinary and fecal samples were estimated with calibration curves (Table 6). Both MMB and butanoic acid were at higher ($p < 0.05$) concentrations in male samples than in female samples (Table 6).

Table 6. Estimated concentrations of candidate molecules in cat urine and feces.

Molecules	Female CI ^a	Male CI ^a	Statistics ^b	p-Value ^c
MMB (µg/mL urine)	0.00–1.63	2.22–10.99	3.18	<0.001
Butanoic Acid (µg/g DM)	5050–18,348	16271–30,090	2.70	0.02

^a Lower and upper value of the 95% confidence interval (CI). ^{b,c} Statistics and significance level of sex difference based on the student *t*-test (MMB, *n* = 7 females and 7 males; butanoic acid, *n* = 8 females and 10 males). DM: dry matter; MMB: 3-Mercapto-3-Methyl Butanol.

3.3. Behavioral Assay

The estimated amounts semiochemicals from one elimination of urine and feces of an intact male cat (i.e., approximately 15 mL urine and 5 g dry matter feces) were dissolved in one milliliter of mineral oil (i.e., 0.1 µg/mL of MMB and 100 µg/mL of butanoic acid) and used as the treatment solution for the behavioral assay. The control solution was one milliliter of mineral oil without volatiles added.

Cats preferred ($p \leq 0.07$) the control scratcher over the volatile-treated scratcher when main treatment effect was tested using Wilcoxon signed rank test. Longer duration of scratching (21.19 ± 3.8 s vs. 6.08 ± 3.8 s; $p < 0.0001$) and interaction (31.54 ± 5.9 s vs. 12.90 ± 5.9 s; $p = 0.0001$) and a trend of higher scratching frequency (1.49 ± 0.3 times vs. 0.82 ± 0.3 times; $p = 0.07$) were observed during the 20min period for the control scratcher compared to the volatile-treated scratcher (Figure 4).

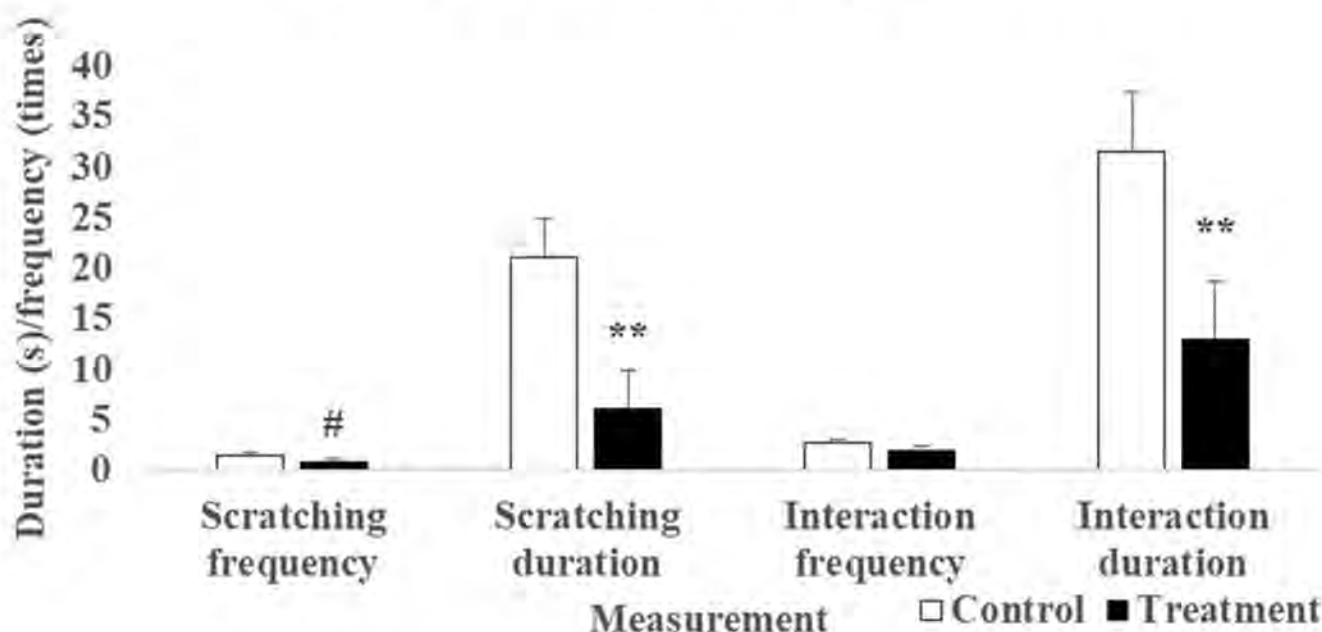


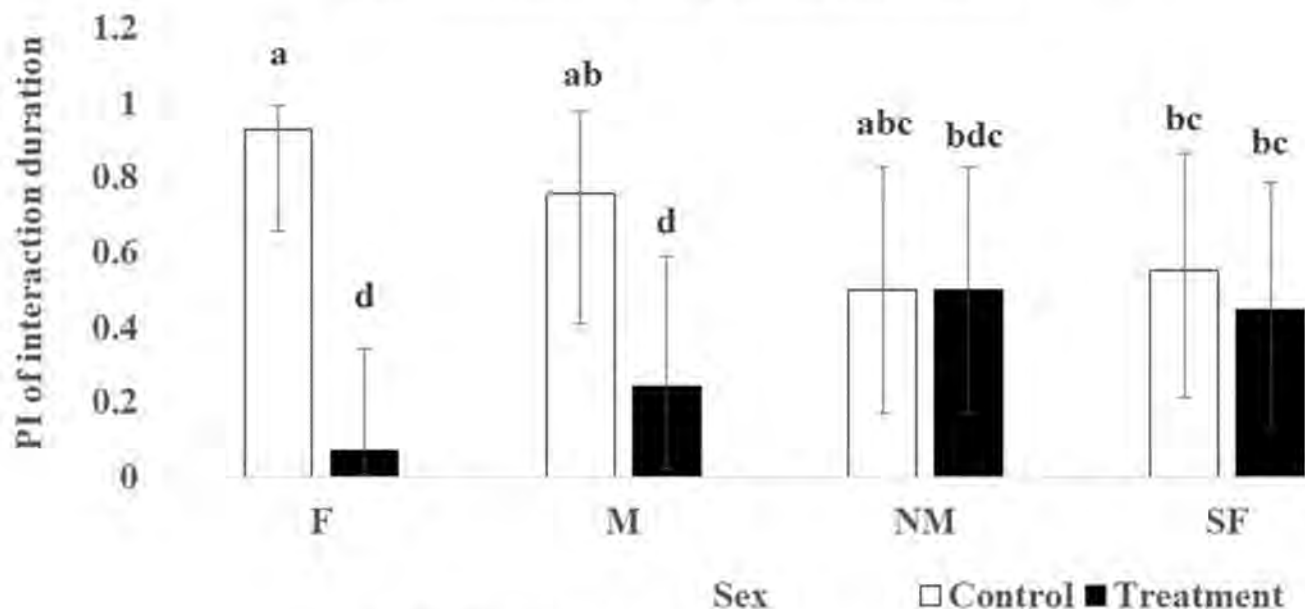
Figure 4. Main treatment effect of treated solution, mix of butanoic acid, and 3-Mercapto-3-Methyl Butanol (MMB) dissolved in mineral oil, compared to control solution (mineral oil) on the behavioral measurements obtained from the standing cardboard scratchers in adult cats (*n* = 28 cats). #, ** Least squares mean differed between treatment groups at $p \leq 0.10$ and $p \leq 0.01$ based on the Wilcoxon signed rank test.

No sex effect was observed for preference index (PI)s of all behavioral measurements (i.e., duration and frequency of scratching and interaction). Focusing on the main treatment effect, PIs for scratching duration and frequency of the control scratcher were higher ($p < 0.05$) than those with the treated scratcher (Table 7), indicating a preference for the control scratcher. The treatment by sex interaction was observed ($p = 0.03$) for the PI of interaction duration, being significantly higher for the control scratcher than the treated scratcher in intact (F, 0.93 vs. 0.07; M, 0.76 vs. 0.24 for control and treatment, respectively) but not among castrated cats ($p > 0.10$; Figure 5).

Table 7. The effects of volatiles (i.e., butanoic acid and MMB) on the use of scratchers based on preference index of behavioral measurements in adult cats.

Measurement Preference Index (PI) ^a	Treatment		<i>p</i> -Value ^b	
	Control	Volatile	TRT	TRT*sex
Scratching duration PI	0.63	0.10	0.0007	0.29
(95% CI)	0.08 ^b (0.41, 0.82)	0.28 ^a (0.01, 0.27)		
Interaction duration PI	0.70	0.30	0.002	0.03
(95% CI)	0.12 ^b (0.53, 0.85)	0.32 ^a (0.15, 0.47)		
Scratching frequency PI	0.56	0.14	0.006	0.14
(95% CI)	0.09 ^b (0.34, 0.77)	0.27 ^a (0.03, 0.33)		
Interaction frequency PI	0.61	0.39	0.08	0.08
(95% CI)	0.17 ^b (0.43, 0.77)	0.29 ^a (0.27, 0.57)		

^a Reverse-transformed least squares means and 95% confidence interval (CI) from arcsine square root transformation. ^b Significant levels of treatment (TRT) effect and treatment by sex interaction (TRT*sex) on the arcsine square root transformed data ($n = 28$, 7 intact and castrated males and females).

**Figure 5.** The effects of the interaction between sex, female (F, $n = 7$), male (M, $n = 7$), spayed female (SF, $n = 7$), and neutered male (NM, $n = 7$), and treatment (i.e., control versus volatile-treated standing cardboard scratcher) on the back-transformed preference index (PI) of interaction duration from arcsine square root transformation. The error bars indicated 95% confidence interval for the back-transformed data. ^{a,b,c,d} Least squares mean of arcsine square root transformed data differed within each measurement with different letters.

4. Discussion

We found fewer volatiles in the urine samples compared to what has been reported by others [10,12,26]. However, software allowed us to subtract male from female data and show the few differences between the sexes. Difference in the methodologies might also contribute to this inconsistency. The extraction method used for feces did not work out for urine samples, and the step of filtration through a reversed phase HyperSep C18 SPE cartridge was added to urine extraction. We identified and quantified 3-Mercapto-3-Methyl Butanol (MMB) and p-cresol (4-Methyl-Phenol). The relative abundance of MMB compared between male and female cats (i.e., approximately 1 to 8) agreed with those reported by Miyazaki et al. (2006) [10]. Few studies have evaluated the concentration of MMB in the

cat urine, but MMB was described as having a typical male cat odor at concentrations of 0.01–1 ppm [27], significantly lower than the MMB estimated in male and female cats in this study. All the samples were collected and transferred to the laboratory on ice and stored at -80°C for no more than a week until analysis. The elapsed time (~4 h) between the shelter and laboratory could contribute to the higher MMB concentrations. Cat urine develops the typical odor peaking in intensity by ~12 to 24 h, which is a result of MMB being decomposed from felinine, a urinary sulphur amino acid by microbial activity and/or oxidation by the air [10,27]. The excretion rate of felinine in the urine was reported to be 95 mg/day in intact male cats, which was higher than intact females (19 mg/day) [28]. The high excretion rate of felinine requires higher intake of dietary sulphur amino acids (e.g., cysteine). Therefore, MMB as the main derivative of felinine may serve as an “honest signal” of hunting skills in the urine of male cats since muscle meat is the main resource for cysteine [28].

Major volatiles identified in cat feces by the current study (e.g., short chain fatty acids, *p*-cresol, indole) were also reported by Miyazaki et al. (2018) [11]. MMB was also identified in the cat feces and in higher concentration in intact males (50 ng/g wet feces) than females [11]. But we could not detect MMB from the feces. Miyazaki et al. (2018) also reported more propanoic acid and 4-methyl pentanoic acid existing in male feces than female feces. Our results disagreed with them in that butanoic acid was found to be 74.5% higher in male feces than in female feces and other fatty acid did not differ between sexes. Cats were able to distinguish between two sets of mixed fatty acids in different ratios that mimic the compositions of fatty acids in the feces of two individual males [11]. This evidence suggests to us that fatty acids may be involved in individual and sexual identification in cats. A role of fatty acids in olfactory communication is also seen with the cat facial and mammary pheromones, both containing a variety of volatile fatty acids [5]. The concentrations of butanoic acid reported in our cat feces were slightly higher but close to what others have reported [29,30], possibly due to a higher starch and fermentable fiber content in the diet for our cats [29,31]. Future studies are needed to better understand the interaction between sex and diet on the volatile fatty acids in cat feces.

The information conveyed by urine spraying in cats is not completely understood but has been suggested to involve sexual and identity communication, territory/route marking, and stress [32,33]. Flehmen response is often induced in a cat after it sniffs cat urine, especially the fresh ones from unfamiliar intact males [12]. Urinary extracts of male cats were shown to attract cats and bobcats, but deterred them from urination and defecation in the testing area [12]. Fecal marking is barely studied in cats. Heavier male cats tend to bury their feces closer to the core area than lighter males, indicating that defecating behaviors in male cats may reveal information of social ranking [8]. Only a few studies have investigated the application of specific urinary or fecal volatiles in modifying cat behaviors, possibly due to their pungent odor. We reported here the application of MMB and butanoic acid reduced scratching and total interactions exhibited to the scratcher in cats. None of the cats in the present study exhibited a flehmen response during the behavioral assay even though they did sniff both treatments. Miyazaki et al. (2016) reported that both male and female cats showed interests in MMB but not to the pure felinine, but information about the dosage of MMB used and whether MMB induced flehmen behavior or just increased sniffing in those cats was not detailed. One study reported that MMB added to the litter at 50 $\mu\text{g}/\text{kg}$ of litter did not alter the use of litterbox in cats [13]. An explanation for the lack of flehmen response in our cats to the solution containing MMB and butanoic acid is that MMB alone was not enough for representing the urine since urine extracts usually contain MMB and other sulphur-containing volatiles [10]. Alternatively, interaction between butanoic acid and MMB blocked the flehmen response of MMB. Because MMB alone was neutral or attractive to cats, we did not test MMB alone, rather we attempted to reconstitute the active molecules that are unique to male cat excretions. Testing MMB and butanoic acid separately in the future may verify this latter hypothesis. The role of MMB in contexts other than sexual and territorial communication also requires further study. In addition, most cats in

the current study finished interacting with both scratchers within the first 10 min of the testing period, therefore suggesting shorter assessments possibly be utilized in the future to make trials easier for both cats and researchers.

The treatment solution with MMB and butanoic acid was assumed to represent a male odor in cats. Cats scratch in the area they live and along the daily pathway to mark a looser home range [7], after sleeping [24], and near feeding and defecation area [5]. Free-roaming cats scratch more often in the presence of other cats [34], but they do not tend to over-mark urinary and fecal marks of others [12]. Cats may also prefer to scratch on the control scratcher nearby but not right on the scratcher treated with male-representative volatiles. Alternatively, cats may avoid the treated-scratcher to avoid confrontation with other cats and a recent hunting area because free-roaming cats whether live a solitary life or form highly gregarious social colonies with one another [35], use urinary and fecal marks to separate themselves apart temporally and spatially during hunting and social communication [34]. Differed responses to the treatment solution between intact and castrated cats were observed, in that interaction duration was reduced in intact but not in castrated cats. Little literature is available for explaining the result. Intact males and anestrus females might show avoidance to the male-representative volatiles to reduce conflicts from confronting a male cat. Castrated animals might exhibit less avoidance due to a lack of reproductive motivation and the hormonal change after gonadectomy [36]. Sex hormones can affect the olfactory sensitivity to multiple odorants [37]. Alternatively, environmental and dietary differences between the two groups of cats may have also played a role since the factors of shelter versus home-reared are completely confounded. A relatively impoverished environment may alter the stress hormones [38] and body chemicals in shelter cats. The castrated cats were tested in homes, at their owner's place, and the intact cats in the play room at the shelter. Shelter cats might be more vigilant and less explorative at the play room as it is a novel environment [39]. This is less possible because the total interaction frequency with both scratchers were not much different between shelter and pet cats (5.36 ± 0.37 times vs. 3.79 ± 2.12 times, $p = 0.32$; Wilcoxon rank sum test). In contrast, pet cats exhibited less interaction in duration with both scratchers compared to cats in the shelter (69.1 ± 70.3 s vs. 21.5 ± 17.8 s; Wilcoxon rank sum test). In addition, no significant sex effects were observed on other behavioral measures (i.e., scratching frequency and duration). During the test, apparent stressful behaviors (e.g., hiding, escape attempts, agonistic vocalization) were not observed for both shelter and owned cats as well [40]. Urinary and fecal samples were collected from shelter cats put on the same diet, and concentrations of chemicals in the treatment solution were determined based on their contents in the samples from shelter male cats. The pet cats in our study were not standardized for their diet, imposing diet as a potential factor for the behavioral difference between shelter and pet cats. In the future, it would be interesting to study how diets with varied ingredients affect urinary and fecal volatiles in cats and if volatiles mimicking body chemicals from cats on one diet will cause behavioral differences in these cats and cats on another diet. Meanwhile, the treatment solution tested in the current study was reported as detectable and aversive upon approaching (<0.5 m) by most owners. Therefore, it would be useful to investigate the minimal concentration of treatment solution that are unnoticeable or acceptable to humans while still protect furniture and other objects from being scratched. From evolutionary considerations, feline social rank and testosterone levels might correlate with the concentration of the semiochemicals in the urine and feces of intact males [10,27]. Future studies could explore the correlation between these variables and test out the intraspecific effects of different combination of candidate volatiles from cat urine and feces in various concentrations.

5. Conclusions

In summary, intact male cats had higher MMB and butanoic acid in their urine and feces compared to intact females. The solution containing the estimated amounts of MMB and butanoic acid from one elimination of a male cat had aversive effects on the use of

scratchers, especially in intact cats. In the future, it will be worthwhile to test the two volatiles separately and investigate their potential behavior-modifying effects in other contexts (e.g., use of litter box). The mixed solution of MMB and butanoic acid may have an application in protecting furniture or other objects or areas from being scratched by cats, which has the potential to inform recommendations that could increase cat welfare and improve human–cat relationships. For example, the semiochemical solution can be applied to a cloth and put over the area where the owner wants to decrease inappropriate scratching (e.g., side of the couch). At the same time, the owner can positively reinforce use of the cat scratcher to redirect the cat’s scratching behavior (e.g., scratcher with catnip/silver vine) [21].

Author Contributions: J.J.M. provided oversight of study design and data analyses. L.Z. was involved with contacting the owners and the local shelter and conducting the trials and data collection and analyses, which was assisted by E.O.A.-R. The first draft of the manuscript was written by L.Z. and Z.B. and K.S. helped with the revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: All research was approved by the Texas Tech University Institutional Animal Care and Use Committee (Protocol 17010-02) prior to the beginning of the work.

Informed Consent Statement: Informed consent was obtained from all the authors and participants in this experiment.

Data Availability Statement: Data is contained within the article.

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Conflicts of Interest: The authors declare that they have no real or perceived conflicts of interests.

Appendix A

Table A1. Information of cats included in the study.

Owner	Cat	Sex ^a	Age (yrs)	Multi-Cat House	Dog in House	Outdoor Access	Kids in House	Sample Collection (SC) or Behavioral Test (BT)
Lingna	Poo	SF	1	No	Yes	No	No	BT
Andrea	Jackie	SF	2	Yes	No	Yes	No	BT
	Midnight	NM	3					
	Harley	SF	1					
	Marble	NM	1					
	Buggy	SF	1					
	Tigers	NM	15					
Neighbor	Mittens	SF	2	Yes	No	No	Yes	BT
	Wisper	SF	5					
	Bonbon	NM	9					
	Fattie	NM	10					
Tristin	Footie	NM	10	Yes	No	No	No	BT
	Bella	SF	1					
	Slick	NM	1					

Table A1. Cont.

Owner	Cat	Sex ^a	Age (yrs)	Multi-Cat House	Dog in House	Outdoor Access	Kids in House	Sample Collection (SC) or Behavioral Test (BT)
Shelter	Smoky	M	5					BT
	Leroy	M	2					BT
	Deb	F	3					BT
	Harvey	F	1					BT
	Alley	M	1					BT
	Mickey	M	3					BT and SC (urine and feces)
	Butterzinger	F	1					BT and SC (urine)
	Fancy	F	3					SC (feces)
	Mickey2	M	1					BT
	Bitty	M	1					BT and SC (urine and feces)
	Carrie	F	3					BT and SC (urine)
	Bengency	M	2					BT
	Shairy	F	1					BT
	Mom	F	3					BT
	Zeke	M	1					SC (urine and feces)
	Karate	M	1					SC (feces)
	Pep	M	2					SC (urine and feces)
	Sundra	M	3					SC (feces)
	Loki	M	1					SC (urine and feces)
	Duke	M	1					SC (urine and feces)
	Bruce	M	3					SC (urine and feces)
	JoJo	F	2					SC (urine)
	Sophira	F	2					SC (urine)
	Jetson	F	2					SC (urine)
	Dean	M	3					SC (feces)
	Cat13	F	1					SC (feces)
	Zoie	F	1					SC (urine and feces)
	Elsa	F	2					SC (feces)
	Heidi	F	1					SC (feces)
	Eleven	F	1					SC (urine and feces)
	Sara	F	2					SC (feces)
	Suize	F	3					SC (feces)

^a F: intact female; M: intact male; NM: neutered male; SF: spayed female.

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Dealing With Stress in Cats: What Is New About the Olfactory Strategy?

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Domestic cats are descended from solitary wild species and rely heavily on the olfaction system and chemical signals for daily activities. Cats kept as companion animals may experience stress due to a lack of predictability in their physical or social environment. The olfactory system is intimately connected to the brain regions controlling stress response, thus providing unique opportunities for olfactory strategies to modify stress and related behavioral problems in cats. However, the olfactory intervention of stress in cats has been mainly focused on several analog chemical signals and studies often provide inconsistent and non-replicable results. Supportive evidence in the literature for the potentially effective olfactory stimuli (e.g., cheek and mammary gland secretions, and plant attractants) in treating stress in cats was reviewed. Limitations with some of the work and critical considerations from studies with natural or negative results were discussed as well. Current findings sometimes constitute weak evidence of a reproducible effect of cat odor therapy for stress. The welfare application of an olfactory stimulus in stress alleviation requires a better understanding of its biological function in cats and the mechanisms at play, which may be achieved in future studies through methodological improvement (e.g., experiment pre-registration and appropriate control setting) and in-depth investigation with modern techniques that integrate multisource data. Contributions from individual and environmental differences should be considered for the stress response of a single cat and its sensitivity to olfactory manipulation. Olfactory strategies customized for specific contexts and individual cats can be more effective in improving the welfare of cats in various stressful conditions.

Keywords: cat stress, welfare, olfaction, pheromone, chemical signals

INTRODUCTION

Domestic cats are one of the most popular pets worldwide. At present, people in cities live a fast-paced lifestyle but are in need of companionship. Cats offer an outlet for nurturing with relatively lower maintenance requirements (e.g., some degree of independence, less space, and social commitment) (1). However, cats are not traditionally kept for companionship, and changes in lifestyle and environmental predictability have exposed, especially indoor cats, to many restraints and aversive stimuli. Stress is an important issue in cats with serious health and behavioral consequences (1, 2). Either being part of the normal reaction to aversive stimuli but considered inappropriate by owners or indeed problematic, behavioral problems are among the top risk factors for cats to be relinquished and euthanized in the shelter (1, 3–6).

In China, escaped or abandoned cats could contribute to the population of free-ranging cats that exert a huge threat to the local wildlife populations and diversity (7). The study of stress and related behavioral problems in cats has the significance of promoting cat wellbeing, human–cat relationship, and healthier ecosystem as a result of reduced abandonment of owned cats. Management of stress in cats often includes the provision of environmental enrichment, such as hiding enrichment (8–11). Dietary supplementation of functional ingredients and prescribed antidepressants were also reported in pet dogs and cats (12, 13) which are beyond the scope of the current review. Similar to many other carnivore species, cats rely heavily on their olfactory system to explore the physical and social environment. Even now considered a facultatively social species, cats often chose to live a solitary life with enough space and resources (14, 15). Chemical communication is, therefore, involved in many inter-cat activities, such as territory marking, reproduction, and individual recognition (16, 17). The olfactory system can serve as a potential target for the modulation of stress response due to its intimate connection with the central limbic system (18). Olfactory stimuli developed with scientific guidance may provide many opportunities for stress management in cats. By integrating current research on olfactory intervention on different stress markers, the goal of the current review is to evaluate the effectiveness of different olfactory stimuli on the regulation of stress in cats and provide insights into future research directions in this field.

STRESS RESPONSE, TRIGGERS, AND BEHAVIORAL SIGNS OF STRESS IN CATS

Triggers of Stress

Cats are constantly adapting to their living environment, detecting and interpreting the various stimuli as either being neutral, positive, or aversive. Aversive stimuli or stressors can be classified into two major categories (i.e., physical and psychological) that are perceived and processed differently in the brain and involve the recruitment of distinctive amygdala and noradrenergic cell groups (19, 20). Psychological stressors, defined as stimuli that exert a threat or are anticipated as threats are indicated as more potent than physical triggers such as body infection or hemorrhage (19). Most of the stressful stimuli in captive cats are situations where either predictability is lacking or the need of a cat is not satisfied (2, 21–23). Some common triggers of stress in cats are summarized in **Table 1**. For example, the exposure to a novel environment and social interaction (21, 42) and change in caretaking routine (32, 33, 44) is controlled by owners or working staff but not the cat. Cats that have no outdoor access or are in lengthy sheltering may not be meeting their needs of expressing natural behavior and social interaction (4, 58).

Stress Response

A real or perceived stressor in the environment triggers the rapid activation of two major components involved in stress response, the sympathetic-adrenal-medullar (SAM) and hypothalamic-pituitary-adrenal (HPA) axis, and the release of mediating hormones to co-ordinate the physiological and behavioral

adaptations and restoring of homeostasis (**Figure 1**) (2). In the SAM axis, the activation of the posterior hypothalamus stimulates the adrenal gland medulla *via* the splanchnic nerve and causes the release of the fast-acting catecholamines, adrenaline and noradrenaline (NA), which activate the “flight or fight” reaction (36) and mediate the first signs of the stress response such as elevated blood pressure (38) and increased heart rate and respiratory rate (39). The sympathetic output will then be decreased by the parasympathetic nervous system. In parallel, the HPA axis is also activated. Corticotropin-releasing hormone (CRH) is secreted together with arginine vasopressin from the paraventricular nucleus (PVN) in the hypothalamus and acts on the posterior pituitary gland, causing the release of adrenocorticotrophic hormone (ACTH) which stimulates the adrenal cortex to release glucocorticoid hormones, predominantly cortisol to the circulation in cats. An increase in cortisol secretion is commonly reported in cats exposed to acute stressors, such as bath (36, 37) and hospital visit (26). The elevated cortisol then exerts negative feedback on the pituitary and hypothalamus to prevent further release of CRH and ACTH. Glucocorticoids affect a vast range of processes pertaining to metabolism, immune function, and brain activity, temporally shutting down systems not emergent for immediate survival, such as digestion and reproduction (2). The response to acute stress declines after successful adaptation and/or the removal of the stressor. In chronic stress where the animal is subjected to prolonged stress response, dysregulation of the HPA axis (33) and pathologies (e.g., ulcer and infection) can occur, resulting in compromised welfare (2, 63). It had been suggested that the common emotions accompanying the stress response include fear and chronic state of anxiety (2). Animals can also exhibit behavioral disorders due to chronic stress. In humans and rodent species, chronic stress is linked to the development of mental disorders such as depression (18). Monoaminergic neurons such as dopamine (DA), NA, and serotonin (5-HT) that project to the pre-frontal cortex are initially enhanced to keep the function of the pre-frontal cortex low during the “flight or fight” response. Sustained or intermittent stress exhausts the monoamine neurotransmitters and causes a decline in neuron function, which has a series of consequences, in the limbic DAergic neurons causing the loss of pleasure, and in the amygdala and hippocampus resulting in memory and emotional dysfunction. Collectively, the incidence of mental and behavioral disorders is increased when the brain function deteriorates under chronic stress. In aging cats, stress contributes to the development or worsening of cognitive dysfunction (64).

Behavioral Signs of Stress in Cats

Physiological and behavioral measures, as well as health indicators, are the most important parameters used for evaluating stress in cats (65). However, a comprehensive and valid welfare tool is not currently available. Methodological restrictions and difficulties (e.g., short of non-invasive, field-verified monitoring devices, or testing methods) in obtaining accurate physiological data in various settings have rendered behavior a particularly common welfare-assessing tool (65, 66). Behavioral exhibition of stress in cats is summarized in **Table 1**. Overall, a preference for

TABLE 1 | Common behavioral signs and triggers of stress in cats.

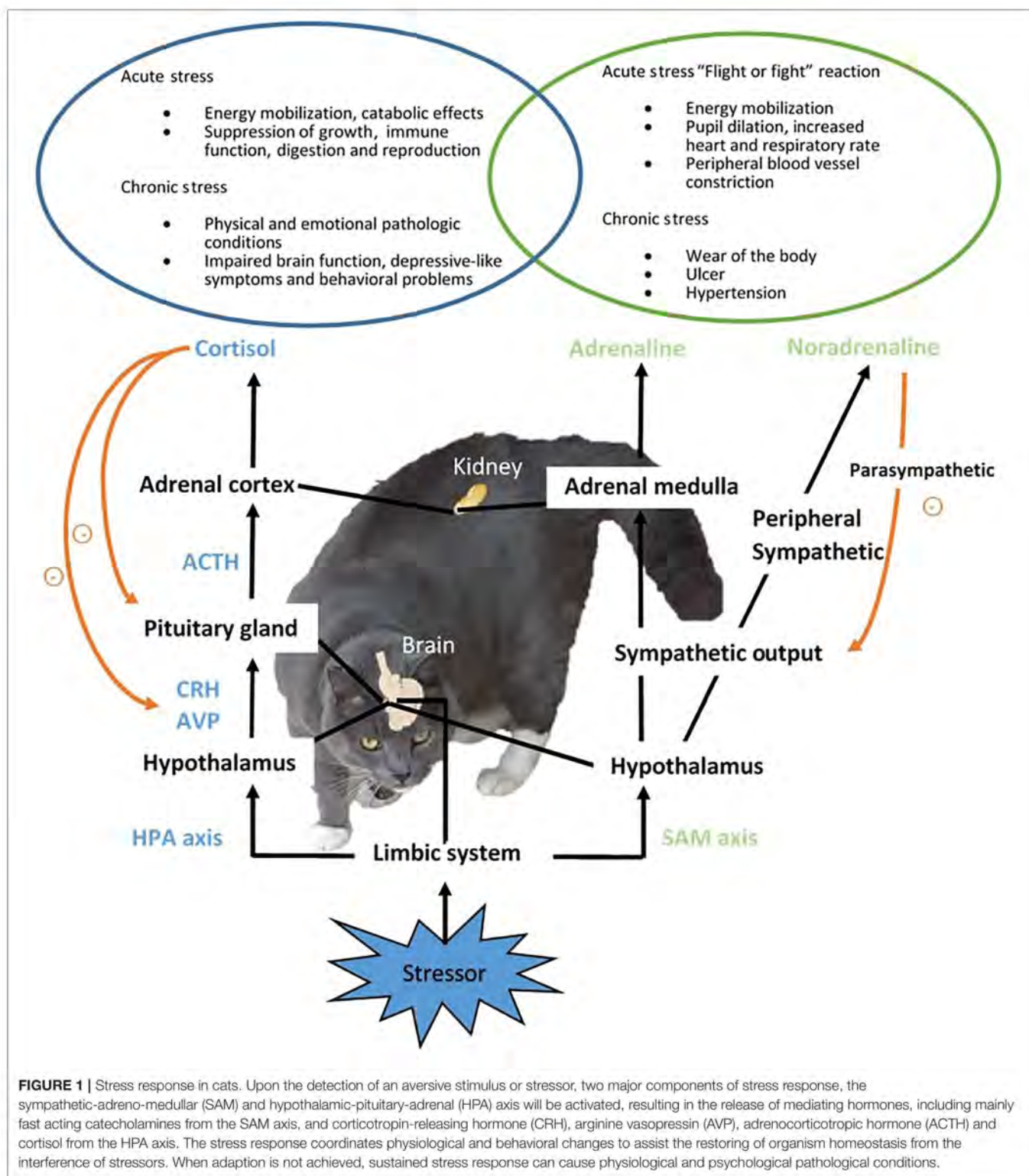
Acute or prolonged stress	Behavioral signs	Triggers
Acute stress	Anxious posture, shaking, fast ventilation, fully dilated pupil and flattened ears, tail close to the body, plaintive vocalization (24–27), struggle, motor activity and aggression (27–29), hiding attempt (8–11) Reduced activity level and diversity, including play, exploration, and maintenance behavior such as feeding, drinking, and elimination (25, 26, 30, 31), reduced social affiliation and facial marking (22, 32), occurrence of feigned sleep (33, 34), increased vigilance (35),	Bath (36, 37) Hospital visit (38–40), handling and restraint practices (28, 29) Confinement (27, 41) Novel environment (e.g., entering shelter) (21, 42, 43) New socialization, such as group housing (21, 42)
Prolonged/chronic stress	Sickness behavior, (e.g., vomiting) Anorexia House soiling problem (45, 46) In appropriate elimination (1, 47) Fecal marking (1) Urine marking (1, 50) Depression-like symptom (e.g., inactivity) Aggression (2, 54–56) Stereotypic behavior (e.g., over grooming or self-mutilation, tail biting, and obsessive vocalization) (60, 61)	Changed caretaking routine (24, 32, 33, 44) Long-term sheltering (31) Social conflict, blocked access to the litterbox (1), changes related to litter (47, 48) Chronic disease, such as feline idiopathic cystitis (49) Outdoor and indoor social conflicts (1) Lower urinary tract disorders (51), substantial changes in the social and physical environment (1, 49, 50, 52) Long-term sheltering (53) Social conflicts (2, 54, 57), high housing density (43), co-residence with dogs (58) and other cats (59), long-term sheltering (53) Stress from chronic disease, environmental and social conflict (1, 60, 61) Frustration from limited outdoor access (58, 61, 62)

concealed areas (i.e., hiding), reduced activity level and diversity, anxious body postures, and aggression are indicative of acute stress in cats (8, 24, 26). Noteworthy, coping style (e.g., reactive or proactive) was reported to impact behavioral responses of individual cats to stressful events or environments such as acute cage confinement (41). Animals with a proactive coping style often exhibit active behavioral responses (e.g., territorial control and aggression) and are characterized by high activation of the SAM axis and low HPA axis activation, while those with a reactive coping style exhibit withdrawal response (e.g., immobility, hiding attempts, and low level of aggression) and had higher parasympathetic reactivity and HPA-axis activation (67, 68), which points out the importance of addressing individual differences when using behaviors to evaluate stress in animals. Behavioral problems developed from prolonged or chronic stress may be less apparent but with increased severity. Depression-like symptoms can occur, such as loss of pleasure seeking, social inhibition, increased aggressiveness, and altered fearfulness, and can negatively impact several sensory perceptions, such as olfaction deficits, blunted taste, and hyperalgesia in humans and other animal species (69). Behaviors indicating learned helplessness (e.g., increased resting/sleeping) have been shown in dogs in lengthy captivity (70). In shelter cats, increased residence time is positively correlated with cats being increasingly inactive and more involved in conflict situations (53). Chronically stressed cats may express behaviors that are either abnormal (e.g., stereotypic behavior) or normal but with altered frequency and/or unwanted (e.g., urine marking) by owners (2, 22). In many cases, several behavioral problems (e.g., elimination disorder,

urine marking, and aggression) were exhibited concurrently in one cat (4, 62). The time frame required for animals to be considered under chronic stress is an unanswered question, one agreed upon definition being that the stress has to occur intermittently and persist for weeks or months (66). There is less information on how individual differences impact the cat's adaption and resistance to future stress and the possibility of developing specific behavioral problems with different types of chronic stress. Future studies addressing these issues can be significant for identifying stress-sensitive cats and the early prevention of developing chronic stress in these cats.

CAT OLFACTORY SYSTEM

Similar to many other mammals, cats have at least two olfactory systems to perceive and process the various chemicals existing in their environment (16). Small volatiles that reach the main olfactory epithelium are often inhaled during breathing and detected by the main olfactory system. These chemicals bind to the receptors on the ciliated dendrites of the first-order olfactory sensory neurons in the olfactory epithelium, the axons of which converge onto glomeruli at the main olfactory bulb (MOB). From here, information is conveyed to the primary olfactory cortex *via* the lateral olfactory tract and further spread to other brain regions (e.g., orbitofrontal cortex) *via* the thalamus. The MOB is also connected to parts of the limbic system to control some hypothalamic activities (18). The perception of chemical signals by the accessory olfactory system occurs through the



vomerolateral organ (VNO), a pair of liquid-filled sacs located at the roof of the mouth and encased within bony capsules in the septum. It is connected to both nasal and oral cavities *via* the nasopalatine canal. The VNO is suggested to be mainly involved

in the detection of water-soluble molecules. Upon investigating the area, cats often sniff and lick the fluid material and exhibit the Flehmen response which opens the nasopalatine canal and allows the passage of fluid-borne molecules to the VNO (16, 71). The

chemosensory neurons lie in the epithelium of VNO and send axons to the accessory olfactory bulb (AOB) located in the dorsal-posterior region of MOB. Information from AOB is not sent directly to the cortex but rather to the emotion-controlling limbic system, where neurons mainly connect to the hypothalamic nuclei to control mostly instinctive behaviors and responses (69, 72). It was previously believed that different chemicals were detected exclusively by the VNO or the main olfactory epithelium, but recent research supports that inputs from both olfactory systems are required for the appropriate processing of some social and predator-related chemosensory stimuli (73, 74). Two systems send inputs to separate but adjacent nuclei in amygdala, and the divergent signals are integrated for relay to the basal forebrain regions to initiate behavioral responses (75–77). Information about how chemical signals detected by the olfactory system are processed in the brain is mainly based on rodent and human studies and is quite limited in cats. Nevertheless, the olfactory system and scent communication play critical roles in many cat activities, such as marking and social interactions (17, 71, 78).

REDUCING STRESS IN CATS: THE OLFACTORY STRATEGIES

Considering its unique intimacy with the limbic system, the olfactory system may serve as a potential target for the intervention of stress response. This is still a matter of debate that warrants further investigation. Olfactory stimuli that induce stress (i.e., from predators and stressed conspecifics) should be avoided (69), while those with comforting or enriching effects may be applied in aversive contexts to reduce stress and improve welfare in cats. The potentially effective stress-reducing and/or enriching scent stimuli for cats are summarized in **Table 2**.

Cat Scent, Signature Mixtures, and Pheromone

Mammalian chemical signals can be classified into pheromones or signature mixtures based on their functions (73). Cats have a number of mechanisms to produce chemical signals, including scent glands throughout the body, and the salivary, fecal, and urinary sources (71). Meanwhile, several marking methods (e.g., rubbing, scratching, and urine marking) that involve the deposit of chemical signals have been described in cats (94). Pheromone is a species-wide chemical signal and belongs to semiochemicals which include also interspecific communicative chemicals (i.e., allomone, kairomone, and synomone). Pheromone is defined as the chemical signal emitted by one individual and elicits a stereotyped behavior or response in the receiving conspecific individual (16, 73). Pheromone communication benefits both emitter and receiver and varies slightly between individuals in the species. Examples of pheromone include the sexual odors given off by queens in estrus (71). Signature mixtures are indicative of an animal's chemical profile, allowing the differentiation between individuals and colonies (73). The anal gland secretions fall into the category of signature mixtures (78). The classification of some chemical signals is debated. For example, it is suggested that the

facial cheek secretions should be considered signature mixtures instead of pheromones because the component and content of facial gland secretions vary in cats (95). A better understanding of the functional properties of different chemical signals of cats can provide opportunities for scent strategies in different settings that can reduce stress and improve cat welfare. A series of synthetic chemical products have been developed for context-dependent use in cats.

Rubbing objects and individuals in the environment is an affiliative behavior in cats that allows the deposit of gland secretions for physical and social marking, organizing the environment, and exchange of scents between individuals (14, 71). So far, several chemical signals have been identified from sebaceous secretions of cat facial area, namely facial pheromone F1–5. The major active components of the cheek secretions are volatile fatty acids, such as oleic acid and palmitic acid. It was suggested that cats use F3 for object marking and organization of their environment, and use F4 for allomarking of other individuals (71). Feliway™, a commercial product mimicking F3, was developed for environmental application to reduce stress in cats, the logic being that the artificial pheromone increases the familiarity of the environmental objects and individuals for the cat. Several studies reported an effect of Feliway™ on reducing stress-related urine spraying in cats, as reviewed by Mills et al. (79). However, only one study followed the randomized controlled design and was double-blinded (96). The F3 analog product was also shown to calm cats at the vet clinic although it did not reduce struggling during handling (30, 97), however, its lack of efficacy in a study including a similar scenario was also reported (98). Another study showed that in a shelter environment, salivary cortisol levels did decrease for the majority of cats (75%; 21/28 cats) following 35 days of F3 analog treatment and male cats responded better to the intervention than female cats (99). However, no control group was included in this study. Stress is related to the contraction and recurrence of certain diseases in cats, including the upper respiratory infections as a result of feline herpesvirus (100, 101) and feline idiopathic cystitis (102). Respiratory tract symptoms caused by feline herpesvirus in shelter kittens were reduced in the pheromone-treated group (100), while another study found no effect of Feliway™ on stress scores or incidence of upper respiratory tract infection in adult shelter cats (101). The synthetic facial pheromone was also shown to improve symptoms and stress behaviors in cats with feline idiopathic cystitis (102). Some researchers suggest that there is insufficient evidence for feline facial pheromone product in calming cats (80, 81), given the lack of positive results and limitations with the experimental design in the aforementioned studies. Collectively, supportive evidence exists for the efficacy of synthetic feline facial pheromone in reducing anxiety and stress-related behaviors, such as urine marking. However, randomized and well-controlled studies with more rigorous methodology are encouraged in the future for validating the use of Feliway™ in additional settings.

Secretions from skin glands of the mammary sulcus by queens during nursing were proposed to have a calming effect and appease both kittens and queens, therefore, called appeasing pheromone (71). The commercial version of appeasing

TABLE 2 | Scent stimuli with potential stress-reducing and/or enriching effect for cats.

Scent name/commercial	Source	Component	Potential function	Application evidence
F3/Feliway™	Cat cheek/sebaceous gland	Oleic acid, azelaic acid, pimelic acid, palmitic acid	Object marking	Putative effect of reducing urine spraying/markings (79), debated efficacy of calming cats at vet clinic and shelter (80, 81)
Appeasing pheromone/Feliway® Multicat or Feliway® Felifriend	Queen mammary sulcus/ skin sebaceous gland during nursing	Oleic acid, palmitic acid, linoleic acid, myristic acid, lauric acid, and stearic acid	Appeasing the queen and kittens	Reduce inter-cat aggression in multi-cat household (82), and also improve cat interaction with co-resident dogs a 6-week testing period (83)
Pedal/ Feliway® Feliscratch™	Cat interdigital area/skin sebaceous gland	Valeric acid, lactic acid and, linoleic acid	Territory marking	Induce scratching when applied to scratching posts (84), more likely due to catnip (85)
Prey odor	Rat, rabbit	Odor mixture	Induce predatory or play behavior	Inconclusive results in captive cats (86, 87)
	Rabbit maternal-neonatal pheromone	2-methyl-2-butenal (2M2B)	Unknown	Improve use of litter box and reduced aggression in pair-housed cats when applied to litter box (88)
Familiar interspecific scent	Mostly from owner	Odor mixture	Comforting effect	Not effective during the strange situation test (89), remains to be tested in other settings
Cat attractant	Plant such as catnip and silver vine	e.g., Neptalactone in catnip; isolindomyrmecin and dihydronepetalactone in silver vine	Chemical defense against mosquitoes	Inducing play behavior in a proportion of adult cats (90–92), calming cats at clinic together with F3 analog (93), cat habituation is common (86)

pheromone, Feliway™, Felifriends or Multicat, was shown to reduce inter-cat aggression in multi-cat households (82) and also improve cat interaction with co-resident dogs over a 6-week testing period (83). With only two studies currently published, much remains to be learned about the efficacy of Feliway™, Felifriends in promoting amiable social behaviors, e.g., the settings for application and individual differences in their responses to the pheromone treatment. It has been suggested that kittens' early experience with the pheromone (hand-raised orphan vs. queen-raised) might influence their response to the appeasing pheromone (103).

Cats under chronic stress show reduced behavioral diversity; therefore, promoting the expression of natural behavior (e.g., scratching) has welfare potential in stimulating behavioral diversity and reducing anxiety. Cats have sweat glands in the planter pads and interdigital skin of the pedal area. Secretions from these glands were suggested to be involved in scent marking and producing alarming messages (71). Scratching is often exhibited on object surface (e.g., furniture in an indoor environment), particularly on vertical surface by male cats (85, 94), and leaves behind physical and chemical marks in the environment (71, 104). Cats tend to return to the same spot for scratching, suggesting that the visual and scent mark served as a reference point (105). The feline interdigital semiochemical (FIS) product, FeliScratch™, contains FIS and catnip extract. Application of FeliScratch™ on the scratching devices successfully directed scratching from furniture to the provided device in 74% (22/29) of cats (106). In another study with a crossover design, cats scratched more of the scratching post that is treated with FeliScratch™ than the placebo post (84). However, our recent study showed that the efficacy of inducing

scratching, if any by FeliScratch™, is more likely due to the ingredient of catnip than FIS (85).

Other sources of chemical signals (e.g., urine, feces, and anal gland secretions) in cats have been identified and their potential functions have been investigated (78, 107, 108). Fecal scents, anal gland secretions, and even facial pheromones are technically all signature mixtures for individual identification (78, 95, 107). It is still debated about which source of chemical signals represents the scent of an individual cat. Social buffering that is the presence of a social companion can moderate HPA responses to stress, and the nature of the relationship between individuals will determine whether or not social buffering of stress response will occur (109). Synthetic analogs that often include mixtures of several representing chemicals in set concentrations are probably perceived by the receiving cat as scents of another cat. The question arises as how cats interpret the scent of another cat, as enriching or threatening? Data included in a recent review paper reported that the scent of conspecifics provided as enrichment did not result in much change in shelter cats (110). Therefore, without a better knowledge of the exact effects (e.g., valence) of these chemicals on cats, their welfare application will remain to be validated.

Other Biologically Relevant Scents

Prey and Food Odor

Predatory behavior/hunting is one of the most important natural behaviors in cats. Segments of predatory behavior are also incorporated in play, such as stalking, pouncing, and kicking prey or toys. Play is probably a means of hunting practice in cats, as play with different-sized toys matched the interaction

pattern with the prey of different sizes (i.e., mice vs. rats) during hunting, and hunger increased the play intensity and interest in larger toys (111, 112). Several studies have investigated the efficacy of prey odor as environmental enrichment in cats, often including other olfactory stimuli for comparison such as the scent of catnip and lavender (86, 87, 110). Shelter cats exposed to cloth impregnated with catnip or rabbit scent, in general, become less active with more time sleeping and reduced exploring of the environment (86). Promoting inactivity may not always be bad as it is important to differentiate between activities that indicate restlessness and anxiety (e.g., stereotypic pacing) and that are indicative of good welfare (e.g., play). In another study, a wooden cube covered with cloth of rat scent induced sniffing and rubbing of the cube in shelter cats (87). The differences in the sources of prey odor (rat vs. rabbit) and measures (instant behavioral responses to the odor vs. general activity) included may contribute to the disagreement between the two studies. Generally, other than catnip, most scents in these studies including prey odor did not induce much interest and predatory behavior in cats. Olfaction plays a minor role during hunting in cats (113). Providing only odor without the sound or visual stimuli of the prey may not be enough to induce predation-related behaviors in cats. Another study added the rabbit maternal-neonatal pheromone, 2-methyl-2-butenal (2M2B), to a cat litter box and found that the use of the litter box was improved and aggression in pair-housed cats was reduced (88). The mechanism underlying this action of rabbit maternal pheromone on cats requires further investigation.

In addition to the pleasure of feeding and the anticipation of food, the stress-reducing effect of cat food could be achieved through functional diets with anti-oxidative or anxiolytic properties (12, 13), and being an element of enriching tools when served together with food puzzles (114). These benefits are more likely to be mediated by the digestive system and crosstalk between the gut and brain rather than by olfactory pathways. Stress inhibits feeding and can cause food neophobia in cats (22, 33). At present, cats are often fed flavored commercial diets. Increasing the attractiveness of food with palatants may promote feeding and recovery from stressful events (115). Most of the food preferences were evaluated in trained or household cats under normal conditions (116). Food odors or the actual preferred food and palatability enhancers are rarely tested for capacities in improving cat feeding in stressful conditions. Nutrient-enriched water with or without poultry flavor was shown to effectively increase cat water intake over a 44-day testing period when compared to control, with the poultry-flavored water that contains more protein and fat being more potent (117). Future research may investigate the stress relief effect of preferred food ingredients and palatants, and odors on cats.

Scent of Familiar Human

Cats kept indoors for companionship can form a close relationship with owners or primary caretakers. Recent studies have shown that cats attach to their owners (118) in a way similar to the relationship between children and parents (119), and that between dogs and owners (120). In the strange situation test, cats on average exhibited less stress-related behaviors in the

strange environment when the owner was present compared to being alone, indicating social buffering of stress response in the presence of the owner; the presence of only objects with the scent of owners (e.g., owner's cloth) was not comforting to the cats (89). This is different from the situation in humans and dogs. The odor of the attached figure reduced stress responses in humans during weak electric shocks (121). An fMRI study reported that the caudate nucleus, a brain region related to rewards and positive experiences in un-sedated dogs, was activated after sniffing the scent of a familiar human, but not of a familiar dog (122). Results from the studies with dogs and cats might not be comparable because of the species differences and also differences in the measured variables and experimental settings. Dogs in the study were well-trained to co-operate and remain still in the fMRI machine; thus, the effect of the scent of a familiar human on dogs was evaluated in a neutral to a positive environment due to extensive training with treats. In the case of the cat study, subjects were exposed to a novel environment, which often induces a strong stress response in cats. The scent from a familiar human may not be as effective as the actual presence of the human. However, this does not exclude the potential application of familiar human scents in other non-testing contexts (e.g., hospital visit and stay in a pet hotel). Future studies may also investigate the effects of scents from other familiar cats or pets on cats from multi-pet households.

Cat Herbal Attractants and Other Plant-Extracted Oil

Cats are naturally attracted by plants such as catnip (*Nepeta Cataria*) and silver vine (*Actinidia polygama*) and react in a euphoric way. Upon sniffing these plants, cats often exhibit the so-called "catnip response," which is comprised of species-specific playing behaviors, such as rubbing, rolling on the ground, and kicking the plant source. The iridoid compounds in the plants (e.g., nepetalactone in catnip; isoiridomyrmecin and dihydronepetalactone in silver vine) are the major active ingredients inducing the "catnip response" (91). Depending on studies, about 20–60% of the cat population was reported to respond to catnip and up to 90% respond to silver vine (85, 90, 92). The response is mediated by the main olfactory system instead of the accessory olfactory system (123), and is independent of sex or the presence of gonads in cats, rather the response increases as the cat matures (90, 92, 124). The existence or absence of specific olfactory receptor(s) for the plant chemicals may explain the diverse responses in cats (125). It is not until recently that researchers started to investigate the biological function and the underlying mechanism of cat responses to these plant attractants. Uenoyama et al. (125) reported that nepetalactol, the chemically synthesized major active component in the silver vine, increased plasma β -endorphin levels in cats, potentially through the activation of the central rewarding system as inhibition of μ -opioid receptors blocked the classic rubbing response. This study provided supportive evidence that plants like catnip and silver vine elicit pleasure in cats, and the lack of response in kittens is due to their immature opioid system (126). Cats are believed to be not addicted to these attractive plants

(127), because the μ -opioid system is not directly stimulated by exogenous opiates, but by elevated endogenous β -endorphin after the activation of olfactory neurons in response to plant odorants (125). These cat-attractive plants, either served alone or together with other stimuli (e.g., toys or scratchers), have been widely used in cats to enrich their environment and increase behavioral diversity. When applied on scratchers, the attractants can increase the use of the scratchers by cats, thus amplifying the enriching effects of these scratching devices (84, 85).

Still, a few things need to be considered when seeking the use of plant attractants for relieving stress in cats. Studies have shown that responses to catnip in captive black-footed cats and shelter cats waned over the 5-day testing period (86, 128), indicating habituation after continuous exposure. Rotation of different plants and limiting free access to the source may help to maintain the attractiveness and effectiveness of these plants. Researchers have proposed that all cats respond to catnip; the active responders exhibit the classic "catnip response," while the passive individuals show "sphinx-like position" and reduced vocalization and activity after exposure (92). Uenoyama et al. (125) included only positive silver vine responders in the study for the measurement of plasma β -endorphin. It is unknown if cats also respond passively to silver vine. Future study may investigate the secretion of β -endorphin in these passive cats or previously classified non-responders to determine if euphoric effects are also elicited despite the lack of behavioral manifestations. Negative emotions (e.g., fear) may inhibit the cat's response to these plant attractants (69, 90); thus, their application in settings involving acute stress may be limited. These attractants may be more effectively applied to reduce boredom and anxiety in long-term confinement, such as sheltering and daily household. Cats also show preferences and response variations to different plants, and repeated testing at different time points and with different plants may be necessary to induce the active response in individual cats, thus helping to expand the population of cats that can benefit from the intervention.

In humans and rodent species, plenty of studies exist for the positive effect of the scent of coffee beans and essential oils of lavender, cypress, α -pinene, and thyme linalool on stress-related behaviors and expression of stress markers in the brain (18). Such study is quite limited to cats and the only study included lavender as odor enrichment showed almost no effects on cats (86).

DISCUSSION AND FUTURE DIRECTIONS

Scent plays an important role in many cat activities and can serve as effective enrichment and stress-reducing tools if properly understood and applied (16). The loss of opportunities for cats to receive and emit chemical signals may affect cat welfare. Cats seem to be naturally comforted by certain conspecific scents, such as the F3 cheek secretions (79) and feline maternal pheromone (82, 83). Therefore, olfactory enrichment is important to both prevent and address stress-related behavioral problems. However, close appraisal of the literature on olfactory manipulation for stress alleviation in cats, especially those focused on commercial chemical signals,

often reveals limitations with methodology. Some issues, such as deficient experiment design and lack of negative control, may have contributed to the inconsistent results in the literature. Current findings constitute weak evidence that there is a reproducible effect of therapy of cat chemical signals for stress. Future studies may benefit from the practice of pre-registration whereby primary outcomes and measures are declared in advance, specifying statistical methods to be applied, and making data openly available, which increases the credibility of the research.

The pre-requisite for appropriate application is a better understanding of the biological functions of the stimuli. The introduction of odors with stimulating properties may be enriching and can promote mental health, but it had been suggested that these stimulating odors may cause increased agitation and result in the development of active types of problematic behaviors (e.g., stereotypy) (129). Long-term research in more depth on the impact of these stimuli is needed before firm conclusions can be drawn about their welfare applications (129). Researchers may rely on more advanced and less invasive technologies to capture accurate physiological and neuroendocrinal data which can be particularly helpful in stress evaluation. For example, high-quality testing kits can benefit the measure of cortisol and its metabolites in other sources (e.g., urine and feces, hair, and saliva), thus minimizing the effect of the sampling procedure. Wearable electronic devices, such as monitoring collars, can help to collect physiological parameters such as respiratory rate, heart rate, and heart rate variability (130), even though their use in cats requires further validation. A cognitive bias test may be applied to reflect the animal's emotional state which is central to welfare studies (131, 132). Integrated information from multiple sources (e.g., behavioral, physiological, and neurofunctional) may provide more fruitful results for the assessment of the stress-reducing or enriching effects of a given olfactory stimulus.

In addition to methodological improvements, research should address individual differences, which has long been recognized by scientists working with animals. Cats with different coping styles are a case in point. Instead of being invariant, the stress response is currently considered an array of different physiological and behavioral patterns when all kinds of aversive stimuli are encountered by animals (41, 67, 68). Meanwhile, genetic factors, early experience (e.g., effect of maternal pheromone), and emotional state (e.g., stress and mood) may all contribute to varied susceptibility to olfactory manipulation in animals (69). The variant responses of cats to catnip and other plant attractants bear genetic and mental contributions (90). Therefore, individuality in stress coping style and susceptibility to olfactory manipulation could be particularly relevant in seeking olfactory strategies for stress management in cats.

Research may consider insights into the mechanical basis of how odors work and their perception by the animals. A few hypotheses for the potential odor effect on stress relief had been proposed (69) and warrant future investigation. Odorants could directly act on the stress centers in the brain or have indirect impacts, such as masking effect and associative learning. Odorant concentrations in the air and the amount

that reaches the olfactory epithelium can be hard to measure and standardize. High doses of odorants in the air may enter the bloodstream and have pharmacological rather than olfactory effects. Positive/neutral odorants can also compete with aversive odorants for binding sites at the sensory epithelium and have masking effects. Odorants considered pleasant and calming for humans can serve as a distraction from the memory recall of negative experiences. Pleasant elements of odorant may also be tested in animals with the assessment of preference variability. The association of odors with a positive emotional state may explain the stress relief action of appeasing pheromone where the animals associate the odors with the maternal environment and early experience. Studies in pigs also showed that when presented with odors that were fed to the sows during late gestation and lactation, piglets exhibited less stress upon weaning (133). The putative effects of food odor and odors of familiar individuals may also be explained by positive association. The social buffering effect of odors from familiar partners is dependent on the quality of the relationship which reflects the accumulative association with experience of former interactions (109). An alternative hypothesis should be tested as well. For example, it is possible that odors act on the owner instead of having direct effects on the cat. The emotional state of the owner has been shown to impact their interaction style with the cat, the cat's stress and emotion, and its behavioral health (134).

Given the importance of olfaction in regulating cat behavior, olfactory strategies hold a huge potential for treating stress-related problems in cats. However, some of the current findings constitute weak evidence for reproducible effects of odor

therapy for stress in cats. A better understanding of the biological functions of the various olfactory stimuli requires a systematic methodological appraisal and the investigation of the mechanisms at play. Future studies should seek improvement in methodology possibly through preregistration of the experiment, take advantage of the advanced measuring techniques, and recognize the importance of addressing cat individuality (i.e., coping style and susceptibility) and the influence of environmental factors.

AUTHOR CONTRIBUTIONS

LZ drafted and wrote the manuscript. BD and QL provided conceptual advice and revised the manuscript. ZB worked on substantial modification at all stages of manuscript preparation. All authors contributed to the article and approved the submitted version.

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Effects of species-relevant auditory stimuli on stress in cats exposed to novel environment

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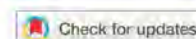


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RESEARCH ARTICLE



Effects of species-relevant auditory stimuli on stress in cats exposed to novel environment

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ABSTRACT

Environmental changes like vet visit could cause stress in cats. Studies have attempted to develop stress management strategies targeting sensory systems. Even though species-appropriate music which includes cat affiliative sound (e.g., cats' purring and suckling sound) has been shown to relieve stress in cats. Little is known whether the cat sound alone works in stress management. This study was conducted to investigate the effects of species-relevant auditory stimuli on stress in cats exposed to a novel environment. During the 28-day experiment periods, 20 cats received four types of sound treatments which included silence (T1), purr of cats (T2), eating sound in cats (T3), and the mixed sound of T2 and T3 (T4) in a novel environment in random orders with intervals of 1 week between treatments. Cats' behaviors were recorded during each 10-min test. Results showed that T4 reduced visual scanning ($P=0.017$) without significantly affecting other behaviors, compared with other treatments. Together, the two types of cat-specific sounds did not exert pronounced effects of relieving stress on cats exposed to a novel environment.

KEYWORDS

Environment enrichment; species-relevant auditory stimuli; animal welfare; cat-behavior

Introduction

Cats are popular companion pets all over the world (Roetman, Tindle, & Litchfield, 2018). Many pet owners see their cats as friends or family members, and increased attention has been drawn to their physical and psychological well-being (Bouma, Reijgwart, & Dijkstra, 2021). Cats are particularly sensitive to changes in their physical and social environment (Conti et al., 2017), therefore novelty and/or unpredictability of the environment can be a source of stress in cats (Amat, Camps, & Manteca, 2016; Mazzotti & Boere, 2009). Previous research has shown that cats isolated in a novel environment exhibit stress behaviors indicative of discomfort and insecurity (Cannas et al., 2020). In general, stressed cats are more prone to show anxious and abnormal behaviors such as aggression, hiding, reduced behavioral diversity and social interaction, and house-soiling problems (Amat et al., 2008; Amat, Camps, & Manteca, 2016). Severe or sustained stress can also increase the risk of developing chronic and reoccurring symptoms, such as upper respiratory infection and urinary tract and gastrointestinal diseases in cats (Enck & Holtmann, 2008; Jones, Sanson, & Morris, 1997; Tanaka, Wagner, Kass, & Hurley, 2012). Therefore, stress management is an important aspect in improving cat welfare and owner-cat relationship in domestic environments.

Environment enrichment (EE) is defined by Ellis (2009) as the physical and/or husbandry strategies applied to relatively impoverished environments, with the intention to encourage the expression of natural behaviors in animals and improve their physical and psychological health (Ellis, 2009).

Although there is still debate over the definition of enrichment but that this is the definition we have chosen to use for this study. Auditory stimulus, as an EE strategy to enrich the sensory environment has been investigated in humans and different animal species, including cats (Hampton, Ford, Cox, Liu, & Koh, 2020; Rochlitz, 1999), dogs (Engler & Bain, 2017), and farm animals such as pigs (Zhao et al., 2021) and hens (Campo, Gil, & Dávila, 2005). Music therapy as a nonpharmacological intervention has been shown to promote physical and mental health in human patients by alleviating anxiety and pain (Bernatzky, Presch, Anderson, & Panksepp, 2011; Phipps, Carroll, & Tsiantoulas, 2010). Specific music might positively affect cognitive capacity and emotion by regulating the levels of neurotransmitters (e.g., dopamine) in the central nervous system (Hao, Jiang, Wu, Yu, & Zhang, 2020) and hormones such as oxytocin (Ooishi et al., 2017). Classical music, especially when compared to heavy metal music has been shown to exert calming effects in different animals, such as to reduce the amount of nervous shaking and vocalization of dogs housed in kennels (Kogan, Schoenfeld-Tacher, & Simon, 2012), relieve stress in hospitalized cats (Paz, da Costa, Nunes, Monteiro, & Jung, 2022), and influence the autonomic nervous system in cats under anesthesia (Mira, Costa, Mendes, Azevedo, & Carreira, 2016). The frequency range and tempo of the music matters for its effectiveness of emotional regulation in the specific species as different sensory and communication systems have evolved within each species (Li et al., 2019; McDermott & Hauser, 2007; Snowden & Teie, 2013). In addition, music that contains species-relevant motivic features seems to have more behavioral effects (Snowdon & Teie, 2010). A study on tamarins showed that tamarins were indifferent to human music but showed greater arousal to sounds based on tamarin threat vocalization and appeared calmer to music based on tamarin affective vocalization (Snowdon & Teie, 2010). Compared with ordinary human classical music, cats showed significant preference and interest in a species-appropriate music which contains melodic lines based on cat affiliative vocalizations (e.g., purring) and rewarding suckling sound (Snowdon, Teie, & Savage, 2015). Recently, cat-specific music was tested at veterinary clinics, and positive effects in reducing cat stress during examination were observed when compared with silence and classical music (Hampton, Ford, Cox, Liu, & Koh, 2020). These findings made us wonder whether playing the recordings of real cat affiliative vocalization and sounds would have positive behavioral effects in cats. Purring is generally perceived as a friendly or soliciting vocalization in cats (Tavernier, Ahmed, Houpt, & Yeon, 2020), while the eating sound is naturally considered rewarding in cats due to its inherent connection to food. Therefore, the purpose of the current study was to determine whether cat-specific affiliative sounds (i.e., purring and sound of eating) could influence cats' behavior in an unfamiliar environment, so as to provide reference for developing new strategies of stress alleviation in cats.

Materials and methods

Animal ethics

All experimental procedures were authorized by the Animal Care and Use Committee prior to animal experimentation (Approval number: 2021a030) and were performed following the guidelines of the Laboratory Animal Center at the South China Agricultural University.

Animal and housing

A total of 20 healthy sterilized British shorthair cats aged 18 months, including 11 males and 9 females with similar life experiences, were selected for this experiment. All cats were purchased at about 8-month-old from a commercial breeding facility (Guangzhou, China) where they were born in cavity and received a same daily care routine. After arriving at our facility, they have also been taken care of in a similar way. Cats were kept in the Laboratory Animal Center of South China Agricultural University (Guangzhou, China) individually with temperature and relative humidity of $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $70\% \pm 3\%$, respectively. The housing cage included separate areas for feeding,

defecation, and resting. All cats were free to eat adequate fresh food and drink clean water in a relatively quiet environment and were allowed to interact and play with volunteers and other cats for 30 min every day. In addition, toys like cat scratch boards and sisal balls were placed in each cage, and two cat scratchers were placed in the room to enrich the living environment.

Experimental design

This experiment was conducted from March to April in 2022 in the Laboratory Animal Center of South China Agricultural University (Guangzhou, China). During the 28-day experimental period, each cat received a total of four types of sound treatments which includes silence (T1), purr of cats (T2), the sound of eating in cats (T3), and the mixed sound of T2 and T3 (T4). Audio of the recorded sound treatments can be found in the supplementary material. Each cat received one of the four treatments once on four separate days (i.e., D1, D2, D3, and D4) with a seven-day interval between treatment tests which let cats recover from the previous exposure (Chadwin, Bain, & Kass, 2017). It was arranged that no cats received the treatments in the same order. The specific test sequence in this experiment is shown in Table 1. During each 10-min test which length of stimulus exposure was referred to previous studies (Pratsch et al., 2018; Quimby, Smith, & Lunn, 2011), the selected cat was quickly moved from its home kennel to another quiet and unfamiliar room (5.2 m × 2.7 m × 2.3 m) which was in the same building as the room where cats lived daily. The cat was placed in an empty cage (1.0 m × 0.6 m × 0.7 m) that was placed in the unfamiliar room. Between tests, alcohol was sprayed in the cage and surrounding area and wiped to eliminate the odor of the previously tested cat. The test room was allowed ventilation for 5 min before the next cat was introduced for testing. The layout of the room is shown in Figure 1. A Bluetooth speaker (Sony SRS-XB13) for playing sound and a video camera (Sony HDR-CX680) for recording cat behaviors were placed near the cage. The volume of sound treatments was set at about 80 decibel which fluctuated between 77 and 84 decibels monitored by a noise meter application on iPhone 11 Promax during the experiment. Cats were returned to their usual living cages for other nutritional experiments after this study was completed. To assess the stress levels of cats in their familiar environment (i.e., in their

Table 1. The sequence of test in this experiment.

Cat ID	D1	D2	D3	D4
1	T1	T2	T3	T4
2	T1	T2	T4	T3
3	T1	T3	T4	T2
4	T1	T3	T2	T4
5	T1	T4	T2	T3
6	T1	T4	T3	T2
7	T2	T1	T3	T4
8	T2	T1	T4	T3
9	T2	T3	T1	T4
10	T2	T3	T4	T1
11	T2	T4	T1	T3
12	T2	T4	T3	T1
13	T3	T1	T2	T4
14	T3	T1	T4	T2
15	T3	T4	T1	T2
16	T3	T4	T2	T1
17	T4	T1	T2	T3
18	T4	T1	T3	T2
19	T4	T2	T1	T3
20	T4	T2	T3	T1

T1, 2, 3, and 4 represent treatments of silence, purr of cats, the sound of eating in cats, and the mixed sound of purr and eating in cats, respectively.

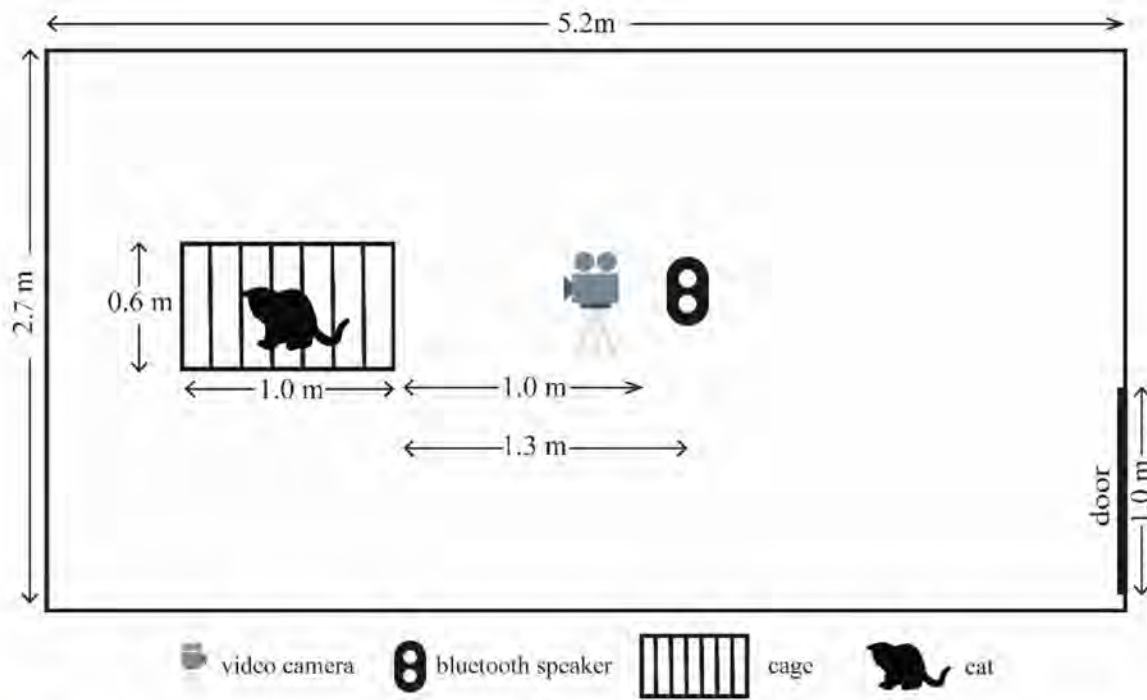


Figure 1. The layout of the experimental room.

usual cages), five cats were randomly selected and 10-min videos of their daily life were recorded on July 13 2023 at the similar time of the day as the experimental period.

Behavior coding and data acquisition

Description of behaviors of interest, as shown in Table 2 was based on previous literature (Gourkow, LaVoy, Dean, & Phillips, 2014). Two research assistants coded the muted videos to obtain most behavioral data (except “Meow”), therefore were blind to the different treatments. Coding for vocalization “Meow” was conducted with videos unmuted. Specifically, one assistant watched the videos of all the cats over 4 days and recorded the data, while the other assistant watched all the videos of five randomly selected cats and recorded the data following the same coding rule. For the events of body movement, visual scan, immobility, and sit, instantaneous sampling of each 10-min footage with 10-s intervals were applied. The recorded data were the times of occurrence of each event over the 10-min testing period. Probabilities of each event were calculated as the times of

Table 2. The definitions of behaviors recorded in cats.

Item of behavior	Definition	Measurement
Meow	Persistent and loud meowing.	Frequency(time)
Lip licking	Cats' tongue licks its upper lip and nose.	Frequency(time)
Visual scan	Cat persistently visual scanning the environment.	Probability of occurrence (%)
Immobility	Including cat remains flat, freezing and crawling: cat body trunk remains motionless while taking a low body posture when lying down, sitting or standing for locomotion.	Probability of occurrence (%)
Body movement	Cat moves its trunk and rotates posture when not taking a low body posture.	Probability of occurrence (%)
Sit	Cats' limbs touch the ground and sits normally with its back being upright.	Probability of occurrence (%)
Escape	Cat stands on hind legs, paws and pushes head against the cage door or bars, pushes paw through cage bars, and bites cage bars.	Duration (s)

occurrence $\times 10$ s/600 s. The event of “Meow”, lip licking, and escape was coded with continuous sampling method and the event frequency or duration (i.e., times or duration of occurrence) in the 10 min-test was recorded. Behaviors of cats in their familiar environment were coded using the same method above. Behavioral differences of the five selected cats in the familiar environment and T1 (i.e., novel environment) were also compared.

Statistical analysis

SPSS 26.0 and GraphPad Prism 8.0 software were used for statistical analysis and graphic presentation. To evaluate the differences between/among familiar and novel environments and sound treatments, generalized Linear Mixed model was used with cat ID as the random effect. Sequence was initially included in the model but was later removed due to non-significant effect. Significant differences were set at $P < 0.05$ and tendencies at $P < 0.10$. Data are presented as mean \pm SEM. Spearman correlation analysis was applied to test the consistency of the behavior data acquired by two research assistants. The inter-observer agreement was high for most coded behaviors ($r > 0.80$ and $P < 0.10$).

Results

The stress level of cats in their familiar environment

Cats had a lower frequency of lip licking (Figure 2b, $P = 0.035$) and spent less time on visual scanning (Figure 2d, $P = 0.001$) and escaping (Figure 2g, $P = 0.067$) in their familiar environment (i.e., their usual cages) compared to the novel environment. In addition, cats spent on average 67.75% and 26.67% of their time in the status of immobility in the familiar and novel environments, respectively, and this difference between environments was also significant (Figure 2e). As shown in Figures 2a, c & 2f, there were no differences in the frequency of “Meow” and the time of body movement and sitting in cats in different environments ($P > 0.05$).

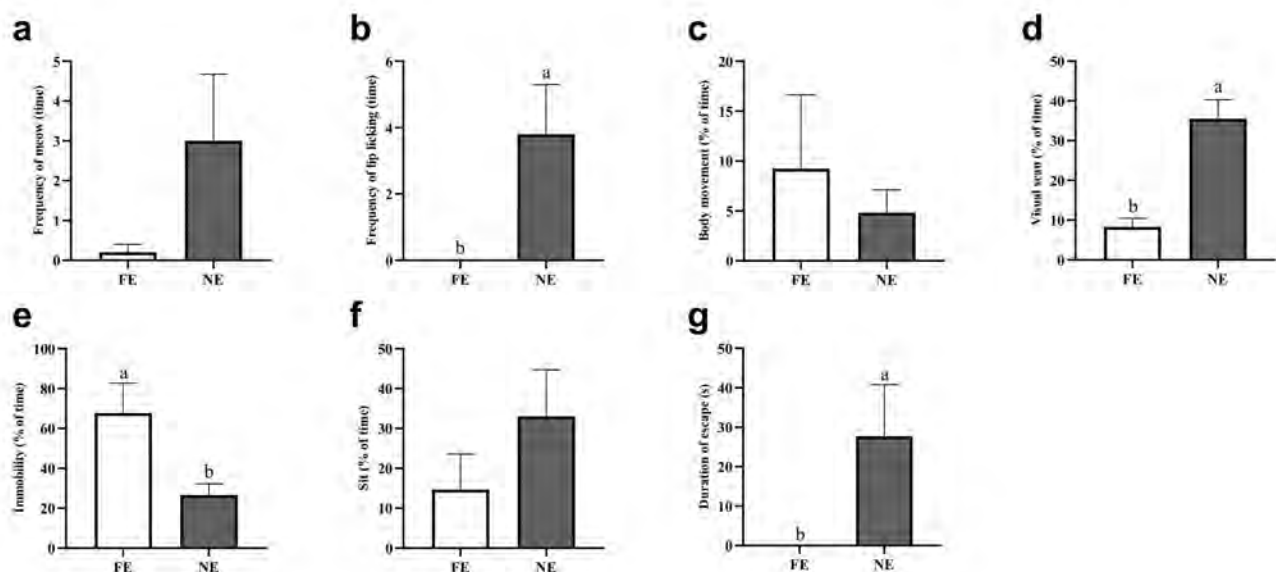


Figure 2. Cat behaviors ($N = 5$) in the familiar environment and novel environment.

FE and NE represent the familiar environment (i.e., cat usual cages) and novel environment respectively. Data are presented as mean \pm SEM. Different letters indicate significant ($P < 0.10$) difference.

Effects of four sound treatments on the behaviors in 20 cats

Different sound treatments did not have significant effects on most behavioral measurements, including the frequency of “Meow” and lip licking, the duration of escape, and the percentage of time cat spent on body movement, relatively immobile, and sitting (Figures 3a–c, 3e–g). On average, the frequency of “Meow” was about 20 times in cats over the 10-min test (Figure 3a, $P > 0.10$). Numerically, cats in T1 licked their lips more frequently than in T2, T3, and T4 (i.e., 23.8 times, 9.90 times, 12.85 times, and 12.50 times in 10 min for T1, T2, T3, and T4, separately), but the difference was not significant (Figure 3b, $P = 0.50$). Cats exposed to different sound treatments exhibited body movement in about 7.21% of time (Figure 3c). As shown in Figure 3e, cats spent a total of approximately 34.07% of time in the status of immobility which were also minimally affected by treatments ($P > 0.10$). Similarly, we did not find a significant difference in the percentage of sitting among the four sound treatments (Figure 3f, $P > 0.10$). As shown in Figure 3g, cats showed escape attempts in about 42.09% of time (i.e., 46.30%, 41.44%, 44.51%, and 36.12% for T1, T2, T3, and T4, separately).

Remarkably, visual scanning which was defined as the behavior of cat visual scanning surroundings in Table 2 was impacted ($P = 0.017$) by the sound treatment in that it was significantly reduced in cats when experiencing T4 compared to other sound treatments, while no differences were observed among T1, T2, and T3 (Figure 3d).

Discussion

Stress response is an adaptive mechanism of animals to defend against real or imagined environmental threats (Dybdall, Strasser, & Katz, 2007), which is mainly mediated by the activation of the hypothalamic–pituitary–adrenal axis and the sympathetic-adrenal system (Carlstead, Brown, & Strawn, 1993). The behavioral changes caused by stress reaction can include reduced general activity level and behavioral diversity, such as reduced play and exploratory behavior, and increased hiding and escape behavior (Iki, Ahrens, Pasche, Bartels, & Erhard, 2011; Nibblett, Ketzis, & Grigg, 2015; Riemer et al., 2021). In addition, vocalization is among the most prevalent behaviors in cats during

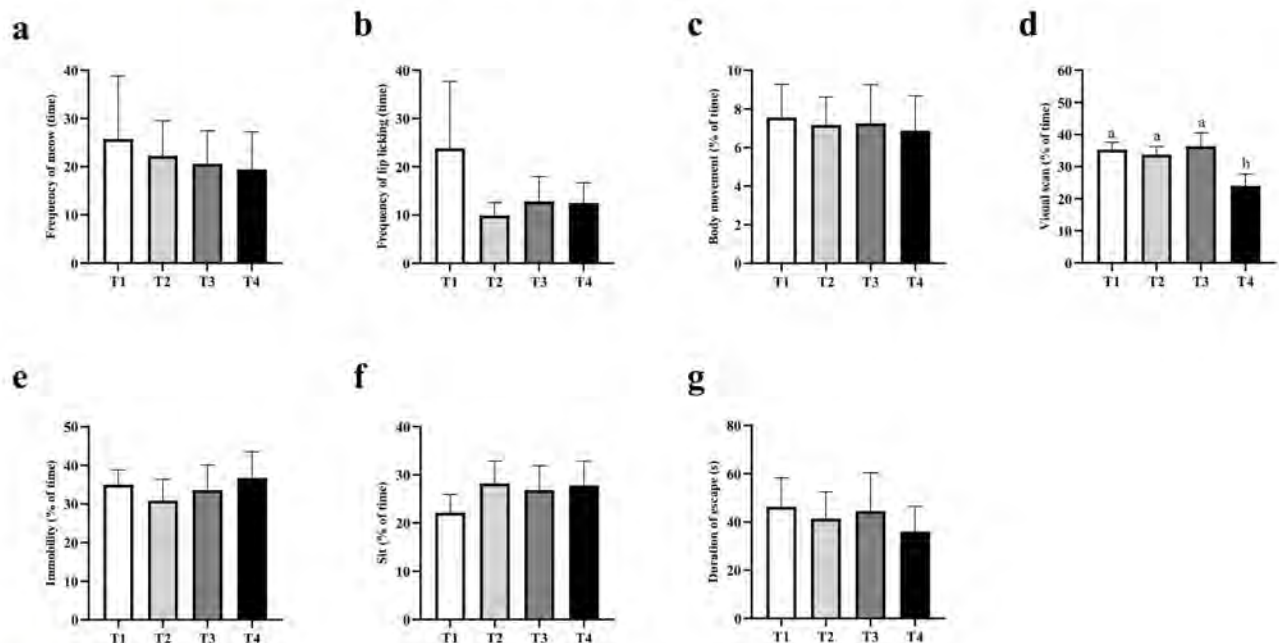


Figure 3. Effects of four sound treatments on cat behaviors ($N = 20$). T1, 2, 3 and 4.

T1, 2, 3, and 4 represent treatments of silence, purr of cats, the sound of eating in cats, and the mixed sound of purr and the sound of eating in cats, respectively. Data are presented as mean ± SEM. Different letters indicate significant ($P < 0.10$) difference.

stressful events (Rand, Kinnaird, Baglioni, Blackshaw, & Priest, 2002), and the rate of vocalization was shown to be positively associated with the peak level of plasma cortisol, a biomarker of stress (Iki, Ahrens, Pasche, Bartels, & Erhard, 2011). Meow, lip licking, immobility (i.e., cat being flat, freezing, and crawling), and escape selected in our experiment were identified as anxious or frustrated behaviors in cats (Gourkow, Hamon, & Phillips, 2014). In a study by Hampton, Ford, Cox, Liu, and Koh (2020), feline specific music, which contained cat-relevant sounds, such as purring and sucking, and with tempo and frequencies that cats prefer, was reported to reduce cat stress score, therefore ameliorate stress of cats in a veterinary visit, while classical music did not show positive effects when compared to the silent control (Hampton, Ford, Cox, Liu, & Koh, 2020). In the current study, cat purring and eating sound were applied based on a similar idea that species-appropriate affiliative vocalization and rewarding sound is likely to calm an agitated cat. Our study reported that visual scanning, a behavior indicative of alert state in cats was reduced by a mixed sound of cat purring and eating. Even though not statistically significant, nervous lip licking was slightly more expressed in cats when receiving no sound treatment than receiving the sound of purring, eating, and the mix of purring and eating. These results implied that species-relevant auditory stimuli might have some soothing effects on cats in novel environment. However, escape behavior which often occurs in stressed cats in unfamiliar environment and immobility as a vigilance behavior were not significantly affected by sound treatments. Therefore, explicit effects of sound treatments on cat stress cannot be concluded in our study due to the lack of differences between/among treatments in most other observed behaviors. The novel environment can be an intense stressor for cats (Buffington, Westropp, Chew, & Bolus, 2006; Gunn-Moore & Cameron, 2004); therefore, we speculate that cat-specific positive sounds may not provide sufficient emotion-soothing effects under this condition.

Limitations and future directions

Some of the limitations might have contributed to the lack of obvious positive effects in the current study. Although key behavioral parameters indicative of cat stress were investigated, other more delicate body postures and facial expressions such as ear position and changes of pupil size (Gourkow, LaVoy, Dean, & Phillips, 2014; Podberscek, Blackshaw, & Beattie, 1991), were not included due to limitations of the quality of footage, and the testing space and schedule. Physiological parameters (e.g., respiratory rate, heart rate and heart rate variability, and cortisol level) that can assist in the evaluation of animal stress (King, Flint, Hunt, Werzowa, & Logan, 2022) were also not included in this study. Besides, alcohol was applied to the testing area between tests to eliminate the odor of other cats. Although a 5-min interval between tests was allowed for the alcohol to evaporate, still there might be a small amount of alcohol left in the room which can be aversive to cats and cause extra stress in cats (Carney et al., 2012; Stella, Croney, & others, 2016). Lastly, the small testing cage could have limited cat ability to move which may affect our results. But this explanation is less likely since our cats are accustomed to a living cage that is similar in size (1.1 m × 0.7 m × 0.7 m) as the experimental cage (1.0 m × 0.6 m × 0.7 m). Besides, we evaluated the stress levels of cats in their usual cages by comparing cat behaviors in familiar and novel environments. Overall, during the 10-min observation, cats showed little stress-related behaviors (e.g., lip licking, escape intentions, and visual scanning) in their familiar environment compared to the novel environment. We consider that cats in their usual cages experienced relatively lower level of daily stress. Therefore, the stress of cats in our study was likely caused by the novel environment rather than the limited cage size.

Future studies that investigate the stress-relieving effects of species-specific sound in cats could benefit more from the combination of physiological measures such as the detection of salivary cortisol, electrocardiographic monitoring, and advanced behavioral analysis using high-resolution recording technique and specific coding software. The observation of subtle feline facial changes like pupillary changes and eye movement could give us a better understanding of the emotional states in

cats. It is also worth exploring the effects of other species-specific sounds and voice of owners on cats under stressful conditions. Cats in the current study were relatively similar in their genetic background and life experience, therefore individual difference was not considered here. Future studies might also explore the individual effects (e.g., socialization experience, personality) on the cat stress response and their reactions to stress relieving strategies.

Conclusion

Under the experimental parameters and settings used in this study, the two types of cat-specific sounds (i.e., purr and sound of eating in cats) did not exert pronounced effects of relieving stress or anxiety on cats in novel environment.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Article

Abrupt Dietary Change and Gradual Dietary Transition Impact Diarrheal Symptoms, Fecal Fermentation Characteristics, Microbiota, and Metabolic Profile in Healthy Puppies

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Simple Summary: In this study, dietary changes in puppies were observed to cause different gastrointestinal responses. Using two change methods, one direct and one gradual, we found that a gradual transition reduced the incidence of diarrhea in puppies throughout the trial period, as well as the concentration of isovaleric acid. Meanwhile, 16S rRNA sequencing showed that the fecal microbiota was changed after different dietary changes. Compared with the bacterial changes after an abrupt dietary change, the relative abundances of beneficial bacteria (i.e., *Turicibacter* and *Faecalibacterium*) in feces were increased after a gradual dietary transition in puppies. Additionally, both change methods caused changes in amino acid metabolism, while an abrupt change also altered lipid metabolism. An abrupt change increased fecal histamine and spermine concentrations, but decreased concentrations of metabolites such as 5-hydroxyindoleacetic acid and serotonin. Our findings indicated that a gradual transition most likely reduced the diarrhea rate in puppies by modulating the composition and metabolism of the gut microbiota.



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Abstract: Dietary changes are inevitable for pets, yet little is known about the impact of different dietary change methods on the gastrointestinal response. The current comparative study evaluated the effects of different dietary changes on the diarrheal symptoms, fecal fermentation characteristics, microbiota, and metabolic profile of healthy puppies. A total of 13 beagle puppies were randomly divided into two groups; puppies in the abrupt change (AC) group were given 260 g of a chicken- and duck-based extruded diet (CD) daily for the one-week transition period, whereas puppies in the gradual transition (GT) group were fed according to a gradual transition ratio of a salmon-based extruded diet (SA) and a CD diets with a difference of 40 g per day for seven consecutive days. Serum samples were collected on D7, and fecal samples were collected on D0 and D7. The results indicated that GT reduced the incidence of diarrhea in puppies throughout the trial period. Dietary change methods had no influence on serum inflammatory factors or fecal SCFAs, but isovaleric acid was significantly reduced after GT. Meanwhile, 16S rRNA sequencing showed that the fecal microbiota was changed after different dietary changes. Compared with the bacterial changes after AC, the relative abundances of beneficial bacteria (i.e., *Turicibacter* and *Faecalibacterium*) in feces were increased after GT in puppies. Additionally, both GT and AC caused changes in amino acid metabolism, while AC also altered lipid metabolism. AC increased fecal histamine and spermine concentrations, but decreased concentrations of metabolites such as 5-hydroxyindoleacetic acid and serotonin. Our findings indicated that GT most likely reduced the diarrhea rate in puppies by modulating the composition and metabolism of the gut microbiota.

Keywords: dietary change; beagle dog; fermentation characteristics; fecal microbiota; metabolomics

1. Introduction

It is well-known that feeding different diets to dogs and cats at different life stages is necessary to meet their nutrient requirements [1]. In other species, dietary changes often induce changes in nutrient digestion, absorption, growth performance, and the gut microbiota (GM) due to different dietary compositions [2–5]. A sudden dietary change often results in diarrhea in pets, but the underlying mechanism remains unknown [6]. Diarrhea is highly associated with gut microbiota alterations. Moon et al. determined colonization of the small intestine as initial evidence pointing to the notion that *Escherichia coli* caused diarrheal disease in newborn pigs now recognized as enterotoxigenic *E. coli* infection [7,8]. Similarly, *Shigella*, *Salmonella*, and *Clostridium difficile* cause diarrhea, and toxic substances produced by these pathogens further cause abnormal gut function and immune responses, leading to the occurrence of diarrhea [8,9]. At the same time, harmful bacteria can compete for resources and implant sites in the gastrointestinal tract, thus inhibiting the number of beneficial bacteria [8–11]. Conversely, beneficial bacteria such as *Lactobacillus*, yeast, and *Bifidobacterium* can be applied to treat pathogen-caused diarrhea by maintaining the balance of GM [12]. Other beneficial bacteria, such as *Firmicutes*, *Faecalibacterium*, and *Coprococcus* bacteria, produce short-chain fatty acids (SCFAs), which serve as the carbon energy for intestinal epithelial cells, and SCFAs help to maintain the colorectum function, as well as the morphology and function of colonic epithelial cells [13]. Recent evidence has begun to link the gut microbiome and its metabolites in mice and humans to gastrointestinal diseases and inflammation [14,15]. Therefore, we hypothesized that the diarrhea problems caused by sudden dietary changes may be caused by changes in the GM.

The current study investigated and compared the effects of two dietary change protocols, namely abrupt change (AC) and gradual transition (GT), on puppies. We evaluated the stool quality, incidence of diarrhea, inflammatory responses, and fecal SCFA content at the beginning and end of the dietary change period. Meanwhile, 16Sr RNA gene sequencing and untargeted metabolomics were adopted to capture changes in the microbiota and metabolic pathways, as well as to identify potential metabolic matters. Therefore, the objective of this research was to determine whether there were key bacteria and/or metabolites to expound the influence of dietary changes on puppies, and whether GT could reduce the changes in GM and metabolism attributed to dietary changes, as well as maintain health in puppies.

2. Materials and Methods

2.1. Animal, Diet, Treatment, and Experimental Design

All processes were approved by the Animal Care and Use Committee prior to animal experimentation (Approval number: 2019188), following the principles of the Center of Laboratory Animal at South China Agricultural University. Animal care staff monitored animal health on a daily basis.

Thirteen 6-month-old beagle puppies were housed individually in a metabolic cage (1.2 m × 1.0 m × 1.1 m kennels) under a relatively constant environment, with a humidity of 70% ± 5%, temperature of 23 ± 1 °C, and 12 h dark/light cycle at the Center of Laboratory Animal of South China Agricultural University. Starting from 1 month before the experiment, none of the animals were dewormed or given drugs that could alter the GM, e.g., antibiotics. All puppies had access to toys at all times and were allowed to socialize outside their cages with humans and other animals for about 1 h at least 3 days a week. Clean water was freely available, and food was offered twice daily throughout the trial.

Two commercial extruded diets, a salmon-based extruded diet (SA) and a chicken- and duck-based extruded diet (CD), were purchased from Foshan Ramical Animal Nutrition and Health Care Technology Co., Ltd. (Foshan, China). Both diets were made from similar ingredients, including corn flour, flour, fish oil, chicken meal, duck meal, beef meal, fish meal, soybean meal, and amino acid, vitamin, and mineral premixes. The chemical and energy compositions of the basal diets are presented in Table 1. Both diets complied with all nutritional recommendations for puppies by the American Association of Feed Control

Officials (AAFCO, 2017). According to the National Research Council (NRC, 2006), each dog had a total feed intake of 260 g, split into two meals per day.

Table 1. The chemical and energy compositions of SA and CD diets.

Items *	SA	CD
DM (%)	91.19	91.68
OM (%)	91.57	92.40
CP (%)	27.69	27.91
EE (%)	11.13	11.58
TDF (%)	3.63	2.94
GE (kJ/g)	16.8	18.49

* All test methods were in accordance with the national standard. DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; TDF: total dietary fiber; GE: gross energy. SA: salmon-based extruded diet; CD: chicken- and duck-based extruded diet.

These puppies first adapted to the SA diet for two months, and then were changed to the CD diet. An outline of the dietary transition and the timeline of events is shown in Figure 1. All puppies were weighed and randomly divided into two groups at D0; there were no differences in body weight (BW) or BCS between the two groups. Puppies in the AC group ($n = 7$, four females and three males) were given 260 g of the CD diet daily for the one-week transition period. Puppies in the GT group ($n = 6$, four females and two males) were fed a gradual transition ratio of SA and CD diets for seven consecutive days as follows: 260:0, 220:40, 180:80, 140:120, 120:140, 80:180, 40:220, and 0:260 g. Body condition scores (BCS) were performed for each beagle on D0 and D7, using the Laflamme method [16]. Fecal scores (FS) were performed for each beagle on D1–D7, using the Middelbos method [17]. A soft stool rate refers to $3.5 \leq FS < 4$; a diarrhea rate refers to $4 \leq FS < 5$.

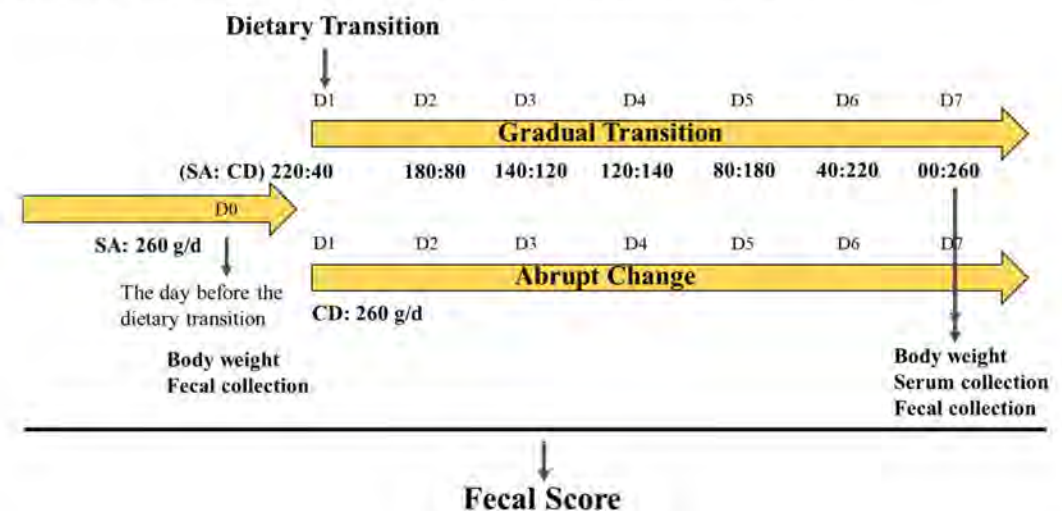


Figure 1. Experiment design and timeline. Beagle puppies were housed individually in metabolic cage. Body weight data and feces samples were collected on D0 and D7. Serum samples were obtained on D7. SA: salmon-based extruded diet; CD: chicken- and duck-based extruded diet. D0: the first day before the dietary transition; D1: the first day of the dietary transition; D7: the seventh day of the dietary transition.

2.2. Chemical Analysis of Diet

The SA and CD diets were collected on D1 and D7 of the dietary transition, and then stored in a dry pot. The feed samples were dried in an oven and pulverized, and then passed through a 1 mm screen for chemical composition analysis. The chemical and energy compositions of SA and CD diets are shown in Table 1.

2.3. Fresh Fecal Sample Collection and Preparation

A clean tray was placed under each dog cage to obtain fresh feces. During the two-week period, fecal samples were scored daily according to Middelbos [17], and fresh feces were collected within 15 min on D0 and D7 of the dietary transition. The feces from one animal were divided into three samples. Among them, two fecal samples were packed into 1.5 mL sterile and enzyme-free EP tubes for metabolomic analysis and measurement of SCFAs and branched-chain fatty acids (BCFAs), whereas one fecal sample was packed into a 5 mL germfree fecal collection tube for microbiota measurement. All samples were snap-frozen using liquid nitrogen and then transferred to a -80°C refrigerator for storage until analysis. The fecal samples were prepared for SCFA and BCFA analysis according to previous research in our laboratory [18]. Briefly, 0.2 g of each fecal sample in a 2 mL tube was combined with 1 mL of ultrapure water, and then vortexed for 2 min. The samples were subjected to ultrasonic crushing at 4°C for 10 min and centrifuged at 13,000 rpm, 4°C for 10 min. The supernatant was transferred into a new 2 mL centrifuge tube, before adding 20 μL of 25% metaphosphoric acid and 0.25 g of anhydrous sodium sulfate. The mix was then vortexed for 1 min, followed by the addition methyl tert-butyl ether in constant volume to 2 mL in the fuming cupboard. After vortexing for 5 min, the tubes were centrifuged at 13,000 rpm, 4°C for 5 min. The supernatant was filtered using a 0.22 μm membrane for GC-MS/MS analysis.

2.4. Blood Sample Collection and Analysis

A 5 mL blood sample was collected via the forelimb vein to determine the serum levels of immune factors on D7. The collected blood was left standing for 30 min and centrifuged at 3000 rpm at 4°C for 15 min. The supernatant of each sample was evenly distributed into three Eppendorf tubes and stored at -80°C . The levels of interferon- γ (IFN- γ , MM-35063O1), tumor necrosis factor-alpha (TNF- α , MM-36988O1), interleukin-4 (IL-4, MM-35084O1), interleukin-2 (IL-2, MM-85058O1), interleukin-6 (IL-6, MM-1546O1), immunoglobulin A (IgA, MM-85082O1), immunoglobulin M (IgM, MM-85090O1), and immunoglobulin G (IgG, MM-2086O1) in the serum of beagle puppies were determined by enzyme-linked immunosorbent assay (ELISA) using commercial kits (MEIMIAN, Jiangsu, China) following the products' instructions.

2.5. Extraction of DNA and High-Throughput Sequencing

Total bacteria DNA was extracted from frozen fecal samples with the Stool DNA Kit following the standard protocol (Tiangen, Beijing, China). The DNA concentration and purity were examined by 1% agarose gel electrophoresis. Then, DNA was diluted to 1 ng/ μL in germfree water.

Quantitative insights into microbial ecology were used to analyze the sequencing data bioinformatics. The 16S V3-V4 rRNA was amplified with region-specific primers (i.e., 341F: CCTAYGGGRBGCASCAG and 806R: GGACTACNNGGGTATCTAAT), where F and R denote forward and reverse, respectively. The PCR reaction was performed using 15 μL of Phusion[®] High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA) with the following conditions: 98°C for 1 min (1 cycle) for initial denaturation, followed by 30 cycles at 98°C for 10 s for denaturation, 50°C for 30 s for annealing, 72°C for 30 s for elongation, and a last step of 72°C for 5 min for final extension. The same volume of 1 \times loading buffer was mixed with PCR products according to equidensity ratios, and the mixture of PCR products was purified using the Qiagen gel extraction kit (Qiagen, Hilden, Germany). Sequences with main band sizes between 400 and 500 bp were selected. The paired-end sequencing was executed by the Illumina NovaSeq 6000 platform (Novogene, Tianjin, China) according to the standard protocol from the manufacturer.

2.6. Bioinformatics Analysis

The bioinformatics analysis of sequencing data was carried out through Quantitative Insights into Microbial Ecology (QIIME, V1.9.1, http://qiime.org/scripts/split_libraries_

[fastq.html](#), accessed on 2 March 2022). Paired-end sequences from the original DNA-overlapped fragment were merged using FLASH (V1.2.7, <http://ccb.jhu.edu/software/FLASH/>, accessed on 2 March 2022). Sequence demultiplexing and stitching, quality filtering, and analysis were performed using the unweighted pair-group method with arithmetic means (UCHIME, http://www.drive5.com/usearch/manual/uchime_algo.html, accessed on 2 March 2022), and the effective tags were obtained.

Operational taxonomic units (OTUs) with a 97% similarity threshold in the sequence were subsequently normalized in order to analyze α and β diversities. The sequence was chosen as a representative for each OTU, and the RDP Classifier (V2.2, <http://sourceforge.net/projects/rdp-classifier/>, accessed on 2 March 2022) was used to label taxonomic information for each representative sequence. A representative sequence was picked for each OUT, and taxonomic information was annotated using the mothur algorithm by the Silva Database (<http://www.arb-silva.de/>, accessed on 2 March 2022). MUSCLE software (V3.8.31, <http://www.drive5.com/muscle/>, accessed on 2 March 2022) was used to carry out multiple-sequence alignment to study the phylogenetic relationship of different OTUs. The anosim index and α diversity (i.e., observed species, Chao1, Shannon, Simpson, ACE, and PD_whole_tree) for the significance of differences within and between groups and the complexity of species diversity for an individual sample were generated with QIIME (V1.7.0) and displayed with R software (V2.15.3). The β diversity for evaluating differences among samples in species complexity was calculated by QIIME software. Cluster analyses containing weighted_unifrac and unweighted_unifrac distances were performed using principal coordinate analysis (PCoA) and displayed using the WGCNA package, stat package, and ggplot2 package in R software (V2.15.3). Linear discriminant analysis coupled with effect size (LEfSe) was adopted to distinguish the bacterial taxa differentially represented between groups at genus or higher taxonomy levels. The default setting of LEfSe software was an LDA score of >4 (<http://huttenhower.sph.harvard.edu/lefse/>, accessed on 25 June 2022).

2.7. Metabolite Extraction

The feces were also used for metabolomics analysis, and the sample preparation began with 60 mg of fecal sample in a 2 mL tube, to which 600 μ L of methanol/water (1:1, *v/v*) and 20 μ L internal standard (L-2-chlorophenylalanine, 0.3 mg/mL, methanol configuration) were added. Sample tubes were homogenized and exposed to 10 min of ultrasonic crushing. Samples were left standing at -20°C for 30 min. Centrifugation was then performed at 14,500 rpm, 4°C for 15 min; then, 200 μ L of supernatant was transferred into a new tube and steamed to dry by a vacuum centrifuge. Next, 200 μ L of methanol/water (1:1, *v/v*) was added to each tube. After vortexing for 30 s, the tubes were exposed to ultrasonic crushing at 4°C for 10 min and centrifuged at 14,500 rpm, 4°C for 15 min. Finally, all supernatant was filtered using a 0.22 μ m membrane for LC–MS/MS analysis. Quality control (QC) was performed by mixing different individual fecal samples at each into a 2 mL tube, referring to the above process.

2.8. UPLC–Orbitrap–MS/MS and Metabolite Profiling Analysis

The process of UPLC–Orbitrap–MS/MS analysis was adapted from [19]. Analytical instruments usually cannot provide undefined and visualized information about metabolites. Sequence preprocessing of the original data is needed to obtain a feasible data matrix, and includes noise filtering and baseline correction, peak detection and deconvolution, alignment, and normalization. Compound Discoverer 2.1 (CD, Thermo Fisher Scientific), a flexible and automated data analysis tool, was used to perform data analysis. CD can identify small molecular metabolites with high accuracy using various tools including mzCloud (online spectral library with >2 million spectra), ChemSpider (chemical structure database with >500 data sources, 58 million structures), mzVault (local spectral libraries), and Masslist (local databases).

2.9. Statistical Analysis

All statistical computation was conducted using SPSS 26.0 (IBM, Armonk, NY, USA). Significances and tendencies were set at $p < 0.05$ and $0.05 \leq p < 0.10$, respectively. Paired or unpaired Student's *t*-tests and chi-square tests were conducted to assess the main treatment effect on different measures, and results were visualized using GraphPad Prism 8.0.2 (GraphPad Software Inc., La Jolla, CA, USA). Data are expressed as the means \pm standard error of the mean (SEM) unless otherwise stated in the text and legends.

Metaboanalyst 5.0 (<https://www.metaboanalyst.ca/>, accessed on 15 October 2022) was used to perform principal component analysis (PCA). Partial least-squares discriminant analysis (PLS-DA) and pathway impact analysis were also performed in this study. The results were visualized with Metaboanalyst 5.0. Spearman's Rho was performed to calculate the correlation between the relative abundance of different microbiota and the relative abundance of metabolites.

3. Results

3.1. FS, Diarrhea Rate

No abnormal feeding behavior, such as changes in dietary intake, or other health problems were observed in the puppies during the dietary transition period. Interestingly, the AC group had significantly higher mean FS than the GT group during the dietary transition period ($p < 0.05$), as well as a higher mean soft stool rate and diarrhea rate than the GT group during the dietary transition period (Table 2).

Table 2. Effects of different dietary change methods on FS, soft stool rate, and diarrhea rate.

Items	GT ¹	AC ²	SEM	<i>p</i> -Value ³
FS	2.607 ^b	2.837 ^a	0.046	0.009
Soft stool rate	7.14%	10.20%	-	0.721
Diarrhea rate	0	10.20%	-	0.059

¹ GT, gradual transition, $n = 6$. ² AC, abrupt change, $n = 7$. ³ Statistical analysis of FS by unpaired Student's *t*-test; statistical analysis of soft stool rate and diarrhea rate by chi-square test. ^{a,b} Means within a row with different superscript letters differ significantly ($p < 0.05$).

3.2. BCS, Fecal pH, and BW

As shown in Table 3, fecal pH in the GT group was not changed after the dietary transition period ($p > 0.05$), while the AC group had significantly reduced fecal pH ($p < 0.05$). BW fluctuated significantly after dietary change ($p < 0.001$), which is logical as the animals were growing, whereas BCS had no significant change ($p > 0.05$). In addition, there was no difference in BW between the two groups because of the same dietary intake ($p > 0.05$).

Table 3. Effects of different dietary change methods on BCS, fecal pH, and total BW.

Groups	Items	Before Change	After Change	SEM	<i>p</i> -Value ³
GT ¹	BCS	5.00	5.00	0	-
	Fecal pH	6.75	6.75	0.109	1.000
	BW (kg)	8.48 ^b	8.85 ^a	0.160	0.000003
AC ²	BCS	5.14	5.07	0.058	0.356
	Fecal pH	6.80 ^a	6.56 ^b	0.060	0.021
	BW (kg)	8.48 ^b	8.86 ^a	0.144	0.000017

¹ GT, gradual transition, $n = 6$. ² AC, abrupt change, $n = 7$. ³ Statistical analysis by paired Student's *t*-test. ^{a,b} Means within a row with different superscript letters differ significantly ($p < 0.05$). BCS, body condition score; BW, body weight.

3.3. Fecal SCFAs and BCFAs

As shown in Table 4, fecal SCFAs had no significant change after dietary change ($p > 0.05$). Isovaleric acid was significantly reduced after GT ($p < 0.05$), while other BCFAs had no significant change after dietary change ($p > 0.05$).

Table 4. Fecal SCFA and BCFA contents between different groups.

Items	GT1 ¹	GT2 ²	SEM	p-Value ³	AC1 ⁴	AC2	SEM	p-Value ⁵
Total acid (μg/g)	3919.500	4066.950	258.612	0.823	4375.781	4117.884	91.880	0.141
SCFAs (μg/g)	3390.796	3593.004	239.989	0.754	3842.939	3665.601	70.025	0.197
Acetic acid (μg/g)	1622.438	1775.754	143.217	0.677	1909.718	1809.504	31.706	0.071
Propionic acid (μg/g)	1230.793	1281.584	85.092	0.828	1370.544	1315.499	30.127	0.333
Butyric acid (μg/g)	537.565	535.666	16.698	0.967	562.678	540.597	14.607	0.518
BCFAs (μg/g)	528.704	473.946	35.865	0.226	532.841	452.284	26.642	0.110
Isobutyric acid (μg/g)	200.018	173.020	21.244	0.398	195.330	165.239	10.978	0.196
Isovaleric acid (μg/g)	289.578	256.938	15.884	0.048	290.874	239.903	14.865	0.059
Pentanoic acid (μg/g)	39.108	43.988	3.729	0.518	46.638	47.141	2.558	0.924

¹ GT1, before gradual transition, $n = 6$. ² GT2, after gradual transition, $n = 6$. ³ AC1, before abrupt change, $n = 7$.

⁴ AC2, after abrupt change, $n = 7$. ⁵ Statistical analysis by paired Student's *t*-test. SCFAs, short-chain fatty acids; BCFAs, branched-chain fatty acids.

3.4. Inflammatory Cytokines

It can be seen from Table 5 that serum inflammatory cytokines had no significant difference between the GT and AC groups after dietary change ($p > 0.05$), indicating that the current dietary change did not induce detectable inflammation in beagle puppies.

Table 5. Inflammatory cytokine serum levels in different groups after dietary change.

Items *	GT	AC	SEM	p-Value
IFN-γ (pg/mL)	115.65	117.81	1.219	0.419
IL-4 (ng/L)	152.25	157.97	1.694	0.108
IL-2 (ng/L)	168.07	169.61	1.545	0.652
IL-6 (ng/L)	45.60	46.12	0.468	0.615
IgA (ng/mL)	7934.36	7993.44	65.065	0.683
IgG (μg/mL)	89.62	89.97	0.866	0.854
IgM (ng/mL)	4217.13	4238.61	39.642	0.808
TNF-α (ng/L)	81.27	81.93	0.610	0.625

* Statistical analysis by unpaired Student's *t*-test. GT, gradual transition, $n = 6$. AC, abrupt change, $n = 7$. IFN-γ, interferon-γ; IL-4, interleukin-4; IL-2, interleukin-2; IL-6, interleukin-6; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; TNF-α, tumor necrosis factor-α.

3.5. Fecal Microbiota Composition

As shown by anosim analysis ($R > 0$; Figure 2A), there was a significant difference between the AC1 and AC2 groups ($p < 0.05$), indicating that AC had a greater influence on the intestinal microbiota than GT. The α -diversity indices Chao 1, Shannon, Simpson, Ace, Goods_coverage, and Observed_species were not significantly different in the GT or AC groups before and after dietary change (Figure 2B) ($p < 0.05$).

β -diversity index analysis was performed to determine similarities between pairs of microbial communities between the two groups, and a PCoA was performed using weighted and unweighted UniFrac distance matrices. The PCoA plots showed no obvious separation of the GT or AC groups (Figure 3).

Column abundance chart analysis of the microbiota with the top 10 abundances at the phylum level testified a distinct microbiota composition among the four treatment groups (i.e., AC1, AC2, GT1, and GT2). The most abundant phyla included *Firmicutes* (49.99%), *Proteobacteria* (19.94%), *unidentified_Bacteria* (14.05%), *Fusobacteriota* (5.75%), *Actinobacteriota* (3.20%), and *Bacteroidota* (2.46%) (Figure 4A). The relative abundance of *Actinobacteriota* and *Fibrobacterota* tended to decrease in GT ($p < 0.05$) (Figure 4B). The column abundance chart analysis of the top 30 abundances of microbiota of the four treatment groups at the genus level is shown in Figure 4C. The most abundant genera included *Ralstonia* (18.82%), *Peptoclostridium* (9.20%), *Lactobacillus* (6.60%), *Turicibacter* (6.50%), *Allobaculum* (5.76%), and *Fusobacterium* (5.20%) in various groups. GT significantly reduced the relative abundances of *Lactobacillus* and *Clostridium_sensu_stricto_1* ($p < 0.05$) (Figure 4D).

AC significantly increased the relative abundances of *Dubosiella*, while reducing those of *Clostridium_sensu_stricto_1* ($p < 0.05$). *Clostridium_sensu_stricto_1* and *Prevotella* were significantly reduced after AC (Figure 4E).

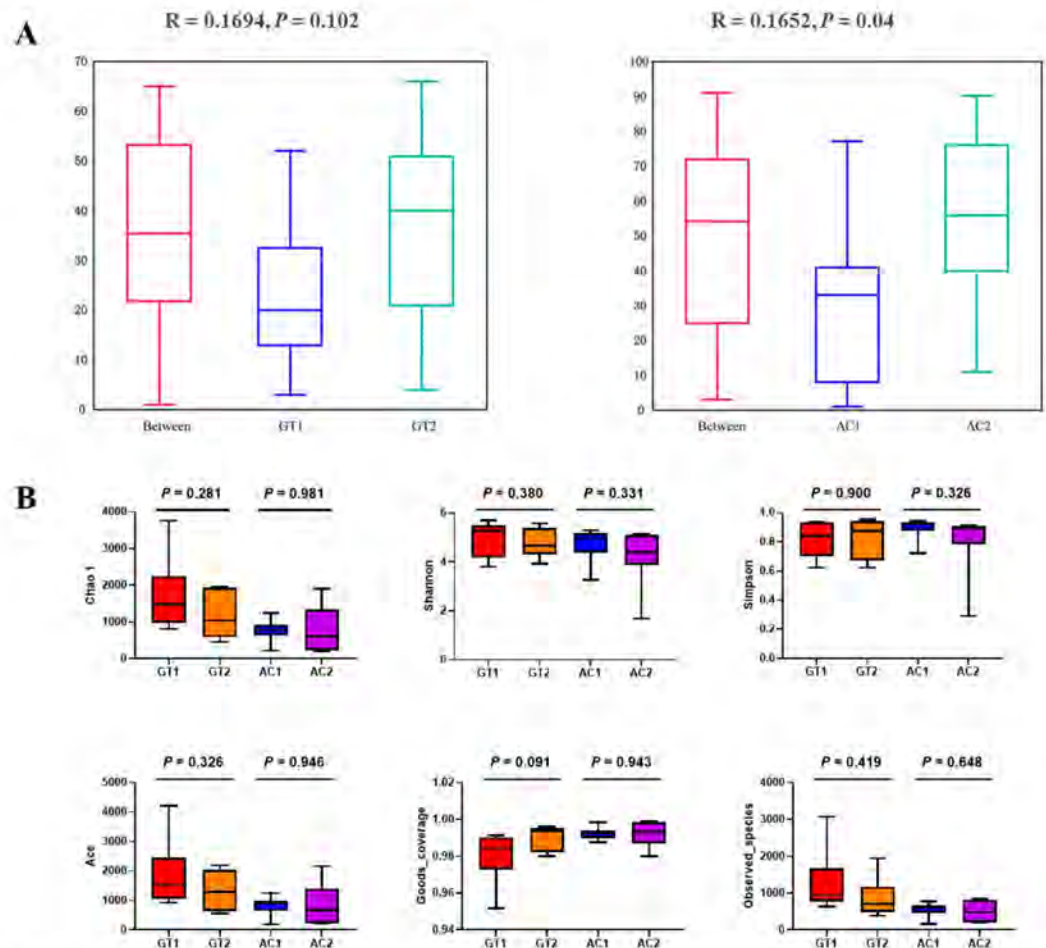


Figure 2. Anosim analysis and α -diversity index analysis of the GT and AC groups. The anosim test box pattern for the GT groups and AC groups (A), and the Chao 1, Shannon, Simpson, Ace, Goods_coverages, and Observed_species indices of the GT and AC groups (B). Values in (B) were analyzed by paired Student's *t*-test and are presented as the means \pm SE. GT1, before gradual transition, $n = 6$; GT2, after gradual transition, $n = 6$; AC1, before abrupt change, $n = 7$; AC2, after abrupt change, $n = 7$.

To further investigate the differential taxa abundances between the two dietary change methods, LEfSe (LDA > 4.0) was used to compare the bacterial taxa abundance in fecal samples between GT1 and GT2 and between AC1 and AC2. Ten bacteria between GT1 and GT2 (Figure 5A) and seven bacteria between AC1 and AC2 (Figure 5B) were identified. All of these identified bacteria had LDA > 4.0 , alpha < 0.01 (according to the factorial Kruskal–Wallis test), indicating that the differences in the abundances of bacterial colonies had biological significance [20]. The relative abundances of *g_Turicibacter*, *s_Turicibacter_sp_h121*, *f_Ruminococcaceae*, *o_Oscillospirales*, and *g_Faecalibacterium* were high in GT2, while *s_Lactobacillus_murinus*, *o_Lactobacillales*, *g_Allobaculum*, *f_Lactobacillaceae*, and *g_Lactobacillus* were rich in abundance in GT1. Meanwhile, the relative abundances of *g_Streptococcus* and *f_Streptococcaceae* were high in AC2, and *g_Allobaculum*, *f_Peptostreptococcaceae*, *o_Peptostreptococcales_tissierellales*, *c_Clostridia*, and *s_Lactobacillus_reuteri* were abundant in AC1.

3.6. Fecal Metabolic Profile and Pathway Analysis

Fecal samples from the GT and AC groups were analyzed by metabolomics. After executing a series of pretreatments to correct the raw data, multivariate statistical analyses of these metabolites were performed, including PCA, PLS-DA, metabolic pathway analysis, and Spearman correlation analysis. This study analyzed the differences before and after dietary change in the GT and AC groups. The PCA score plots and PLS-DA model of GT1 and GT2 had different clusters of metabolites (Figure 6A,C). In the AC group, metabolites from AC1 and AC2 were completely separated into distinct clusters (Figure 6B,D), indicating that AC caused more dramatic changes than GT. In this study, we adopted MetaboAnalyst 5.0 to investigate the potential metabolic pathways influenced by GT and AC. Metabolites identified in the GT ($n = 29$) and AC ($n = 50$) groups were analyzed for pathways, and the charts in GT and AC are shown in Figure 6E,F. Four metabolic pathways (thiamine metabolism, aminoacyl-tRNA biosynthesis, alanine, aspartate, and glutamate metabolism, and purine metabolism) were affected after GT. Additionally, a series of metabolic pathways were affected after AC, including amino acid metabolism (i.e., aspartate, tryptophan, beta-alanine, histidine, methyl-histamine, methionine, glycine, and serine metabolism) and lipid metabolism (i.e., steroidogenesis, and the biosynthesis of phospholipid, phosphatidylcholine, and phosphatidylethanolamine).

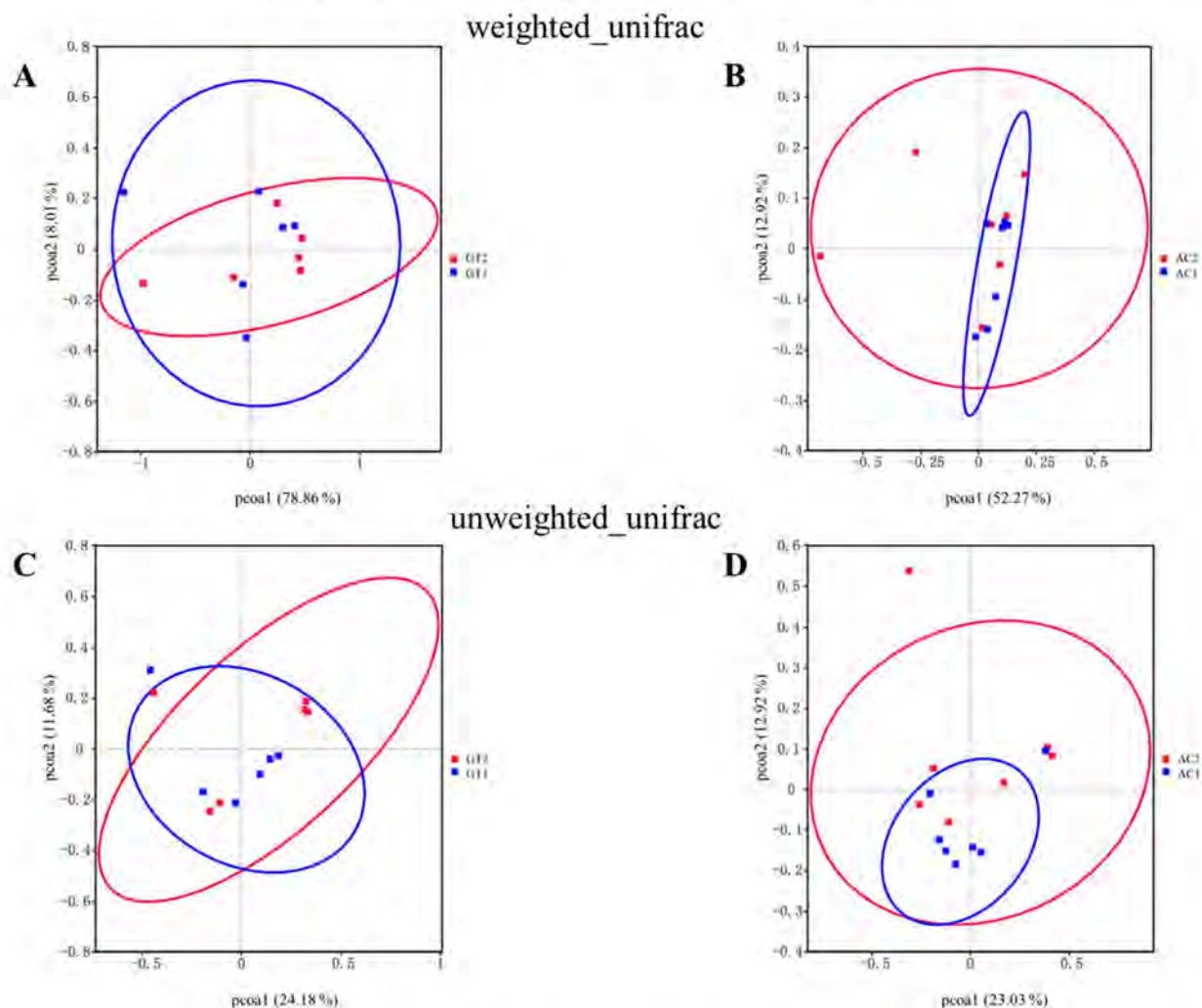


Figure 3. The β -diversity index analysis of GT and AC groups. The principal coordinate analysis (PCoA) of weighted_unifrac (A,B) and unweighted_unifrac (C,D) of GT groups and AC groups. GT1, before gradual transition, $n = 6$; GT2, after gradual transition, $n = 6$; AC1, before abrupt change, $n = 7$; AC2, after abrupt change, $n = 7$.

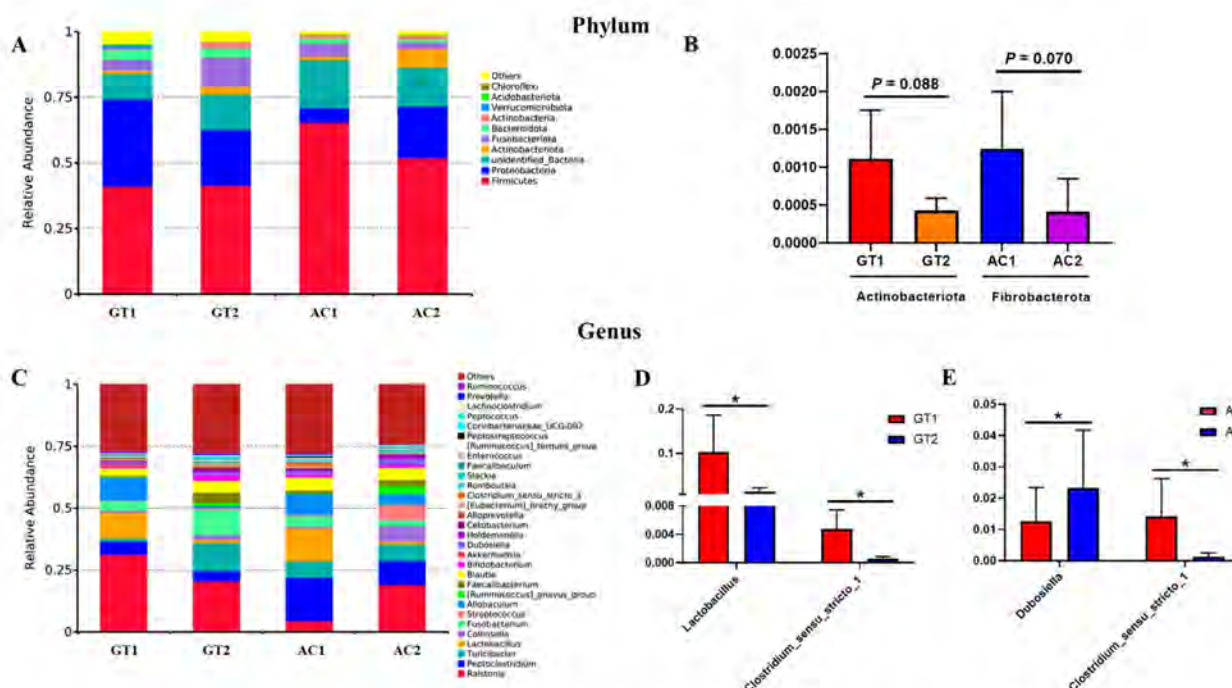


Figure 4. Abundance distribution and column chart of the fecal microbiota. Abundance distribution of the top 10 phyla in GT and AC groups (A); column chart in in GT and AC groups (B). Abundance distribution of the top 30 genera in GT and AC groups (C); column chart in GT (D) and AC groups (E). Values in (A–D) were analyzed by paired Student's test and are presented as the means \pm SE. GT1, before gradual transition, $n = 6$; GT2, after gradual transition, $n = 6$; AC1, before abrupt change, $n = 7$; AC2, after abrupt change, $n = 7$. * Significant difference between two groups (* $p < 0.05$).

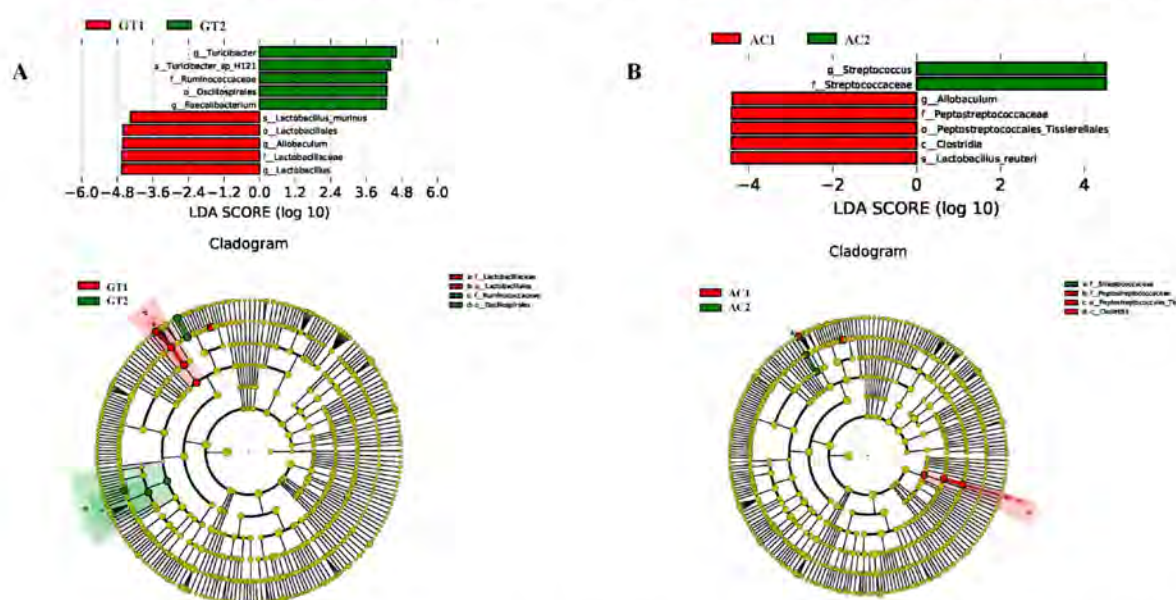


Figure 5. LEfSe analysis identified fecal bacteria of GT (A) and AC (B) groups. The histogram of the LDA score shows that the abundance of species differed significantly between different groups. The LDA score represents the size of the effect. In the cladogram, the circles radiating from the inside to the outside represent the classification level from the phylum to the genus (species). The diameter of each circle is proportional to the relative abundance of taxa. Red nodes refer to the bacteria that contributed greatly in GT1 or AC1, whereas green nodes refer to the bacteria dominant in GT2 or AC2. GT1, before gradual transition, $n = 6$; GT2, after gradual transition, $n = 6$; AC1, before abrupt change, $n = 7$; AC2, after abrupt change, $n = 7$.

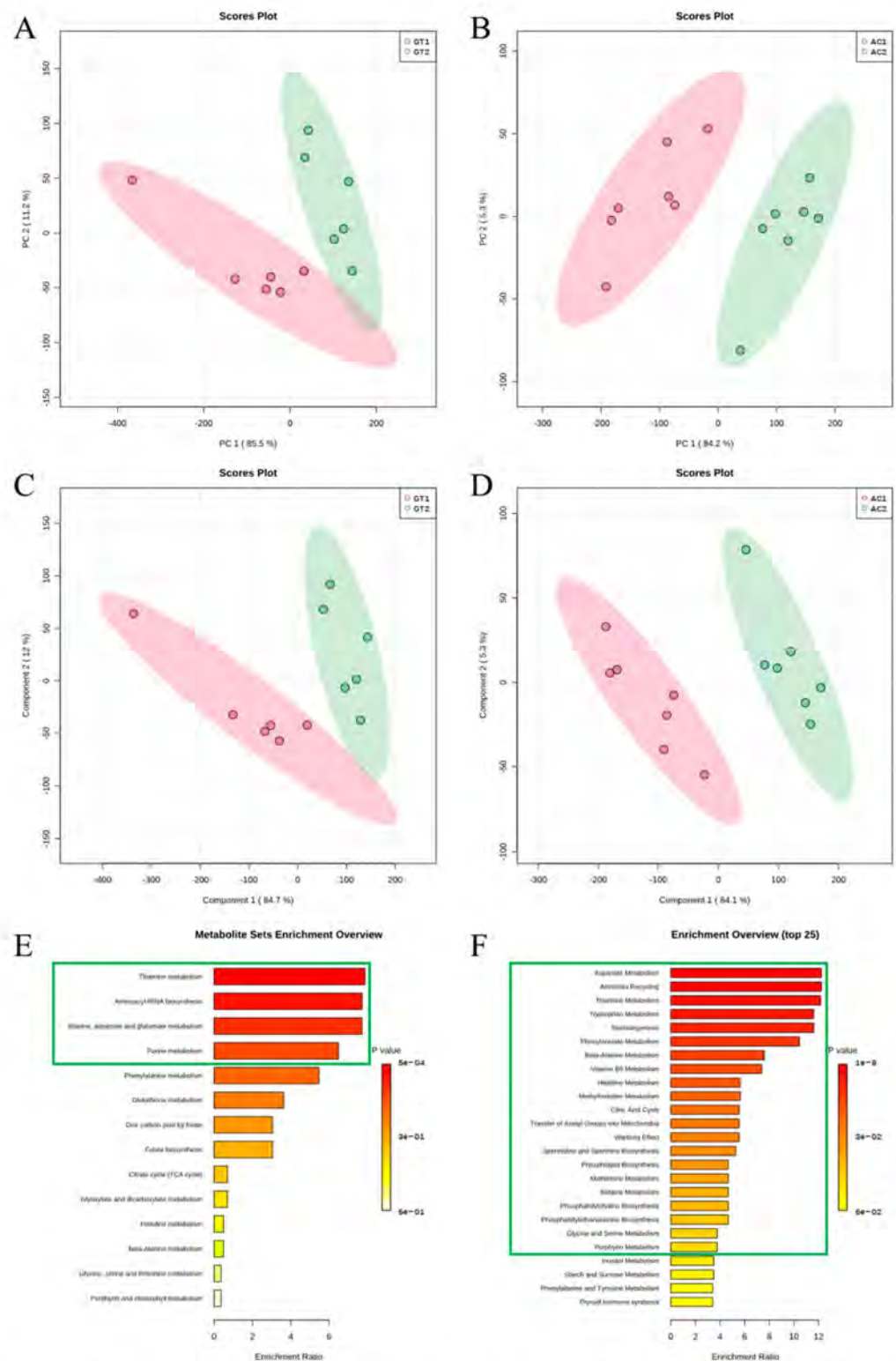


Figure 6. Multivariate statistical analysis of GT and AC groups. Score plots from the PCA model in GT groups (A) and AC groups (B). Score plots from the PLS-DA model in GT groups (C) and AC groups (D). Metabolism pathway analysis in GT groups (E) and AC groups (F). For (E,F), the x axis is the pathway impact, and the y axis is the pathway enrichment. Larger sizes and darker colors mean greater pathway enrichment and greater pathway impact values. GT groups, GT1 and GT2; AC groups, AC1 and AC2. GT1, before gradual transition, $n = 6$; GT2, after gradual transition, $n = 6$; AC1, before abrupt change, $n = 7$; AC2, after abrupt change, $n = 7$.

Changes in metabolites are shown in Figure 7. Overall, AC had more metabolite changes than GT. The contents of thiamine, L-asparagine, L-histidine, and citric acid were significantly upregulated, whereas those of phenylacetic acid and hypoxanthine were significantly downregulated (Figure 7A). Eight compounds (i.e., L-asparagine, L-histidine, thiamine, carnosine, histamine, citric acid, spermine, and 5-aminolevulinic acid) were significantly upregulated, while seven compounds (i.e., indoleacetic acid, serotonin, 5-hydroxyindoleacetic acid, corticosterone, phenylacetic acid, 4-pyridoxic acid, and choline) were significantly downregulated after AC (Figure 7B).

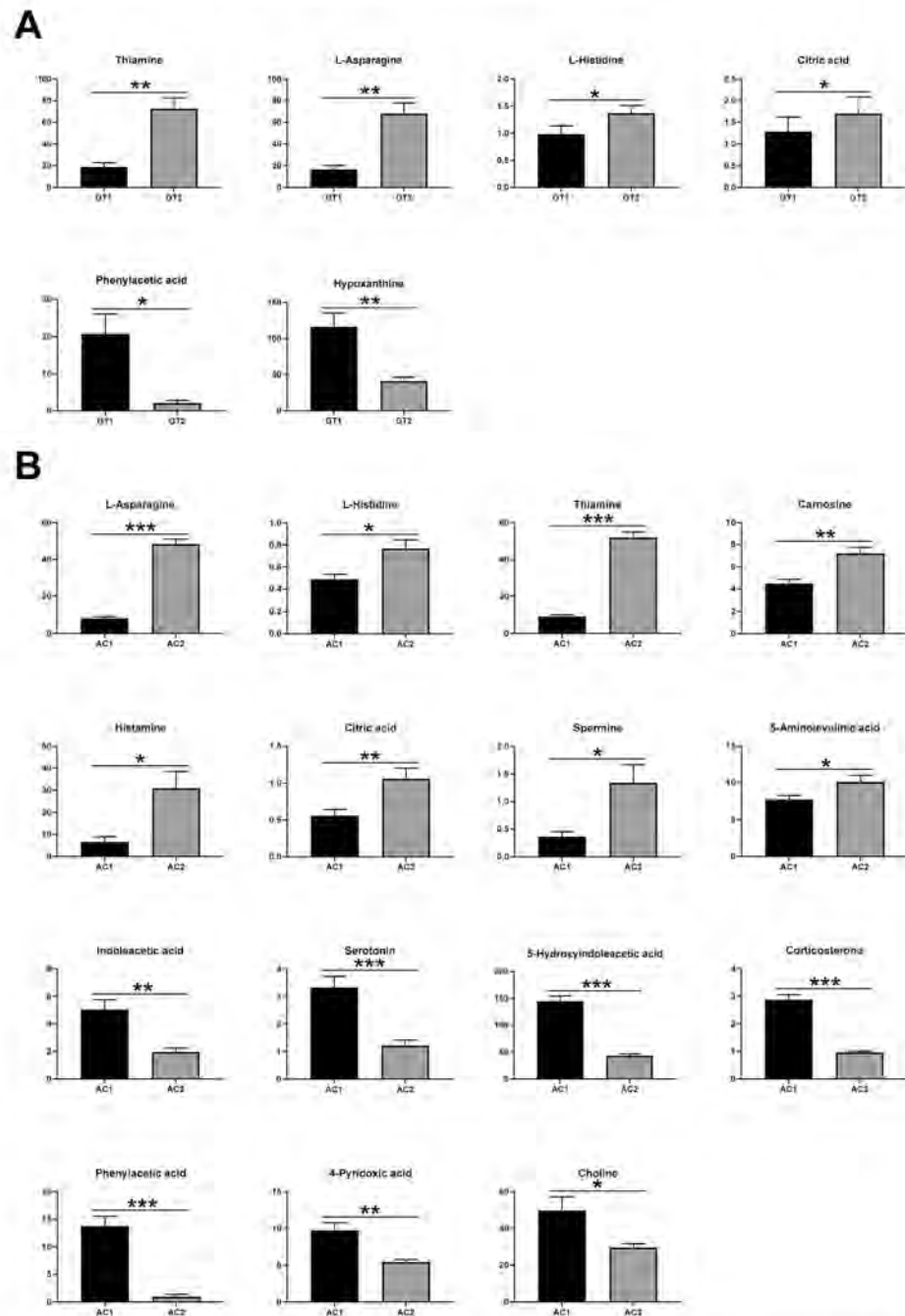


Figure 7. Boxplot of key metabolites of GT (A) and AC (B) groups. The *p*-values in (A,B) were analyzed by paired Student's *t*-test and are presented as the means \pm SE. GT1, before gradual transition, *n* = 6; GT2, after gradual transition, *n* = 6; AC1, before abrupt change, *n* = 7; AC2, after abrupt change, *n* = 7. * Significant difference between two groups (* *p* < 0.05, ** *p* < 0.01, and *** *p* < 0.001).

3.7. The Correlation Analysis of Fecal Metabolites and GM

Spearman correlation analysis was used to determine the relationship of the differential metabolites and microbiota. The results of GT are shown in Figure 8A; hypoxanthine was positively correlated with beneficial bacteria such as *Allobaculum*, *Clostridium_Sensu_Stricto_1*, and *Lactobacillus*, but negatively correlated with *Ruminococcaceae* and *Turicibacter*. Phenylacetic acid was positively correlated with *Clostridium_Sensu_Stricto_1* and *Lactobacillus*, but negatively correlated with *Faecalibacterium*. L-asparagine and thiamine were negatively correlated with *Allobaculum*, *Clostridium_Sensu_Stricto_1*, and *Lactobacillus*, but positively correlated with *Turicibacter*, *Holdemanella*, *Faecalibacterium*, and *Ruminococcaceae*. Hippuric acid was negatively correlated with *Lactobacillus_murinus*. In AC (Figure 8B), *Streptococcaceae* and *Streptococcus* were positively correlated with L-histidine, spermine, citric acid, carnosine, L-asparagine, and thiamine, but negatively correlated with 5-hydroxyindoleacetic acid, phenylacetic acid, corticosterone, and serotonin. In addition, *Lactobacillus_reuteri* and *Clostridium_sensu_stricto_1* had a positive correlation with choline, 5-hydroxyindoleacetic acid, phenylacetic acid, 4-pyridoxic acid, indoleacetic acid, corticosterone, and serotonin. Simultaneously, *Clostridium_sensu_stricto_1* was negatively correlated with 5-aminolevulinic acid, spermine, histamine, citric acid, L-asparagine, and thiamine. Moreover, *Allobaculum* was negatively correlated with spermine, carnosine, L-asparagine, and thiamine, but positively correlated with 5-hydroxyindoleacetic acid, phenylacetic acid, and indoleacetic acid. *Peptostreptococcaceae* and *Peptostreptococcales-tissierellales* were negatively correlated with citric acid, carnosine, L-asparagine, and thiamine, but positively correlated with 5-hydroxyindoleacetic acid.

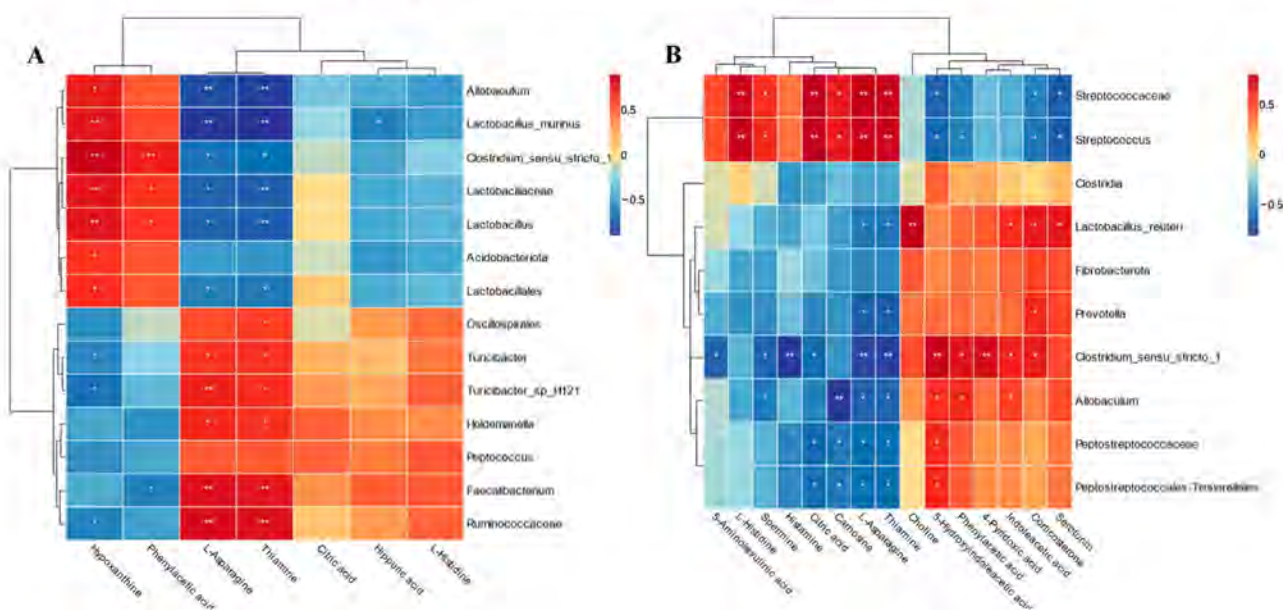


Figure 8. Spearman correlation analysis between the differential fecal metabolites and fecal microbiota in GT groups (A) and AC groups (B). GT groups, GT1 and GT2; AC groups, AC1 and AC2. GT1, before gradual transition, $n = 6$; GT2, after gradual transition, $n = 6$; AC1, before abrupt change, $n = 7$; AC2, after abrupt change, $n = 7$. * $p < 0.05$, ** $p < 0.01$.

4. Discussion

Dietary changes can cause alterations in pets' bodily functions such as diarrhea, metabolic pathways, and the GM. Previous research has shown that a gradual transition can make ruminants more resilient to highly fermented diets [21,22]. In this study, we explored whether a 7-day gradual transition could alleviate the harm of dietary change on pets. Our data showed that GT significantly reduced FS, which was closer to the normal value of 2.5 points, while GT had a lower soft stool rate and did not cause diarrhea in puppies. The results indicated that, to an extent, GT could reduce the gastrointestinal

responses caused by dietary change. Moreover, AC rather than GT significantly lowered fecal pH. Lin et al. found that diarrhea in beagle dogs decreased on the second day following a rapid dietary transition [23].

Although responses to dietary change can be complex, gastrointestinal symptoms are among the most observed symptoms; hence, the GM could play an important role [24]. The current study showed that dietary change also affected the GM composition.

Both AC and GT had no significant effect on the diversity of GM, but AC reduced the relative abundance of *Fibrobacterota*, which is the main bacteria that degrades fibers and is potentially involved in the production of SCFAs [25]. Both GT and AC decreased the relative abundance of *Clostridium_sensu_stricto_1*; Liu et al. found the relative abundance of *Clostridium_sensu_stricto_1* was higher in human patients with irritable bowel syndrome [26].

In the LEfSe analysis, *Turicibacter* and *Faecalibacterium* were significantly enriched after GT. It has previously been shown that low levels of *Turicibacter* can affect the gut ecosystem [27], also being associated with diseases such as depression [28]. Studies have indicated that *Faecalibacterium* can produce SCFAs, particularly butyric acid in the intestine [29], inhibit the secretion of IL-6 and IL-8 in cells, and help prevent breast cancer [30,31]. After AC, *Streptococcus* was significantly enriched, which was shown to be negatively correlated with the production of butyric acid and valeric acid [32]. It is also noteworthy that *Lactobacillus* was reduced in both methods; one explanation for this may be the different composition of the SA and CD diets, while another explanation is that the puppies were still growing. Wells found that the amount of *Lactobacillus* in the feces of weaned piglets decreased over the growing period, which could be the basis for our second explanation [33].

Beneficial bacteria such as *Peptostreptococcaceae* were reduced after AC, which may have contributed to the increase in the rate of soft stool and diarrhea in puppies. In contrast, GT increased *Ruminococcaceae* and *Faecalibacterium*. The former mainly degrades various polysaccharides and fibers to produce SCFAs [34,35], while *Faecalibacterium* has strong anti-inflammatory properties and can produce butyrate [35,36]. *Clostridium* also produces butyrate [37]. The fact that SCFAs have anti-inflammatory effects in the intestine [38,39] may help to explain why GT could alleviate soft stool and diarrhea when changing diet. Thus, as observed in the AC group, an abrupt dietary change changed the structure of the gut microbial community and resulted in intestinal discomfort in beagle puppies, but a gradual dietary transition could minimize the disturbance of the microbiota and reduce negative intestinal symptoms.

Furthermore, fecal untargeted metabolomics revealed that GT affected 4 metabolic pathway changes; surprisingly, AC affected 11 metabolic pathways, mainly including amino acid and lipid metabolisms. Both dietary change methods altered amino acid metabolic pathways, possibly due to the different dietary compositions of SA and CD diets. When compared with AC, GT caused fewer changes in metabolic pathways, indicating that GT could reduce the impact of dietary change on metabolic changes. We also found that AC significantly increased the levels of two biogenic amines, histamine and spermine. Histamine and spermine, when present in low concentrations, are considered markers of a healthy gut [40]. However, elevated levels of histamine and spermine are considered to be putrefactive and potentially carcinogenic in the gut [41,42]. Histamine is involved in the mechanism of headache from food intolerance by releasing nitric oxide from the vascular endothelium [43]. In the present study, histamine and spermine levels were significantly elevated after AC, implying that proteins were abnormally fermented in the gut, which may endanger the health of beagle puppies.

In addition, 5-hydroxyindoleacetate (5-hydroxyindoleacetic acid) declined dramatically after AC. In human studies, Shen et al. found that patients with colorectal cancer had a significant decline in fecal 5-hydroxyindoleacetate [44,45]. 5-Hydroxytryptamine (5-HT) exerts a significant role in mammalian central nervous system embryogenesis and brain ontogeny. Higher 5-HT concentrations would decrease the severity and frequency of episodes

of Scottie cramp; moreover, the serum 5-HT levels of puppies affected by canine parvovirus type II increased after treatment [45,46]. Roles of 5-HT include scavenging free radicals and exerting anti-oxidative, anti-inflammatory, and analgesic effects [47–49]. Decreased serotonergic activity is observed in patients with neuron disorders such as depression, mania, phobia, post-traumatic stress disorder, and generalized anxiety disorder [50–52]. The current study showed that 5-HT declined significantly after AC. Simultaneously, 5-HT can induce the secretion of corticosterone [53]. In this study, both serotonin and corticosterone were decreased after AC. Briefly, AC raised the content of biogenic amine in the intestine, but it reduced the content of some metabolites such as 5-HT, which may have caused adverse reactions in the organism.

Next, we conducted a correlation analysis between fecal metabolites and fecal microorganisms. Results showed that hypoxanthine was positively correlated with *Lactobacillus* and *Clostridium_sensu_stricto_1*, while 5-HT was positively correlated with *Clostridium_sensu_stricto_1* but negatively correlated with *Streptococcus*. Conversely, histamine and spermine were positively correlated with *Streptococcus*, but negatively correlated with *Clostridium_sensu_stricto_1* and *Allobaculum*. Collectively, changes in the population and diversity of fecal microbiota may have contributed to the alterations of fecal metabolism resulting from different dietary change methods.

In summary, this study is the first to report that GT can reduce changes in hindgut-related metabolites and the microbial community structure caused by dietary changes in beagle puppies, thereby reducing the risk of diarrhea.

5. Conclusions

This study comprehensively evaluated the serum and fecal change caused by different dietary change methods in beagle puppies, committed to revealing the related mechanisms underlying the metabolism and microbiota responses of GT to alleviate the adverse reactions of dietary changes. GT reduced diarrhea induced by AC throughout the dietary transition period. Moreover, the fecal microbiota was changed after different dietary changes, and the relative abundances of beneficial bacteria (i.e., *Turicibacter* and *Faecalibacterium*) in feces were increased after GT in puppies. Metabolomics analysis revealed that AC resulted in more metabolic disorders in the gut. In addition, AC increased fecal histamine and spermine levels, but decreased 5-hydroxyindoleacetic acid and serotonin levels. In summary, GT most likely attenuated diarrhea in puppies by modulating the composition and metabolism of gut microbiota. This study can be used as a basis for dietary change in pets; the physiological mechanisms involved need to be further studied.

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Review

Dietary Strategies for Relieving Stress in Pet Dogs and Cats

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Abstract: A variety of physical, emotional, and mental factors can induce a stress response in pet dogs and cats. During this process, hypothalamus–pituitary–adrenal (HPA) and sympathetic–adrenal medulla (SAM) axes are activated to produce a series of adaptive short-term reactions to the aversive situations. Meanwhile, oxidative stress is induced where there is an imbalance between the production and scavenging of reactive oxygen species (ROS). Oxidative damage is also incorporated in sustained stress response causing a series of chronic problems, such as cardiovascular and gastrointestinal diseases, immune dysfunction, and development of abnormal behaviors. In this review, the effects and mechanisms of dietary regulation strategies (e.g., antioxidants, anxiolytic agents, and probiotics) on relieving stress in pet dogs and cats are summarized and discussed. We aim to shed light on future studies in the field of pet food and nutrition.

Keywords: stress response; oxidative stress; dog and cat; dietary strategy; pet food and nutrition



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1. Introduction

With the improvement of living standards and changes in population structure (i.e., increasing single and geriatric populations), the number of domestic pets has largely increased in recent years, accompanied by the rapid growth of the pet industry and pet-related economy [1]. Meanwhile, welfare concerns for pets have become increasingly prominent [2]. Stressors exist ubiquitously along the pet industry chain, such as exposure to transportation and novel environments, and inappropriate caretaking strategies [3]. Diseases, behavioral problems, and even death can occur in animals if stress is not properly managed [4]. Being part of the stress response, oxidative stress is an important factor in the pathogenesis of many diseases, such as neural dysfunction and inflammatory bowel disease [5], and there is a complicated interaction between oxidative stress and disease progression [6]. In turn, aversive consequences from the stress response can challenge the animal's welfare, damage the pet–owner relationship, and increase the abandonment of pets, which could exert a threat to public safety [7] and biodiversity [8]. Appropriate management of stress in pets is therefore necessary and urgent.

The current paper attempts to comprehensively describe stress in pet dogs and cats, summarizing its causes, mechanisms, and potential consequences, and mainly focusing on dietary strategies for relieving pet stress. The hypothesis is that dietary ingredients that can address physiological and behavioral changes of stress response may serve as effective modifying strategies for stress management. The aim of this review was to identify the potential of some substances in relieving stress in pet dogs and cats and to provide a reference for the development of new functional pet foods targeting stress management.

2. Causes of Stress in Pets

Causes of stress (i.e., stressors) can be classified as physical (e.g., infection, hemorrhage) or psychological (e.g., restraint and threat). Stress in pets rises most commonly in situations when predictability is lacking or when the animal's needs are not met.

2.1. Environmental Factors

Uncomfortable environments can cause chronic stress in dogs and cats. Extreme temperature may lead to cold and heat stress [9]. At the same time, interruption of daily routines [10,11], vet visits [12], and novel environments [13] can also cause stress and anxiety, especially in cats. Abrupt environmental accidents such as sudden noise [14] and falling objects [15] usually result in panic and fear in pets. Even some common feeding practices, such as water-softened dry food can present stress in pets [16]. Psychological stress can occur when space allowance for activity and behavioral needs are not met [17,18]. Dogs and cats kept mostly or strictly indoors with little environmental enrichment may not be able to fully perform natural behaviors such as playing and hunting, the frustration from which can cause anxiety and depression, and the exhibition of behavioral and physical problems [19,20].

2.2. Social Conflicts

Pets living with humans can be exposed to imbalanced power because we are the ones that control their physical and social environment. Good human–pet relationships and the forming of bonds between pets and owners can provide mutual benefits [21,22]. Inappropriate or aversive interactions with pets can result in compromised or even broken relationships and cause additional stress in pets. Examples include the use of punishment [23,24], social deprivation [25,26], and some seemingly normal or intimate owner behaviors such as restraint [27] and forced interaction [4].

Pets may also encounter other inter- or intraspecific social conflicts, such as territorial disputes and miscommunication [28]. Conditions of limited space or resources (e.g., food) further increase the possibility of conflict outbreak [29]. The introduction of a new cat to a stable colony may interrupt the original social dynamic and cause fighting [30,31]. In addition, non-contact aversive social stimuli, such as exposure to dog barking, can also cause stress in cats [28].

3. Mechanisms of Stress

Stress response is elicited when an actual or potential threat to the homeostasis of the organism is perceived [32]. The process involves the activation of the hypothalamus–pituitary–adrenal (HPA) and sympathetic–adrenal medulla (SAM) axes as shown in Figure 1 [33,34]. As a result, changes in various physiological processes and behaviors are induced [33,34]. Oxidative damage is also incorporated in sustained stress response causing a series of chronic problems [6].

3.1. SAM and HPA Axis

Excitement of the sympathetic nervous system in the SAM axis promotes the release of acetylcholine from preganglionic fiber endings and the postganglionic neurotransmitter noradrenaline, which acts on the adrenal medulla that is located above the kidneys on both sides of the spine in the retroperitoneal space, thereby promoting the release of catecholamine (i.e., adrenaline and noradrenaline) into the bloodstream [35]. Blood redistribution occurs after SAM axis activation, leading to vasoconstriction in many microvascular networks and vasorelaxation in skeletal muscle and liver [36]. This accelerates cardiac contraction, thereby increasing blood output and blood pressure. The SAM axis responds to stress rapidly to get the animal ready for the “flight or fight” reaction.

The HPA axis includes the hypothalamic paraventricular nucleus (PVN), a hollow funnel-like region located inside the supraoptic area of the hypothalamus, the pituitary (an oval body located in the ventral hypothalamus), and the adrenal gland. After exposure to stress stimulation, the PVN secretes corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) to the portal circulation of the median eminence [37], where CRH quickly reaches the pituitary gland and promotes its secretion of adrenocorticotrophic hormone (ACTH). ACTH acts on the adrenal cortex to promote the secretion of glucocorticoids (GCs), such as cortisol and corticosterone. Glucocorticoid secretion negatively regulates

CRH and ACTH secretion. The activation of the HPA axis, and the subsequent increased content of GCs, modulates energy reserve mobilization and catabolic processes, such as to promote gluconeogenesis and increase protein and fat metabolism through proteolysis and lipolysis. Meanwhile, certain physiological processes are temporarily inhibited, leading to immune suppression and the inhibition of digestion, reproduction, and growth [38,39]. The activation of the HPA axis is relatively slow [39,40]. Although the negative feedback mechanism will restore it to its normal level, excessive cortisol from long-term chronic stress brings serious health risks to the body [39,40].

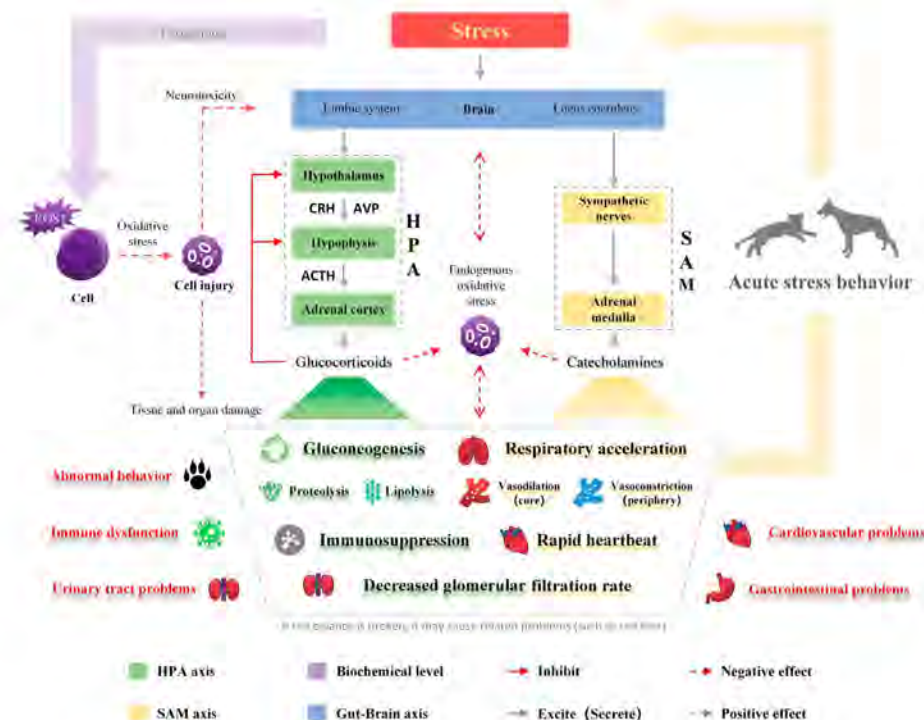


Figure 1. Regulatory mechanism of stress response. The components highlighted in green are mainly affected by the hypothalamus–pituitary–adrenal axis, and those in yellow are mainly affected by the sympathetic–adrenal medulla axis. It is worth noting that only the main impact is presented, and there is a broader and more complex relationship between the two systems. The elements in red indicate the possible harm of stress. Stress can induce oxidative stress to cause ubiquitous damage to cells, tissues, and organs. ROS, reactive oxygen species; CRH, corticotropin-releasing hormone; AVP, arginine vasopressin; ACTH, adrenocorticotrophic hormone.

3.2. Oxidative Stress

Oxidative stress refers to when the production of oxidants exceeds the antioxidative capacity of the body, leading to the disruption of redox (i.e., oxidation/reduction reactions) homeostasis and ubiquitous damage to cellular, tissue, and organ systems [41]. The mechanism of oxidative stress is shown in Figure 2.

Reactive oxygen species (ROS) widely refers to oxygen-derived free radicals and non-free radicals. In normal cellular activities, oxygen in the mitochondrial inner membrane will gain electrons under the action of the respiratory chain and produce ROS with high chemical reactivity due to unpaired electrons [42]. Other main sources of ROS include enzymes such as NADPH enzyme oxidation [42], cytochrome P450 in the endoplasmic reticulum, lipoxygenase, xanthine oxidase, and cyclooxygenase [43].

Meanwhile, the body is endowed with a defensive reducing system to combat ROS, which consists of antioxidant proteins, antioxidant enzymes, and small-molecule antioxidants. Antioxidant proteins include mainly albumin, haptoglobin, ferritin, ceruloplasmin, etc. [44]. Antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT),

glutathione peroxidase (GPx), and some coenzymes. Small-molecule antioxidants are divided into lipid-soluble and water-soluble antioxidants [6].

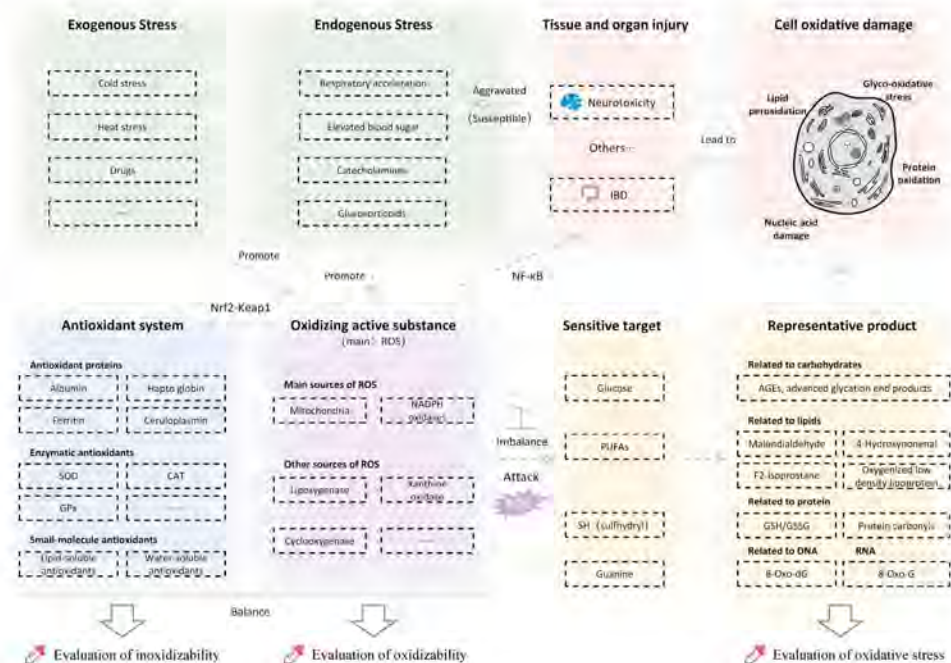


Figure 2. Mechanism and influence of oxidative stress. SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; 8-oxo-dG, 8-oxo-2'-deoxyguanosine; 8-oxo-G, 8-oxo-guanine; GSH, reduced glutathione; GSSG, oxidized glutathione disulfide; IBD, inflammatory bowel disease.

Under normal circumstances, ROS and the antioxidant system maintain a relative balance. However under stressful conditions, ROS will be overproduced, which may lead to oxidative stress [45]. The increased respiratory rate [46], blood glucose [47], and the secretion of glucocorticoids [48] and catecholamines [49] during stress response are all proven to induce ROS production. On one hand, the production of ROS promotes the activities of antioxidant enzymes through the Nrf2-Keap1 pathway [41]. On the other hand, an inflammatory reaction is induced, mainly through the NF-κB pathway [50].

The level of oxidative stress can be reflected by the typical byproducts from the process of oxidative damage. For example, malondialdehyde, 4-hydroxynonol, F2-Isoprostane, and oxygenated low-density lipoproteins are derived from polyunsaturated fatty acids during lipid peroxidation [6,41]. The sulfhydryl group in protein is also easily attacked by ROS, which is converted to carbonyl protein [51]. Reactive carbon groups, such as advanced glycation end products (AGEs), may be generated during glycosylation of protein under the action of glucose ROS [52]. With nucleic acid, ROS may also attack guanine to generate 8-oxo-2'-deoxyguanosine (8-oxo-dG) in DNA and 8-oxo-guanine (8-oxo-G) in RNA [53]. Reduced glutathione (GSH) and glutathione disulfide (GSSG) are two forms of glutathione that play important roles in protein redox [54]. GSH is oxidized to form GSSG [55], and an increase in ROS usually leads to a loss in GSH; therefore, the ratio of GSH/GSSG also can serve as a measure of oxidative stress [55].

The brain has high oxygen consumption and is rich in lipid content, which makes it vulnerable to oxidative stress [56]. As a result, chronic ROS accumulation presents a threat to the integrity of brain cells and neural functions, disrupting neural circuits, impairing connections between the hippocampus, amygdala and cortex, and ultimately leading to behavioral and cognitive deficits [57].

In addition, oxidative stress can induce inflammatory responses through the NF-κB pathway [50]. Inflammatory responses can induce inflammatory bowel disease (IBD) and affect the kynurenine pathway to worsen neurological and intestinal health, thus

aggravating stress or increasing sensitivity to stressors [58]. IBD is a chronic gastrointestinal disease that is usually associated with stress [59]. Although the exact mechanism remains to be explored, more and more studies have shown that oxidative stress plays a crucial role in the pathogenesis and progress of IBD [50]. Oxidative stress caused by excessive ROS may stimulate the initial inflammatory reaction and lead to additional ROS production which may result in further damage to the intestinal tissue [50]. In the intestinal tract, tryptophan can be used to synthesize 5-hydroxytryptamine and kynurenine (KYN); the latter can be further broken down to produce kynurenic acid (KYNA) and neurotoxic quinolinic acid (QUIN) [60,61]. Under inflammatory conditions, increased QUIN synthesis results in the depletion of gamma-aminobutyric acid (GABA) and adenosine triphosphate (ATP), which further aggravates damage to nerve cells [62].

Generally speaking, damage caused by oxidative stress has a negative impact on many tissues and organs, and if it is not alleviated, it may cause or mediate the progression of a series of problems or diseases [63].

4. Adverse Consequences of Stress

4.1. Gastrointestinal Diseases

Studies have shown that the number of gastroenteritis cases in cats during the SARS-CoV-2 pandemic was higher than in the pre-pandemic period, due to stress as a result of the changes especially influencing the daily routine of cats [64]. Chronic stress may lead to gastrointestinal ulcers (i.e., lesions of the gastric and duodenal mucosa), which manifest as mucosal erosion, bleeding, and even perforation [65]. Secretion of catecholamines during stress response decreases the blood flow to the gastrointestinal system causing mucosal ischemia [66]. Meanwhile, intestinal hypoxia can lead to ATP depletion, acidosis, and the destruction of the gastric mucosal barrier [67]. The H^+ in the gastric cavity diffuses reversely into the mucosa and further aggravates the gastrointestinal injury. Studies have also shown that glucocorticoids can increase gastrointestinal permeability [68]. Although varied in different stress models and species, it is generally believed that acute stress will lead to the delayed gastric emptying and accelerated transport of the large intestine [69], resulting in diarrhea, vomiting, and other digestive tract problems [70]. The effect of chronic stress on the gastrointestinal tract seems to be sustained even after the stressor is removed. Some studies show that when the stressors are eliminated, the colon still accelerates transport of digesta [69,71], which may be closely related to sustained diarrhea observed in chronic stress. In addition, oxidative stress is considered to be involved in different gastrointestinal diseases in pets, such as feline panleukopenia [72] and inflammatory bowel disease (IBD) in dogs [5].

4.2. Immune Dysfunction

Acute stress can enhance innate immunity in order to better cope with adverse changes. The underlying mechanism may be that norepinephrine and other stress hormones induce the recruitment of dendritic cells and the increase in macrophages at antigen exposure sites, thereby enhancing the primary immune response [73]. Under acute stress conditions, the total number of white blood cells also increases [74]. However, continuous activation of the HPA axis will lead to leukopenia [75]. Increased glucocorticoids have been shown to exert a strong immunosuppressive effect by inhibiting cytokine production, macrophage function, lymphocyte proliferation and differentiation, and natural killer cell activity [76,77]. Therefore, chronic stress can lead to immunosuppression and increase the risk of pathogen invasion. For example, contraction of feline infectious peritonitis due to feline coronavirus has been linked to oxidative stress and decreased antioxidant status in cats [78].

4.3. Urinary Tract Diseases

Stress can lead to urinary tract problems such as dysuria, hematuria, pollakiuria (i.e., increased frequency of urination), and periuria (i.e., urination in inappropriate locations). On the urethral side, the activation of the renin–angiotensin–aldosterone system and

the secretion of catecholamines from the SAM axis lead to renal vasoconstriction and reduced glomerular filtration rate and urine output. Moreover, the increase in the secretion of antidiuretic hormone enhances the reabsorption of water and further reduces urine volume. In the lower urethra, feline idiopathic cystitis is mostly of type I neurogenic origin [79]. It was found that plasma catecholamine concentration at rest in cats with idiopathic cystitis was significantly higher than that in healthy cats [80]. In addition, plasma catecholamine concentrations decreased with stress adaption in healthy cats but remained high in cats with idiopathic cystitis [81]. Collectively, stress can affect urinary tract health through neuroendocrine pathways.

4.4. Cardiovascular Problems

The cardiovascular system often reacts to stress with accelerated myocardial contraction and heart rate, and increased blood pressure and cardiac output. The reaction is induced through catecholamines interacting with their β -receptors on myocardial cells [82]. In the long run, the threshold of ventricular fibrillation is reduced due to over-secretion of catecholamines, causing abnormal myocardial activity and arrhythmia [83]. An earlier study in infarcted dogs showed that stressful stimuli provoked diverse ventricular arrhythmias including ventricular tachycardia and early extrasystoles [17]. The more worrying situation with chronic stress is that prolonged secretion of GCs can lead to a permanent increase in cardiac sympathetic tension and hypertension, resulting from elevated blood cholesterol levels and sodium retention in vascular smooth muscle cells [84]. Therefore, high-intensity, high-frequency, or long-term hypertension induced by stress can have adverse effects on the cardiovascular system and even lead to heart disease [84,85]. Meanwhile, oxidative stress seems to be highly correlated with cardiovascular disease. The activity of SOD in cats with hypertrophic cardiomyopathy was decreased significantly [86]. The serum antioxidant capacity of dogs with heart failure also decreased [87].

4.5. Acute Stress Behavior and Behavioral Abnormalities

4.5.1. Acute Stress Behavior

When facing acute stress, cats or dogs often exhibit “flight or fight” responses. Cats will try to hide or flee. The typical hiding posture in cats is freezing while squatting and crouching their body [88]. If avoidance of the threat is not achieved, cats will exhibit intimidating and aggressive behaviors, such as hissing, growling, slapping, scratching, and biting [89]. When dogs suffer from acute stress, there will be body shaking, lowering of the posture, mouth licking, and restless walking and standing [15]. They will even show aggressive behaviors, such as barking, lunging, growling, and biting/snapping [90]. Fortunately, mild, transient acute stress does not cause substantial damage to the body. If not alleviated, acute stress may evolve into chronic stress [91], which in turn leads to abnormal behaviors (e.g., stereotypic behavior, urinary marking, aggression).

4.5.2. Behavioral Abnormalities

Stress can cause anorexia nervosa, leading to decreased appetite and food intake in dogs and cats [92]. The neural circuits regulating food intake converge on the paraventricular CRH-releasing nuclei and neurons containing urocortin [93]. CRH exerts an anorexigenic effect by inhibiting the release of neuropeptide Y and other hypothalamic neuropeptides, such as growth-hormone-releasing hormone and somatostatin. The orexigenic effects of glucocorticoids are counteracted by a steroid-induced rise in leptin levels that close a regulatory loop regarding food consumption [94,95]. On the other hand, studies in rats and humans show that stress may also lead to overeating [96,97], an eating disorder involves the brain reward system [98].

Some common obsessive–compulsive behaviors in pets include feline hyperesthesia syndrome, psychogenic alopecia and pica in cats [99], and acral lick dermatitis in dogs [100]. Studies have shown that stress can lead to obsessive–compulsive behavior in dogs and cats, which may be related to the dysfunction of neurotransmitters (e.g., 5-hydroxytryptamine

and dopamine). Mami Irimajiri et al. partially confirmed this in dogs and showed that 5-hydroxytryptamine reuptake inhibitors (e.g., clomipramine and fluoxetine) exhibit reliable mitigation effects on obsessive–compulsive disorder [100].

Urine marking is considered a territorial behavior in dogs and cats as urine contains odor information for individual and sex identification [101]. Inappropriate urine marking is especially common in multi-cat households where incompatible or unfamiliar individuals live together [4]. The exhibition of urine marking in response to social conflict is the attempt of cats to gain control of the environment by leaving behind familiar odors [4]. Urine marking is often accompanied by other behavioral problems, such as aggression in cats [24], indicating a close relationship between a general stressful environment and behavioral problems [102].

5. Dietary Strategies for Relieving Stress in Pets

Current strategies for relieving stress in cats and dogs commonly include managing their environment, training techniques [103], pheromone therapy [101,104], and some other olfactory stimuli such as plant-extracted essential oils [105]. Pharmacotherapy may be necessary when the case is severe, but drug administration itself may provoke stress [4]. In recent years, more and more studies have focused on relieving stress through nutritional regulation, which have been mainly focused on effectiveness in anti-oxidation, anti-anxiety, and/or maintaining intestinal health. Studies on the nutritional management of stress in cats and dogs have been summarized in Table 1.

Table 1. Dietary strategies for stress alleviation in cats and dogs.

Active Ingredients	Resources	Mechanism	Species	Dosage	Measurements	Supportive/Negative	Reference
Gallic acid	Fruits, vegetables, and medicinal plants	Antioxidants; intestinal health	Dog	500 mg/kg	SOD and CAT ↑; TNF- α ↓; IL-1 β ↓; diarrhea rate ↓; SCFAs-producing bacteria ↑; serum cortisol and HSP70 ↓	Regulate intestinal flora to alleviate oxidative stress and inflammatory reaction	[32]
Tannic acid	Gallnut	Antioxidants; intestinal health	Dog	2.5 g/kg	Serum COR ↓; GC ↓; ACTH ↓; HSP70 ↓; beneficial bacteria ↑; pathogenic bacteria ↓; fecal butyrate ↑	Regulate intestinal flora to alleviate stress injury	[106]
<i>Pinus taeda</i> hydrolyzed lignin (PTHL)	<i>P. taeda</i> (tree)	Antioxidants	Dog	/	SOD, CAT, and GPx activity ↑	Antioxidation	[107]
Curcumin	<i>Curcuma longa</i>	Antioxidants	Dog	32.9 mg/kg	ROS ↓; CAT, SOD and GPx ↑; total antioxidant capacity ↑; lymphocytes and globulin levels ↓	Enhance antioxidant capacity and alleviate inflammatory reaction	[108]
A blend of essential oils and vitamin E	Essential oils (cloves, rosemary, and oregano)	Antioxidants	Dog	/	Non-protein self-sustaining group ↑; glutamate S-transfer ↑; ROS ↓	Antioxidation	[109]
Vitamin E	Commercial sources	Antioxidants	Dog	500 mg	Prevent the decrease in PON1 activity and EMF, and the increase in plasma MDA.	Alleviate oxidative stress	[110]
Vitamin C	Commercial sources	Antioxidants	Dog	/	SOD, GPx, and CAT ↑; ROS ↓; improve the blood flow distribution, promote the synthesis of catalamine and arginine vasopressin, regulate immunity, and inhibit the activity of cytotoxic T cells	Antioxidation	[111]
			Dog/cat	/		Relieve the damage caused by oxidative stress and inflammatory reaction	[112]

Table 1. Cont.

Active Ingredients	Resources	Mechanism	Species	Dosage	Measurements	Supportive/Negative	Reference
VE and VC and beta-carotene	Commercial sources	Antioxidants	Cat	VE: 742 mg/kg; VC: 84 mg/kg; beta-carotene: 2.1 mg/kg	Serum 8-OHdG ↓	Reduce DNA oxidative damage	[113]
Selenium	Selenium yeast	Antioxidants	Dog	0.3 mg/kg	MDA ↓; GPx, SOD, and CAT ↑	Antioxidation	[114]
Radioiodine	Commercial sources	Antioxidants	Cat	/	Urinary free 8-isoproteneates ↓	Alleviate lipid peroxidation	[115]
<i>Saccharomyces cerevisiae</i> fermentation product	<i>S. cerevisiae</i> fermentation	Antioxidants	Dog	0.13%	Serum MDA and 8-isoproteneates ↑; the expression of blood COX-2 and MPO mRNA ↓	Inhibit innate immune activation to alleviate inflammation	[116]
Fish-oil-based foods	Commercial sources	Antioxidants	Dog	/	GPx and CAT activity ↑; blood glucose and total and LDL cholesterol ↓	Antioxidation and reduce blood sugar and blood lipid	[117]
Melatonin	Commercial sources	Antioxidants	Dog	0.3 mg/kg	Serum SOD, GPX, and CAT ↑; MDA ↓	Enhance antioxidant capacity to relieve oxidative damage	[118]
α-casozepine	A tryptic bovine αs1-casein hydrolysate	Anxiolytic agents	Dog	/	Anxiety behavior ↓; serum cortisol ↓	Relieve anxiety and improve behavior; reduce stress hormone secretion	[119]
			Dog	Closely 15 mg/kg BW	Score of emotional disorder evaluation in dogs ↓; anxiety score ↓; different items (fear of strangers, contact with familiars, general fears, fear-related aggressions, and autonomic disorders) ↓	Relieve anxiety and improve behavior	[120]
			Cat	15 mg/kg BW		Relieve anxiety and improve behavior	[121]
α-casozepine and tryptophan	Commercial diet	Anxiolytic agents	Cat	α-casozepine: 15 mg/kg; tryptophan: 3.6 g/kg DM	The ratio of plasma tryptophan to large neutral amino acids ↑; urinary cortisol ↓	Promote tryptophan utilization and reduce stress hormone secretion	[122]
			Cat	/	The duration of cat inactivity decreases when placed in unfamiliar positions	Relieve anxiety and improve behavior	[123]
Tryptophan	Commercial sources	Anxiolytic agents	Dog	5.7 g/kg DM	Plasma Trp ↑; Trp/(large neutral amino acids) ↑	Promote tryptophan utilization; the impact on anxiety and behavior remains to be determined	[124]
			Dog	Trp: LNAA = 0.075:1	Serum serotonin ↑; improved stool scores	Relieve anxiety and reduce diarrhea	[125]
			Dog	Add extra 1.45 g/kg	Attacks related to territorial domination ↓	Reduce the stress of territorial competition	[126]
			Dog	/	Stress-related abnormal behavior ↓	Relieve anxiety and improve behavior	[127]

Table 1. Cont.

Active Ingredients	Resources	Mechanism	Species	Dosage	Measurements	Supportive/Negative	Reference
L-theanine	Commercial sources	Anxiolytic agents	Dog	50 mg (less than 10 kg), 100 mg (10–25 kg), 200 mg (more than 25 kg)/day	Anxiety scores ↓; drooling, following people, pacing, panting, and hiding ↓	Relieve anxiety and improve behavior	[128]
			Dog	50 mg (less than 10 kg), 100 mg (more than 10 kg)/day	Interactive behavior ↑	Relieve anxiety and improve behavior	[129]
			Cat	50 mg/day	Stress score ↓; inappropriate urination/defecation, fear-induced aggressiveness, hypervigilance/tenseness, or physical/functional manifestations of stress ↓	Relieve anxiety and improve behavior	[130]
Medium chain triglyceride diet	Commercial diet	Anxiolytic agents	Dog	5.5%	ADHD-related anxiety behavior ↓	Relieve anxiety and improve behavior	[131]
Medium chain triglyceride and Brain Protection Blend (BPB) Fish hydrolysate and melon juice concentrate Lemon balm, fish peptides, oligofructose, and L-tryptophan <i>Bacillus amyloliquefaciens</i> CECT 5940	BPB including B vitamins, antioxidants, omega-3 fat acids, and arginine	Anxiolytic agents	Dog	6%/9%	Blood DHA, EPA, total omega-3 PUFAs, and omega-3/omega-6 ratio ↑; symptoms of cognitive dysfunction syndrome ↓	Promote brain health and improve behavior	[132]
	Commercial sources	Anxiolytic agents	Dog	F: 500 mg, M: 11 mg; double (BW more than 10 kg)	Interactive behavior ↑; stress behavior ↓	Relieve anxiety and improve behavior	[133]
	Commercial diet	Anxiolytic agents	Cat	L: 0.1%; F: 0.1%; O: 0.5%; Trp: 0.08%	Average 24 h urinary cortisol/creatinine ratio ↓	Reduce stress hormone secretion	[134]
	Commercial bacteria	Intestinal Health	Dog	1×10^6 CFU/g DM	The bacillus ↑; the coliforms ↓	Regulate intestinal flora	[135]
Polyphenols and omega-3 fatty acids	Fish oil and a polyphenol blend (citrus pulp, carrot, and spinach)	Intestinal Health; anxiolytic agents	Dog	/	Plasma 4-EPS ↓; anxiety-related metabolites ↓; Blautia, Parabacteroides, and Odoribacter ↑	Regulate intestinal flora to relieve anxiety	[136]
<i>S. boulardii</i>	Commercial bacteria	Intestinal Health	Dog	1×10^9 CFU di/kg of feed	Fecal calprotectin ↓; IgA ↓; fecal cortisol ↓	Reduce intestinal inflammation and stress hormone secretion	[137]
A fiber-prebiotic-probiotic blend	Commercial sources	Intestinal Health	Dog	/	Fecal score ↓; blood lipid ↓; fecal IgA ↑	Enhance intestinal immunity and improve stool quality	[138]
<i>Enterococcus faecium</i> SF68	Commercial bacteria	Intestinal Health	Cat/Dog	2.1×10^9 CFU/day	Diarrhea rate ↓	Reduce diarrhea	[139]

↑, increase; ↓, reduction; IgA, immunoglobulin A; NO, nitric oxide; COR, cortisol; GC, glucocorticoid; ACTH, adrenocorticotrophic hormone; HSP70, heat shock protein 70; SOD, superoxide dismutase; CAT, catalase; GST, glutathione-S-transferase; GPx, glutathione peroxidase; ADHD, attention-deficit/hyperactivity disorder; ROS, reactive oxygen species; GSH, reduced glutathione; MDA, malondialdehyde; PON1, paraoxonase-1; EMF, erythrocyte membrane fluid; 4-EPS, 4-ethylphenyl sulfate; PUFA, polyunsaturated fatty acids; COX-2, cyclooxygenase-2; MPO, myeloperoxidase; LNAA, large neutral amino acids; Trp, tryptophan.

5.1. Antioxidants

Exogenous antioxidants are substances that can improve immune function, boost the endogenous antioxidant system, and balance the cellular oxidative status by scavenging free radicals and by interrupting the lipid peroxidation process [140]. The protective role of different natural antioxidants in chronic diseases has been documented in various animal species and humans [140].

5.1.1. Polyphenols and Other Plant Extracts

Antioxidant phytochemicals are commonly found in fruits (e.g., berries, apples, grapes, and pomegranates), cereal grains, vegetables, and plants. The main group is polyphenols, the chemical structure of which contains one or more aromatic rings and can act as free radical scavengers and metal chelators [141].

Gallic acid (GA) is a naturally occurring polyphenol commonly exist in fruits, vegetables, and herbal medicines. GA can positively affect intestinal health and immune response [142], and may alleviate stress through the brain–gut axis [32]. In humans, GA has been reported to reduce the formation of free radicals and enhance innate immune activation [143], inhibit the production of ROS, nitric oxide, and the release of pro-inflammatory cytokines [144], and increase macrophage phagocytosis to improve immune regulation activity [145]. In addition, GA can induce a shift of intestinal microbial groups toward more favorable composition and promote the production of short-chain fatty acids (SCFAs) [146], which can serve as neuroactive substances further affecting the nervous and immune systems of the body [147]. Collectively, these activities of GA have positive significance for reducing the damage from oxidative stress. Yang et al. (2022) verified this in dogs by showing that GA markedly reduced diarrhea and caused a moderate decline of serum cortisol and heat shock protein (HSP) 70 levels in puppies after transportation [32]. The same study also reported that GA alleviated the oxidative stress and inflammatory response induced by transportation, and maintained the stability of intestinal flora and the content of short chain fatty acids [32]. In addition, the fecal and serum metabolomic analyses revealed that GA markedly reversed the abnormalities of nutrient metabolism caused by stress [32]. Tannic acid extracted from gallnut (a widely used traditional medicine in China) inhibited the secretion of serum stress hormones (i.e., COR, GC, and ACTH) and the expression of heat shock protein 70 to protect dogs from stress-induced oxidative damage and inflammatory response [106]. Dietary supplementation with pomegranate peel extract (PPE) had a positive impact on the antioxidant status in dogs, improving indices of erythrocytic antioxidants, namely, reducing glutathione, catalase, glutathione peroxidase, and glutathione S-transferase, together with a reduction in lipid peroxidation [148]. Resveratrol, a natural phytoalexin contained in wine, can reduce the level of ROS and MDA, improve the activities of SOD, GPX, and CAT activities, and improve the ratio of reduced glutamate to oxidized glutamate in cat models in which hepatotoxicity was induced [149]. Pinus taeda hydrolyzed lignin is a polyphenol mixture that can increase the activity of SOD, CAT, and GPx to improve antioxidant capacity in healthy dogs [107]. Curcumin extracted from curcuma longa can also enhance total antioxidant capacity by improving the activities of ROS, CAT, SOD, and GPx, and relieve inflammation by reducing lymphocytes and globulin level in dogs [108].

In vitro experiments with canine and feline cells have also revealed the antioxidative potential of some other plant or seed extracts. For example, quercetin is a natural occurring bioflavonoid that can increase GSH and decrease ROS in methimazole-induced oxidative stress in feline kidney epithelial cells [150]. Morin, also a flavonoid, can enhance the antioxidant capacity of hydrogen-peroxide-induced oxidative-stressed canine kidney cells by increasing the activities of SOD and CAT, and reduce mitochondrial oxidative damage and apoptosis [151]. Grape seed proanthocyanidin extract, alone or together with resveratrol, has also been proved to reduce ROS production in canine lens epithelial cells [152]. However, the antioxidative effects of some substances require further verification through in vivo studies.

5.1.2. Vitamins

Vitamin C has a strong antioxidant capacity that can reduce the damage of free radicals to cells by actively removing superoxide and other ROS [112]. Decreased vitamin C levels have been detected in dogs with naturally occurring gastric dilatation–volvulus [153]. However, in dogs with chronic heart failure, the concentration of vitamin C increases, which is considered to be a compensatory increase induced by chronic oxidative stress [154]. One study on kidney transplant dogs showed that the activities of SOD, GPx, and CAT were increased after feeding vitamin C, indicating improved antioxidative capacity [111]. However, another study showed that when fed an adequate diet, additional vitamin C supplementation had no significant impact on the antioxidant capacity and immune function of healthy dogs [155]. Lipid-soluble vitamin E is a chain-breaking antioxidant that reacts with lipid oxygen or lipid peroxide free radicals [156]. A study on dogs showed that vitamin E can prevent the increase in plasma malondialdehyde caused by exercise, which indicates that vitamin E has a positive role in preventing lipid peroxidation [110]. When vitamin E and C, and beta-carotene, were fed together to cats with renal insufficiency, the concentration of serum 8-OHdG decreased, indicating alleviated DNA damage from oxidative stress [113]. Vitamin B plays an important role in the health of the central nervous system [157]. Some mixed foods rich in vitamin B, fish oil, and other antioxidants have been shown to improve the cognitive function of cats [158] and dogs [159]. However, the direct effect of vitamin B on stress in dogs and cats remains to be explored. Taken together, the addition of vitamins B, C, and E may have a positive effect on the antioxidative capacity and health of the nervous system in pets [6,113].

5.1.3. Minerals

The antioxidant and anti-stress abilities of minerals have long been investigated and applied, especially in combination with vitamins [160,161]. Representative trace elements include Fe, Zn, Se, and Mn. The role of these elements has been widely verified in a variety of species [162,163]. Studies have also shown that some dog skin diseases may be related to oxidative stress and zinc deficiency [164]. Organic selenium can reduce blood malondialdehyde levels and improve the activities of glutathione peroxidase, superoxide dismutase, and catalase, thus enhancing the antioxidant capacity of dogs with induced renal calculi [114]. In hyperthyroid cats, radioiodine can reduce urinary isoprostane, the high level of which reflects renal oxidative stress [115].

5.1.4. Polyunsaturated Fatty Acids

Polyunsaturated fatty acids (PUFAs) are fatty acids with more than one double bond in their backbone. Omega-3 PUFAs are among the most commonly used in dogs and cats. The antioxidative effect of PUFAs is achieved through either the component of cell membranes to decrease their sensitivity to free radicals, or boosting the endogenous antioxidative system (e.g., increasing cellular concentration of super oxide dismutase or glutathione peroxidase) [165]. Feeding fish oil, which is rich in omega-3 PUFAs, to police dogs can promote the activities of GPx and CAT, and reduce the levels of blood glucose, and total and LDL cholesterol, indicating that fish oil can improve the antioxidant capacity and alleviate oxidative stress caused by strenuous exercise in dogs [117]. Additionally, due to its critical role in the development and function of the central nervous system, PUFAs are often included in brain protection formulae, which have been shown to improve the cognitive function and behavioral health of cats [158] and dogs [132,159,166]. In rodent models, it has been approved that omega-3 PUFAs can reduce anxiety-like behaviors and improve cognition in animals subjected to early life stress [167], possibly through the regulation of intestinal microbiota and the function of the brain–gut axis, including HPA [168]. Even though not verified in pet dogs and cats, we suggest similar mechanisms involved with the behavioral improvements and dietary intake of PUFAs exist as in rodents.

5.1.5. Thiols

Thiols or mercaptans are a class of organic compounds with antioxidative capacity because their chemical structure contains a sulfhydryl group that is easily oxidized. The representative ones are N-Acetylcysteine and α -lipoic acid [169]. Studies on cats showed that feeding N-Acetylcysteine can increase the important cytosolic antioxidant, reduced glutathione, under the oxidative stress induced by onion powder [170]. N-Acetylcysteine can also protect liver tissue from the oxidative damage induced by acetaminophen in cats [171].

Lipoic acid is a small molecule of both animal and plant resources that contains two thiol groups that may be oxidized or reduced. Lipoic acid and its reduced form, dihydrolipoic acid, are powerful antioxidants with amphiphilic character [172]. They can easily quench radicals, chelate metals, interact with and regenerate other antioxidants, increase endogenous glutathione activity, and attenuate the release of free radicals and cytotoxic cytokines by regulating the second messenger nuclear factor κ B [172]. The powerful antioxidant properties of α -lipoic acid make it helpful in the ancillary treatment of many human diseases, such as cardiovascular diseases and neurodegenerative diseases [172]. As summarized in a review study, supplementation of α -lipoic acid in appropriate doses (i.e., 1–5 mg/kg/day) can be beneficial in dogs, helping to reduce and delay lens opacities in diabetic dogs, reduce biomarkers of osteoarthritis, and when supplemented together with other antioxidants, reduce cognitive dysfunction and improve learning in senior dogs [173]. Even though α -lipoic acid can be safe and well tolerated by humans or animals, the recommendation of use in cats is rare because they are extremely sensitive to the toxic effect of α -lipoic acid compared to other species [174].

In humans and other animal species, additional substances and/or dietary formulae have been identified and investigated for their antioxidative function, such as sacchariterpenin, which is a new natural additive mainly extracted from *Camellia* plants [175], and the methionine/lysine proportion in the diet [176]. This evidence indicates that there are still many nutritional strategies with antioxidant potential that remain to be developed, and their application in relieving oxidative stress in cats and dogs requires further verification. Additionally, studies have suggested the use of a combination of different ingredients to achieve better antioxidative effects. For example, dietary supplementation of an antioxidant mixture containing quercetin (Q), resveratrol (R), curcumin, and vitamin E was shown to counteract both the oxidative stress and the related side effects elicited by methimazole treatment in hyperthyroid cats [177].

5.2. Anxiolytic Agents

5.2.1. Gamma-Aminobutyric Acid and Its Receptor Agonists

Gamma-aminobutyric acid (GABA) is a small non-protein amino acid that is produced in the brain and other parts of the body (e.g., β cells of the pancreas, gastrointestinal tract, and endothelium) [178]. In the mammalian brain, GABA acts as the main inhibitory neurotransmitter and is widely known for its effect on anxiety- and stress-related disorders [179]. Peripheral administration of GABA is not effective in increasing its concentration in the brain due to the high polarity of the structure which limits its passage through the blood–brain barrier (BBB) [180]. Alternatively, many anxiolytic drugs/substances were developed to target GABA receptors [181]. Studies have shown that oral administration of alpha-casozepine, a milk-sourced lipophilic decapeptide that can cross the BBB and act on GABA receptors as an agonist, was effective in the management of anxiety disorders such as social phobias in domestic cats [121]. Alpha-casozepine was also shown to decrease the score of emotional disorder evaluation in dogs (EDED) [120] and reduce anxiety behavior and serum cortisol in anxious dogs [119]. The mixed addition of alpha-casozepine and L-tryptophan in diet reduced the urinary cortisol of cats [122] and reduced the inactive time of cats in unfamiliar environments [123]. The above evidence shows that alpha-casozepine, as a GABA receptor agonist, can alleviate anxiety and reduce stress in dogs and cats.

5.2.2. L-Tryptophan

Tryptophan is the precursor for the synthesis of neurotransmitter 5-hydroxytryptamine/serotonin (5-HT), and the central serotonergic system is associated with fear- and anxiety-related states and stress responses [182]. An anxiolytic effect of a dietary addition of tryptophan is likely achieved by facilitating central 5-HT synthesis and signaling [58]. In cats, L-tryptophan is often tested together with alpha-casozepine [122]; therefore, the effect of tryptophan on stress management in cats requires further verification [123]. In dogs, the addition of tryptophan to the diet can increase the plasma tryptophan concentration and the ratio of tryptophan to large neutral amino acids [124]. Tryptophan will compete with large neutral amino acids (LNAAs) for transporters to cross the BBB [183]. When tryptophan and LNAAs were supplemented at a ratio of 0.075:1, the serum serotonin increased and the stool score improved in training dogs [125]. In addition, tryptophan supplementation can reduce attacks related to territorial domination [126] and stress-related anxiety behaviors [127] in dogs. Taken together, the dietary intake of tryptophan has the potential to alleviate anxiety in cats and dogs, but other factors that are involved in regulating tryptophan synthesis of 5-HT need to be considered, such as the ratio of tryptophan to LNAA, the alternative kynurenine pathway, and the activity of key enzymes including tryptophan hydroxylase [58].

5.2.3. Theanine

Theanine, chemically named N-ethyl-L-glutamine, is an amino acid unique to green tea leaves that can compete with L-glutamic acid for the binding of glutamate receptors in the brain to exert its anti-stress effect [184]. Relevant studies in rat models have shown that theanine intake increases the concentration of 5-HT and dopamine in the brain [185]. In humans, theanine was shown to reduce the heart rate and relieve elevated blood pressure during stress, and weaken the stress response of the autonomic nervous system induced by physical and psychological stress [184]. In cats, theanine was shown to be effective in improving undesirable manifestations of stress, especially inappropriate elimination [130]. Theanine can also reduce the global anxiety scores in storm-sensitive dogs, as reflected by reduced anxious behaviors (e.g., drooling, following people, pacing, panting, and hiding) and latency to return to a baseline behavioral state after the storm ends [128]. In addition, a study suggests that theanine is effective for reducing fearful behavior toward unfamiliar human beings in dogs [129]. The above research shows that dietary administration of appropriate theanine may serve as a promising strategy for relieving stress and improving anxious behaviors in dogs and cats.

5.2.4. Diet with Differed Macronutrient Composition

The composition of certain nutrients in the diet may also impact animal behavior. An earlier study revealed that incorporating more protein in the diet in exchange for an isoenergetic amount of fat resulted in a trend toward decreased dominance aggression but increased territorial (fear) aggression in dogs [186]. This change of dietary nutrients on behavior may be associated with tryptophan concentrations since diets with different protein contents (11.8, 16.9, and 22.2 g/MJ) are linearly correlated with their tryptophan levels (67, 105, and 115 mg/MJ). However, a ketogenic diet high in fat, but low in protein and carbohydrate content, was shown to reduce the attention-deficit/hyperactivity disorder and fear/anxiety of dogs with idiopathic epilepsy [131]. A possible mechanism is that the high content of medium chain triglyceride in the diet alters the energy metabolism in the brain which may contribute to behavioral changes [131]. More studies are required to determine the mechanisms underlying the connection between dietary nutrient composition and animal behaviors.

5.3. Probiotics and Prebiotics

Animal and human studies have shown that gut microbiota can be involved in the regulation of stress/emotion factors such as serotonin synthesis [187], brain-derived neu-

rotrophic factor [188], and cortisol [189], thereby participating in the management of an individual's stress level and related psychiatric symptoms. A microbial metabolite converted from tyrosine, 4-ethylphenyl sulfate (4-EPS) has recently been shown to contribute to the mechanism involving gut–brain interaction [190]. The metabolite can enter the brain, damage oligodendrocytes and reduce myelination of neuronal axons, thus inducing anxiety behavior [190]. The mixture of prebiotics, fish oil, and polyphenols can reduce the content of plasma 4-EPS and anxiety-related metabolites in dogs [136]. Meanwhile, the relative abundance of *Blautia*, *Bacterioides*, and *Odoribacter* was decreased, which are found to be decreasing in patients with anxiety [136]. A recent study in dogs reported that *S. boulardii* (1×10^9 CFU di/kg of feed) reduced fecal calprotectin, IgA, and cortisol, indicating that *S. boulardii* may play an active role in alleviating intestinal inflammation and reducing stress hormone secretion [137]. Therefore, we can infer that improving the composition of intestinal flora may have therapeutic potential in relieving anxiety and stress. In terms of probiotics and prebiotics that can benefit gut health in pets, there are more studies in the literature that have provided evidence [135,138,139,191] but their direct effects on regulating stress and related behaviors are yet to be determined. However, probiotics and prebiotics have many positive effects on intestinal and neural health, which is expected to also play a role in relieving stress and related symptoms [192].

6. Conclusions

In the modern domestic environment, dogs and cats are regularly faced with various stress problems. Causes of stress include uncomfortable environments and conflicts in social life. When dogs and cats perceive stress, a series of physiological changes occur in the body, mainly mediated by the HPA and SAM axes. At the same time, oxidative stress has also been proved to be highly correlated with stress response. However, intestinal health is of great significance, especially in regulating dog and cat behaviors via the gut–brain axis. If stress is not alleviated, it may cause gastrointestinal diseases, urinary tract diseases, decreased immunity, abnormal behavior, and some cardiovascular problems. Dietary supplementation (e.g., antioxidants, anxiolytic agents, and probiotics) is conducive in alleviating the systemic changes associated with pet stress. Through this review, we provided insight into potential future research directions. Some small peptides and amino acids, such as alpha-casozepine and theanine, may act as agonists for receptors in the neuron system and thus show anxiolytic effects. Plant extracts (e.g., gallic acid and tannic acid) may have great potential in alleviating oxidative damage and promoting intestinal flora, which may be of great significance in improving intestinal stress symptoms. However, much remains unclear about how to apply the different dietary strategies into stress management (e.g., exact functions, side effects, and application guidelines). Overall, stress management and control in pets through dietary strategies is a systematic project that requires multifaceted efforts and sustained research.

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Wet-food diet promotes the recovery from surgery of castration and control of body weight in adult young cats

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ABSTRACT

Inappropriate dietary management may lead to delayed recovery from castration surgery and significant weight gain in cats after castration. Wet canned food often exhibits more advantageous characteristics than dry food (e.g., higher palatability and digestibility, and lower energy density). This study compared the effects of canned and dry food on surgical recovery and weight management in cats after castration. Eighteen healthy cats (weighed 4.33 ± 1.04 kg and aged 18-months old) were allocated to one of the two dietary treatments ($N = 9/\text{group}$), dry (CON) and canned food (CAN) balanced for sex and initial BW. Cats were fed *ad libitum* for 7 weeks, including one week before surgery (week 0) and 6 weeks after surgery (week 1–6). Daily dry matter intake (DMI), and weekly body weight (BW) and body condition score (BCS) was obtained. Feces were collected for measuring nutrient digestibility and concentrations of short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA). Physical pain and wound surface assessment were performed at week 1. Blood was also collected intermittently for measuring biochemical indices and untargeted metabolomics analysis. Results indicated that BW, BCS and daily DMI in CON group increased ($P < 0.05$) over time after castration, but were maintained relatively stable in CAN group. Cats in CAN group exhibited less pain-related behavior as reflected by lower score of comfort ($P < 0.05$) and vocalization ($P < 0.10$), improved wound surface assessment ($P < 0.10$), lower level of lipase ($P < 0.10$) and ratio of blood urea nitrogen/serum creatinine (BUN/SC; $P < 0.05$), and higher level of superoxide dismutase (SOD; $P < 0.05$) in week 1 than CON cats. Meanwhile, the CAN group had significantly higher concentration of immunoglobulin G (IgG) on days 5 and 7, and higher level of high-density lipoprotein cholesterol (HDL-C; $P < 0.10$) but lower triglyceride (TG; $P < 0.05$) than CON group on day 20 and 48. Fecal total and most individual SCFA increased significantly from week 1 to week 6 regardless of diet, but the increase of butyric acid over time only occurred in CON group ($P < 0.05$). Also, serum metabolomic analysis revealed differential metabolic pathways between the two groups. Overall, compared with the dry food, the canned food tested in our study promoted cat wound recovery by reducing pain and increasing immune and antioxidative capacity after sterilizing surgery, and helped to maintain healthy body condition in cats after castration.

Lay Summary

Castration is a surgical operation common in pet cats and dogs, and weight gain is often observed a period after castration. Nutritional management can be important for animal health in both processes. Due to differences in manufacturing techniques and nutrient composition, wet canned food generally exhibits higher palatability and lower energy density than dry food. Till date, few studies have explored if compared to dry kibbles, canned diet can have advantages in promoting recovery from castration surgery and maintaining normal body condition after castration in cats. In our study, dry and canned diets were fed to cats experiencing castration surgery with a free-feeding method. During the one week after surgery, cats fed canned food exhibited less pain and discomfort, and improved inflammation and antioxidative capacity than cats fed dry food. During the four weeks after surgery, cats fed dry food showed significantly more weight gain and change of body condition, meanwhile their blood and fecal measures resembled more of those observed in overweight and/or obese individuals than cats fed canned food. Collectively, canned food with high palatability and low energy density promoted the recovery of cats from the castration surgery and reduced their weight gain after castration.

Key words: cat, castration, pet food, surgery recovery, weight management, wet-food diet.

Abbreviations: A/G, albumin/globulin; ALB, albumin; ALP, alkaline phosphatase; BC, body condition; BCFA, branched-chain fatty acids; BCS, body condition score; BUN/SC, blood urea nitrogen/serum creatinine; BW, body weight; CAN, wet canned diet; CF, crude fiber; CON, control diet (i.e., dry extruded food); CP, crude protein; DE, digestible energy; DM, dry matter; DMI, dry matter intake; EE, ether extract; ELISA, enzyme-linked immunosorbent assay; FC, fold change; GE, gross energy; GT, glutamyl transferase; HDL-C, high-density lipoprotein cholesterol; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IL-10, interleukin 10; IL-8, interleukin 8; OM, organic matter; OPLS-DA, orthogonal partial least-squares discriminant analysis; PPS, physical pain score; PRT, response permutation testing; SCFA, short-chain fatty acids; SOD, superoxide dismutase; T-AOC, total antioxidative capacity; TBA, total bile acid; TG, triglyceride; TNF- α , tumour necrosis factor- α ; TP, total protein; VIP, variable importance in projection; WSS, wound surface score

INTRODUCTION

Cats are among the most popular pets worldwide (Foreman-Worsley and Farnworth, 2019). Many owners con-

sider their cats as family members (Ines et al., 2021) and pay increased attention to the cat physical and mental health. Meanwhile, pet overpopulation could result in pet

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abandonment and relinquishment, causing overwhelming burden to animal shelters and euthanasia of unwanted pets (Scarlett et al., 2002; Kustritz, 2007). Castration is one of the most common technique for surgically sterilizing dogs and cats, which signifies the removal of reproductive organs (Howe, 2006). Early statistics from 2007 showed that 92.0% of cats aged between 6 and 12 months were castrated in the United Kingdom (Murray et al., 2009), while the prevalence of cat castration was 82.1% in the United States in 2007 (Trevejo et al., 2011). There are behavioral and health benefits to castration, such as decreased urine spraying, roaming, and aggression in male cats, and reduced incidence of reproductive tract neoplasms in females (McKenzie, 2010).

The procedure of castration (e.g., stress from transportation, change of environment and the surgical procedure) poses challenges to cat welfare. Physiological and behavioral indicators of pain, wound healing process, and the overall effects of surgery on cat well-being should be closely monitored and evaluated (Väisänen et al., 2007). Nutritional management is critical in postoperative care (Collins, 2016; Vendramini et al., 2020). Relevant studies in dogs and cats have been focused on nutritional support (Brunetto et al., 2010; Corbee and Kerkhoven, 2014) and the supplementation of bioactive ingredients, such as ginger rhizome powder (Javdani et al., 2021). For example, adequate energy support, even if modest and close to resting energy requirement of dogs and cats during the hospitalization could reduce the length of the hospital stay (Brunetto et al., 2010). This indicates the importance of nutritional support in speeding the recovery of pet cats and dogs from treatment procedures of illness, including surgery. Hyporexia (i.e., reduced appetite) and in the more severe case anorexia (i.e., complete loss of appetite) can occur due to surgery, therefore stimulation of spontaneous food intake shortly after surgery by providing food with high palatability (e.g., canned food with raised moisture, protein and fat content) is important (Corbee and Kerkhoven, 2014). Cats were shown to prefer wet food with a moisture content close to that of raw meat (Zaghini and Biagi, 2005). In addition, food with high water content can contribute to rehydration of animals and reduce the chance of food being vomited or regurgitated due to quicker movement of digesta from stomach to intestinal tract (Sachdeva et al., 2013). Nutrient digestibility of canned food has been shown to be higher than that of dry kibbles in cats (Bermingham et al., 2013, 2018). Collectively, canned food may promote the body recovery for cats after surgical procedure due to its advantages in promoting rehydration and better nutrient support (Corbee and Kerkhoven, 2014).

Castration has been associated with weight gain and obesity in pet cats (Larsen, 2017). The sexual and appetite-related hormonal changes after castration was shown to impact feeding behavior and general activity (Fettman et al., 1997). Castration may induce significant weight gain and obesity by reducing energy expenditure and increasing food intake (Martin et al., 2001). A significant increase in feed intake was observed as early as three days after neutering, along with a body weight (BW) gain of about 28% by week 7 in male cats (Kanchuk et al., 2003). Nutritional management can also be important for the maintenance of normal BW after castration, and existing studies mainly concentrate on the adjustment of energy and nutrients. For instance, previous experiment suggests that diet of low energy and fat can effectively control excessive weight gain in castrated dogs

(Schauf et al., 2016). Excessive BW gain can be controlled by limiting the amount of energy supply in food. One major difference between wet and dry pet food is the water content, which contributes to the dilution of energy density in wet food compared to the dry food. A survey on feline obesity identified feeding mainly dry kibbles as a major risk factor for cats at age of 12.5–13 months (Rowe et al., 2015). One study also reported that adding 40% water to commercial dry food could increase voluntary activity and ameliorate regain of BW after caloric restriction in cats (Cameron et al., 2011). Therefore, the diluted energy density of wet food due to higher water content may positively regulate cat BW and body condition (BC) through mainly reducing dry matter and energy intake, and increasing activity to maintain energy balance.

Till date, few studies have compared the effects of dry and wet pet food on the control of BW and body condition score (BCS) in cats after castration. Accordingly, we hypothesized that high-moisture canned food which is often more palatable, and with better nutrient digestibility and lower energy density, might speed the recovery from castration surgery and the long-term BC control after castration in cats when compared to dry food.

MATERIALS AND METHODS

Animal ethics

All experimental procedures were authorized by the Animal Care and Use Committee prior to animal experimentation (Approval number: 2021a030) and were performed following the guidelines of the Laboratory Animal Center at the South China Agricultural University.

Animals and housing

A total 18 healthy Ragdoll cats, 6 males and 12 females, with the mean BW of 4.33 ± 1.04 kg and aged 18 months old were included in this experiment. Cats were housed individually at the laboratory in Qingke Biotechnology Co., Ltd (Guangzhou, China) with relative humidity and temperature of $70\% \pm 3\%$ and $22^\circ\text{C} \pm 2^\circ\text{C}$, respectively. The housing cage (1.1 m \times 0.7 m \times 0.7 m) included separate areas for feeding, defecation and resting. Cats were also allowed to interact with people for 10 minutes daily and access to toys. All cats were vaccinated and dewormed and no drugs such as antibiotics that may influence the results of this experiment were given one month prior to the trial. Before the experiment, cats were free to eat adequate fresh food and drink clean water.

Diets and experiment design

Cats were allocated to one of the two dietary treatments (N = 9/group), dry food (CON group, 3 males and 6 females) and wet canned food (CAN group, 4 males and 5 females), according to their sex and initial BW. All cats were offered respective diet *ad libitum*. Specifically, the CON group were fed excess amount of dry food (i.e., 200 g) once daily at 9:00 am. Meanwhile, wet food was provided to the cats in CAN group for four times daily at around 8:00 am, 13:00 pm, 18:00 pm, and 23:00 pm, respectively, every time with a new can that contains 160 g wet food opened. After five days of adaption to the experimental diets, the CON and CAN group were castrated in the same veterinary hospital. All the experimental subjects were then transported back to the lab for the 6 weeks of postoperative recovery and BW monitor

experiment. The timeline and sampling time points of this experiment are shown in Figure 1.

Recording of Daily Dry Matter Intake (DMI), BW, and BCS

The daily DMI of all experiment subjects was recorded every morning and the BW and BCS which referred to the five-point BCS system in cats (Shoveller et al., 2014) were recorded weekly after overnight fasting during the whole period. Cats all started with a normal BCS that ranged from 2.7 to 3.2.

Diet composition and apparent digestibility of nutrients

The CON group was fed a commercial extruded pet food (Qingke Biotechnology Co., Ltd, Guangzhou, China) and the CAN group was fed a canned pet food manufactured in Guangdong Munchkin Biotechnology Co., Ltd (Shantou, China). Both diets meet the nutrient recommendations of Association of American Feed Control Officials for adult cats. The ingredients, analyzed chemical and energy composition of two diets tested are listed in Table 1.

Total feces from every cat were collected for 5 consecutive days before morning feeding at week 2 to determine the apparent digestibility. The process was conducted in week 2 because cats at week 1 after surgery ate little and feces excreted during this period was not enough for the analysis of nutrient digestibility. The diets and feces samples collected were oven-dried at 65°C until dry and grounded for chemical analysis. Samples were analyzed for dry matter

(DM, method No. 934.01), organic matter (OM, method No. 942.05), crude fiber (CF, method No.962.09), crude protein (CP, method No. 954.01) and ether extract (EE, method No.920.39) according to Association of Official Analytical Chemists methods (Horwitz and Latimer, 2007). Gross energy (GE) of diets and feces were determined by oxygen bomb calorimeter (IKA C 200, IKA Guangzhou Instrument Equipment Co., Ltd, Guangzhou, China). Apparent digestibility of certain nutrient was measured using the following formula: apparent digestibility (%) = $(1 - A1/A) \times 100\%$, where A is the content of a given nutrient in the diet, A1 is the content of the same nutrient in the feces. And the digestible energy (DE) was measured using the following formula: $DE (J/g) = B - B1$, where B is the GE (J/g) of diets and B1 is the GE (J/g) of feces.

Evaluation of physical pain and wound surface

A composite pain scale was adopted to evaluate the state of somatic pain in cats with minor modifications (Brondani et al., 2011). The rationality is that cats experiencing different levels of stress and pain would exhibit differences in pain-related behaviors (e.g., vocalization, aggression, body posture) and response to human social interaction (Brondani et al., 2011). The most relevant items selected from the tool were vocalization, posture, and comfort. Besides, wound healing scale containing items of inflammation, swelling, and color of the wound surface was applied to evaluate the wound recovery in cats (Drudi et al., 2018). Results were presented as physical pain score (PPS) and wound surface score (WSS), respectively. Evaluations were performed within week 1 by two experimenters who were

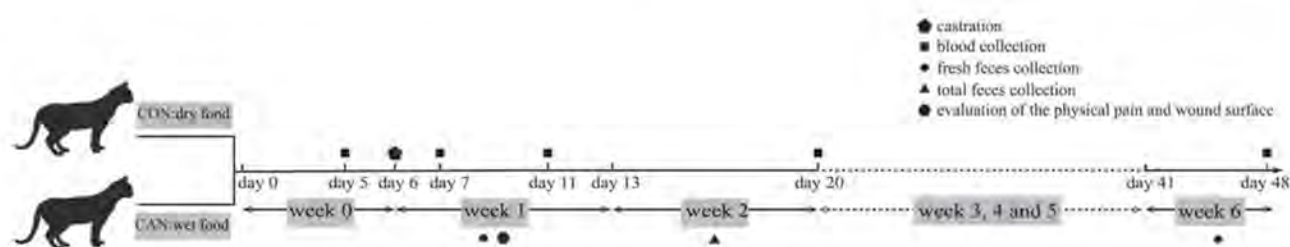


Figure 1. Timeline and time points of sample collection.

Table 1. Major ingredients, analyzed chemical and energy composition of two diets tested

Item	CON	CAN
Ingredients	Chicken (40%), chicken meat powder (33%), chicken fat (6%), tapioca flour (4%), sweet potato flour (3.5%), chicken liver (2%), chicken heart (2%), Brewer's yeast powder (2%), alfalfa powder (2%), fish oil (1.5%), chicken liver powder (1%)	Water, chicken (55%), swine liver (16%), cornmeal (1.7%), soy protein isolate (0.25%), fish oil (0.2%), chicken offal powder (0.4%)
Analyzed composition		
DM (%)	93.43	34.23
OM (% DM)	91.39	94.24
CF (% DM)	1.70	1.90
CP (% DM)	47.09	52.23
EE (% DM)	16.50	20.74
GE (J/g)	21943.00	9375.96
GE (J/g, on DM basis)	23486.03	27391.06

DM, dry matter; OM, organic matter; CF, crude fiber; CP, crude protein; EE, ether extract; GE, gross energy. CON, cats fed dry food; CAN, cats fed wet food.

blind to the treatments, and an agreed score was given for each item by the two experimenters. The scoring criteria were shown in Tables 2 and 3.

Blood collection and serum biochemical analysis

As shown in Figure 1, on the day before the surgery, day 1, day 5, day 14 and day 42 after the surgery (i.e., day 5, day 7, day 11, day 20 and day 48), 4 mL blood was collected from each cat via forelimb vein after overnight fasting. The blood samples were then transferred to a pre-cooled serum separator tube, left to stand for 30 minutes before centrifugation at 3,500 rpm at room temperature for 15 minutes. After centrifugation, the supernatants were aliquoted into microcentrifuge tubes and stored at -80°C for further analysis.

Serum albumin (ALB), total protein (TP), globulin (GLOB), albumin/globulin (A/G), glutamyl transferase (GT), alkaline phosphatase (ALP), total bile acid (TBA), lipase, blood urea nitrogen (BUN), and ratio of BUN/SC (blood urea nitrogen/creatinine) on day 5, day 7, and day 11 were measured with commercial kits using an automatic blood biochemical analyzer (Chemray 800, Shenzhen Redu Life Technology, Shenzhen, China). Total antioxidative capacity (T-AOC) and superoxide dismutase (SOD) on day 5, day 7 and day 11 and triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) on day 20 and day 48 were determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's protocols. Tumour necrosis factor- α (TNF-

α), interleukin 10 (IL-10), interleukin 8 (IL-8), and immunoglobulin (Ig) A, G, and M on day 5, 7 and 11 were evaluated using commercial cat enzyme-linked immunosorbent assay (ELISA) kits (MEIMIAN, Jiangsu Meimian Industrial Co., Ltd., Jiangsu, China). These parameters were chosen to reflect the inflammatory state, and the antioxidative and immune capacity of the body, which can contribute significantly to postoperative recovery (Moldal et al., 2012; Mogheiseh et al., 2019), and might be differentially impacted by the two diets if the wet food showed advantages in rehydrating the body and nutrient support than dry food.

Collection of fresh feces and analysis of short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA)

Fresh feces were collected at week 1 and week 6 for consecutive 3 days as shown in Figure 1, and stored at -80°C for further analysis. Upon processing, samples of feces were placed on ice for thawing, and 1 mL of ultra-pure water was added to 0.2 g fecal sample and vortexed for five minutes. The samples were placed in ice bath for ten minutes of ultrasonic crushing. Then samples were centrifuged at 13,000 rpm for ten minutes at 4°C , whereafter 20 μL of 25% metaphosphoric acid solution and 0.25 g anhydrous sodium sulfate was added to the collected supernatant. After mixing for 2 minutes, 1 mL of methyl tert-butyl ether was added into each sample. The samples were then centrifuged at 13,000 rpm for 5 minutes at 4°C after five minutes of mixing. Supernatant was harvested and

Table 2. Evaluation standard of physical pain after surgery

Item	Assessment	Score
Vocalization	Cats purr and interact with humans when they are touched.	0
	Cats hiss or groan when people approach. When people petted the cat, it calms down.	1
	Cats hiss or groan when they are petted.	2
	Cats hiss or groan spontaneously.	3
Posture	Cats are relaxed and comfortable.	3
	Cats lie on their sides, stretch limbs and tense muscles.	
	Cats lie on their backs with muscular tension and low activity.	2
	Cats are nervous, moving around and trying to find a comfortable place.	1
Comfort	Cats are interested in their surroundings and have exploratory behavior.	3
	Cats are relatively quiet and not interested in external stimuli.	2
	Cats keep lying down and getting up and feeling restless.	1

Table 3. Evaluation standard of wound surface after surgery

Item	Assessment	Score
Inflammation	A large amount of tissue fluid or pus ooze from the wound with tissue proliferation.	3
	A small amount of fluid oozes from the wound.	2
	The wound is dry without fluid exudation.	1
Swelling	The wound is markedly swollen.	3
	The wound is slightly swollen.	2
	There was no swelling on the wound.	1
Color	The wound is bright red.	3
	The wound is pink.	2
	The wound has the color of normal skin.	1

filtered through 0.22- μ m Millipore pore membrane filter to a sample vial.

The quantitative analysis of SCFA and BCFA of the preprocessed samples were carried out using gas chromatography-MS-QP2020 system (Shimadzu, Tokyo, Japan) following the method previously used in our laboratory (Yang et al., 2022). The gas chromatography was equipped with an auto-injector AOC-20i (Shimadzu) and coupled to a flame ionization detector. The chromatographic separation was performed on a DB-FFAP capillary column (30 m \times 0.25 mm \times 0.25 mm). Sample (0.6 μ l) was injected with a 30:1 split ratio using an autosampler. The injection port was set to a temperature of 250 $^{\circ}$ C. The initial temperature of the column was 80 $^{\circ}$ C for 2 minutes. Then the temperature was increased to 150 $^{\circ}$ C at a rate of 10 $^{\circ}$ C/minute and maintained for 2 minutes. Finally, the temperature was increased to 180 $^{\circ}$ C at a rate of 15 $^{\circ}$ C/minute and maintained for 5 minutes. The total run time was 18 minutes. Helium (He; 99.999%) was the carrier gas with a flow rate of 3 ml/minute. The MS parameters were electron impact mode at ionization energy of 70 eV. The ion source and interface temperatures were 230 $^{\circ}$ C and 250 $^{\circ}$ C, respectively. The solvent delay time was 1 minute at the temperature of 230 $^{\circ}$ C. The acquisition mode was selected at ion monitoring mode with a scan interval of 0.3 second. Fecal SCFA and BCFA were measured with the intention to provide additional explanations for the potentially different impacts of dry and wet food on BW and BC of cats, as studies have correlated gut microbiota, along with their products (e.g., SCFA) with weight gain and the development of obesity in different species (Kielar et al., 2017; Riva et al., 2017; Wei et al., 2021).

Untargeted serum metabolomics analyses

As an advanced method widely applied in the research field of nutrition and metabolism (Carlos et al., 2020), serum metabolomics analyses allows the identification of changes in almost all the small molecules involved in the metabolic processes in serum, and can help to determine the underlying metabolic pathways that mediate the potential differences in the observed physiological responses caused by the two diets.

Serum samples were thawed and 200 μ l of each sample was mixed with 800 μ l methanol. After two minutes of vortex, the samples were centrifuged at 14,500 rpm for 15 minutes at 4 $^{\circ}$ C. The supernatant was blow-dried with nitrogen. Each sample was then re-dissolved with 200 μ l methanol and

mixed for two minutes. Ultrasonic crushing was performed at a low temperature for ten minutes and samples were centrifuged at 14,500 rpm for 15 minutes at 4 $^{\circ}$ C. Finally, all samples were filtered through 0.22- μ m microporous membranes for UPLC-Orbitrap-MS/MS analysis using the method described previously (Xin et al., 2018).

The Compound Discoverer 2.1 (Thermo Fisher Scientific) data analysis tool was employed to complete raw data preprocessing automatically and was applied to identify metabolites by searching the mzCloud library and mzVault library. Orthogonal partial least-squares discriminant analysis (OPLS-DA) of metabolites was performed with the SIMCA-P 14.1 software. Response permutation test (RPT) was performed to test the accuracy of OPLS-DA model. The metabolites with variable importance in projection (VIP) > 1 and fold change (FC) > 2 or < 0.5 were deemed as the differential metabolites. To explore the changes of metabolic process further, a KEGG pathway analysis of differential metabolites was performed by using the enrichment analysis module on MetaboAnalyst 5.0.

Statistical analysis

SPSS 26.0 and GraphPad Prism 8.0 software were used for statistical analysis and graphic presentation. Independent samples Student's *t*-test were performed to compare the difference between the two groups. Two-way repeated measure analysis of variance with Bonferroni adjustment for multiple comparisons was performed to analyze the differences within two groups (i.e., diet) at different time points (i.e., time). Significant differences were set at $P < 0.05$, and tendencies at $P < 0.10$.

RESULTS

BW, BCS, and daily DMI

Time and the interaction between diet and time (diet \times time) affected BW and daily DMI of cats ($P < 0.05$; Figure 2). Time but not diet \times time significantly affected BCS of cats (Figure 2). No significant differences of BW, BCS, and daily DMI were observed between the two diet-treated groups (Figure 2a).

Briefly, the increase of BW, BCS, and daily DMI over time occurred in both diet-treated groups, but were at greater rates in the CON group than the CAN group. Specifically, cats of both groups lost weight at week 1 compared with week 0 ($P < 0.05$, Figure 2a). Increase of BW can be noticed in the

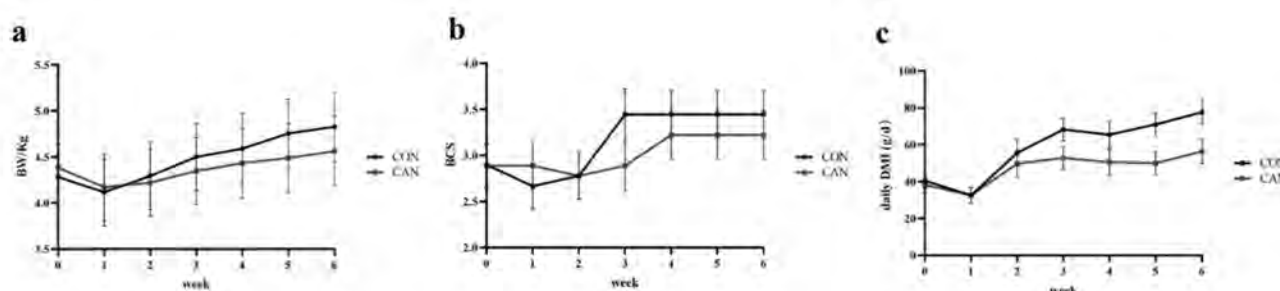


Figure 2. The effect of diets and experiment duration on BW, BCS and daily DMI in cats: (a) BW, body weight, (b) BCS, body condition score, (c) daily DMI, daily dry matter intake. CON, cats fed dry food; CAN, cats fed wet food. Week 0 represents the five days before the surgery, week 1, 2, 3, 4, 5 and 6 represent the following six weeks after surgery. Data are presented as mean \pm SEM. The increase of BW, BCS, and daily DMI over time occurred in both diet-treated groups, but were at greater rates in the CON group than the CAN group.

CON group at week 5 and 6 when compared with week 0 ($P < 0.05$, Figure 2a), but no such difference was observed with cats in the CAN group ($P > 0.10$, Figure 2a). Compared with the CAN group, which showed no significant difference in BCS at different time points, BCS in the CON group at week 3, 4, 5 and 6 was significantly higher than week 1 but not week 0 (Figure 2b). For the CON group, daily DMI from week 3 to week 6 was significantly higher than week 0 ($P < 0.05$, Figure 2c). Diversely, daily DMI of CAN group maintained at a stable level over the whole period ($P > 0.10$, Figure 2c).

Apparent digestibility of nutrients and DE

The apparent digestibility of DM, CF, CP, and EE were relatively higher ($P \leq 0.06$) in CAN group than CON group, while digestibility of OM and GE between the groups were not different (Table 4). After calculation, DE of wet and dry food was 20022.11 J/g and 8565.80 J/g (as is), respectively.

PPS and WSS

Cats in the CAN group had lower score of vocalization than CON group ($P < 0.05$, Figure 3a), while the CON group had a moderately higher score of comfort than CAN group during the first week after the surgery ($P < 0.10$, Figure 3a). With regard to the WSS, the score of inflammation and color of the

wound in the CAN group is slightly lower than that of the CON group ($P < 0.10$, Figure 3b).

Serum chemistry

The main effect of diet was significant for lipase and BUN/SC ($P < 0.05$), while the effects of time and diet \times time were not significant ($P < 0.10$; Figure 4). It's evident that CON group had higher concentration of lipase and higher ratio of BUN/SC than CAN group on day 5, 7 and 11 ($P < 0.10$, Figure 4a, b). The effects of diet and diet \times time on BUN were not significant ($P > 0.10$), while time significantly affected BUN. The level of BUN of both groups displayed a conspicuous reduction after surgery on day 7 compared with day 5 ($P < 0.001$, Figure 4c).

Inflammatory cytokines, immunoglobulins and antioxidant parameters

There was significant time effect but no diet and diet \times time effect on TNF- α , IL-8 and IgM. Specifically, the level of TNF- α increased on day 7 ($P < 0.10$, Figure 5a) and decreased on day 11 ($P < 0.001$, Figure 5a) in both groups. The level of IL-8 decreased on day 7 ($P < 0.05$, Figure 5b), when compared to day 5. As for immunoglobulins, the level of IgM on day 11 was significantly lower than it was on day 5 and day 7 ($P < 0.05$, Figure 5c). The effects of diet and diet \times time on IgG was significant ($P < 0.05$). The CAN group had higher level of IgG than CON group on day 5 and day 7, but not on day 11 ($P < 0.05$, Figure 5d). On day 5, 7 and 11, diet significantly impacted the concentration

Table 4. Effects of diets on apparent digestibility in cats

Item	CON	SEM	CAN	SEM	P-value
DM (%)	87.04	0.01	89.14	0.01	0.02
OM (%)	87.64	0.01	89.17	0.01	0.13
CF (%)	30.08	0.04	61.39	0.05	0.01
CP (%)	90.62	0.01	91.52	0.01	0.06
EE (%)	94.03	0.01	96.60	0.01	0.01
GE (%)	91.25	0.01	91.36	0.01	0.82

DM, dry matter; OM, organic matter; CF, crude fiber; CP, crude protein; EE, ether extract; GE, gross energy. CON, cats fed dry food; CAN, cats fed wet food. Data are presented as mean \pm SEM. Statistical difference was calculated by Student's *t*-test.

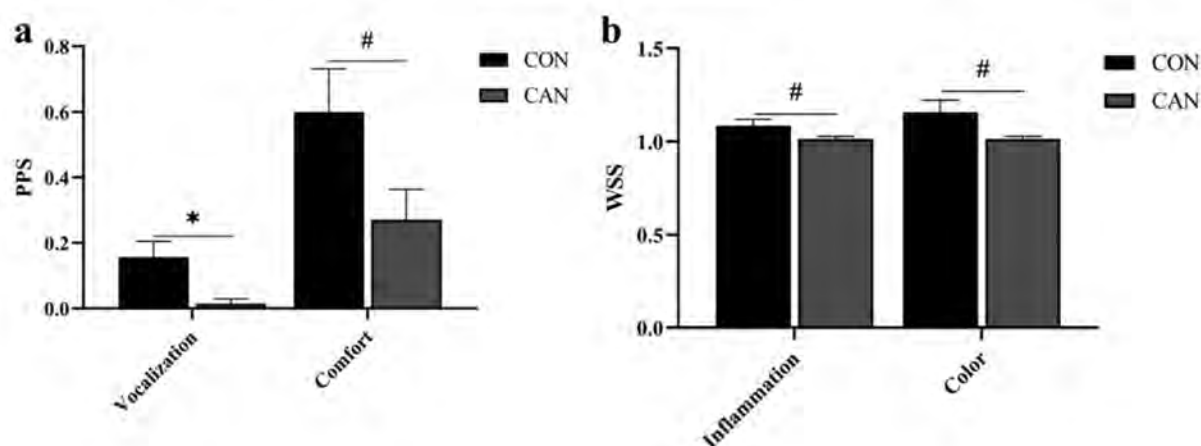


Figure 3. The effect of diets and experiment duration on the PPS and WSS in cats at week 1: (a) PPS, physical pain score, (b) WSS, wound surface score. CON, cats fed dry food; CAN, cats fed wet food. Week 1 represents the first week after surgery. Data are presented as mean \pm SEM. The symbol (*) indicates statistically significant differences between two groups (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$), and the symbol (#) represents difference tendency ($P < 0.10$).

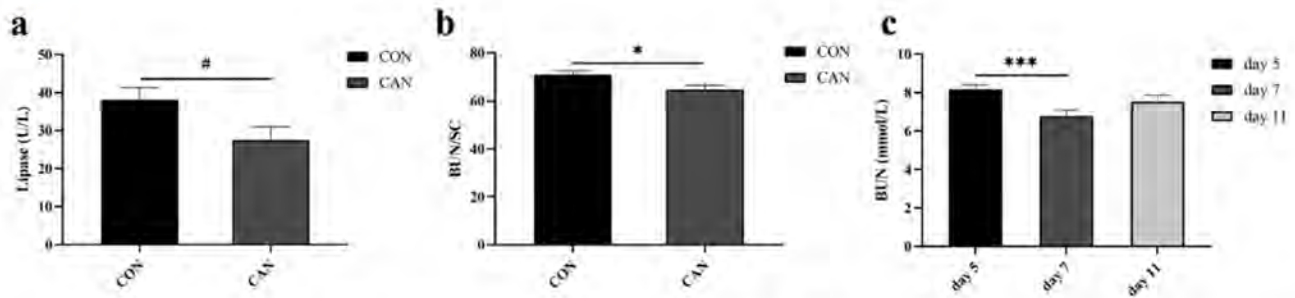


Figure 4. The effect of diets and experiment duration on serum chemistry in cats on day 5, 7 and 11: (a) lipase, (b) BUN/SC, blood urea nitrogen/serum creatinine, (c) BUN, blood urea nitrogen. CON, cats fed dry food; CAN, cats fed wet food. Day 5 represents the day before the neutering surgery, and day 7 and day 11 represent the first and fifth day after the surgery. Data are presented as mean \pm SEM. The symbol (*) indicates statistically significant differences between two groups (* P < 0.05, ** P < 0.01, and *** P < 0.001), and the symbol (#) represents difference tendency (P < 0.10).

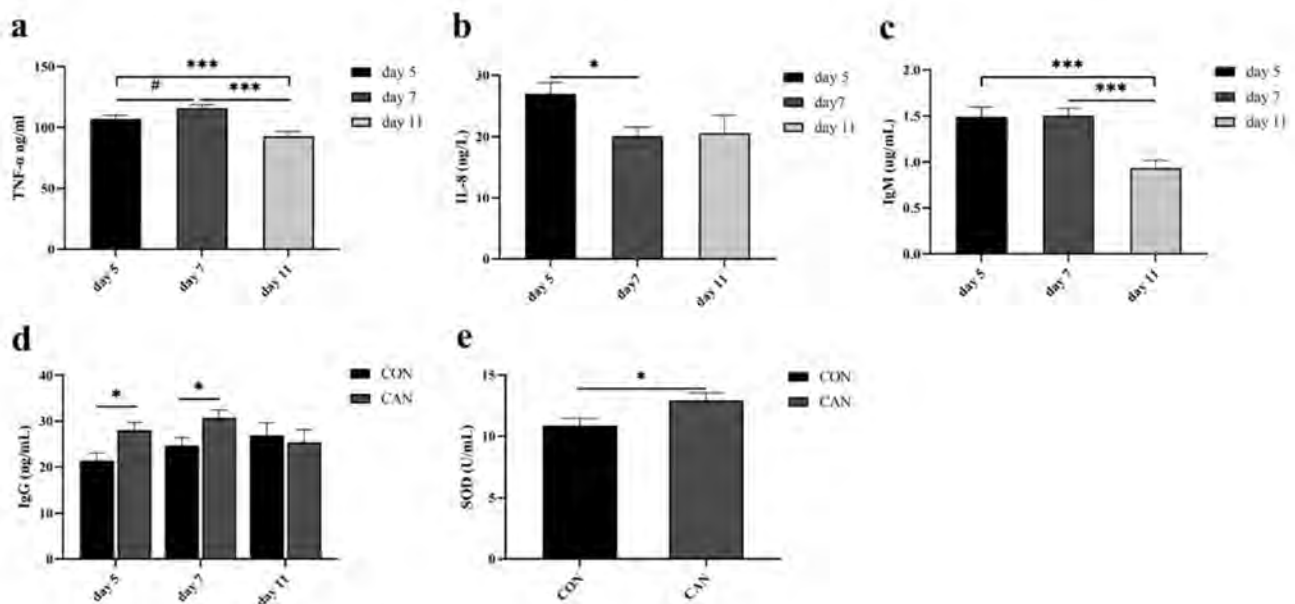


Figure 5. Effects of diets and experiment duration on the levels of inflammatory cytokines, immunoglobulins and antioxidant parameters in cats on day 5, 7 and 11: (a) TNF-α, tumor necrosis factor-α, (b) IL-8, interleukin 8, (c) IgM, immunoglobulin M, (d) IgG, immunoglobulin G, (e) SOD, superoxide dismutase. CON, cats fed dry food; CAN, cats fed wet food. Day 5 represents the day before the neutering surgery, and day 7 and day 11 represent the first and fifth day after the surgery. Data are presented as mean \pm SEM. The symbol (*) indicates statistically significant differences between two groups (* P < 0.05, ** P < 0.01, and *** P < 0.001), and the symbol (#) represents difference tendency (P < 0.10).

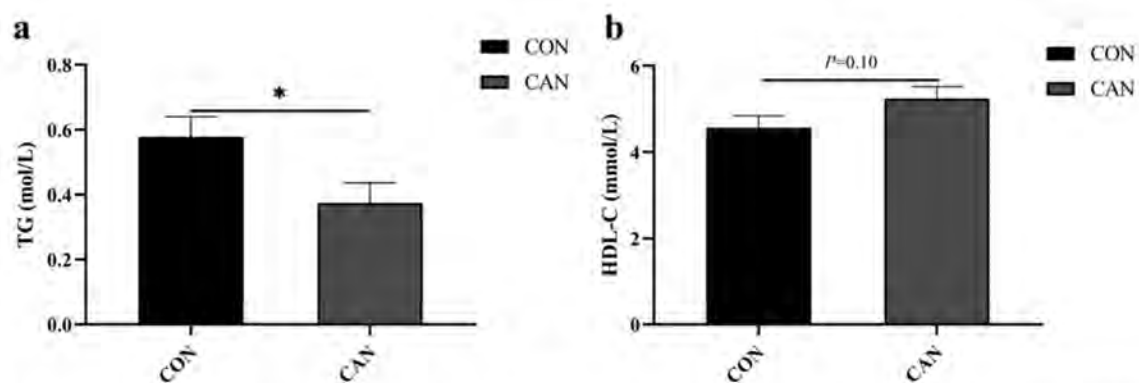


Figure 6. The effect of diets and experiment duration on the levels of obesity-related indices in cats on day 20 and 48: (a) TG, triglyceride, (b) HDL-C, high-density lipoprotein cholesterol. CON, cats fed dry food; CAN, cats fed wet food. Day 20 and day 48 represent the 14th day and 42th day after the surgery. Data are presented as mean \pm SEM. The symbol (*) indicates statistically significant differences between two groups (* P < 0.05, ** P < 0.01, and *** P < 0.001), and the symbol (#) represents difference tendency (P < 0.10).

of SOD in that the SOD level in the serum of CAN group was higher than that of CON group ($P < 0.05$, Figure 5e).

Obesity-related indices

As shown in Figure 6, there was a significant effect of diet on the concentration of TG ($P < 0.05$), but time and diet \times time had no effect ($P > 0.10$). The concentration of TG in CAN group was lower than that in CON group on day 20 and 48 ($P < 0.05$, Figure 6a). Only the effect of diet on HDL-C was obvious ($P < 0.10$). Concentration of HDL-C in CAN group tended to be higher than CON group on day 20 and 48 ($P = 0.10$, Figure 6b).

Fecal SCFA and BCFA

The data of SCFA provided is on wet feces basis. Only time had significant effect on the level of total SCFA, acetic acid, and propionic acid ($P < 0.05$), and diet \times time affect butyric acid ($P < 0.10$; Figure 7). The concentration of total SCFA in feces increased from week 1 to week 6 in both groups ($P < 0.05$, Figure 7a), and a similar trend could be observed with acetic acid ($P < 0.01$, Figure 7b) and propionic acid ($P < 0.10$, Figure 7c). The level of butyric acid was significantly higher at week 6 than week 1 in CON group but not in CAN group (Figure 7d). Besides, the content of butyric acid was marginally lower in CAN group than CON group at week 6 ($P < 0.10$, Figure 7d).

Serum metabolome on day 5, day 7 and day 11

Untargeted serum metabolome was monitored to further explore the effects of time and diet in cats (Figure 8). Results showed that a total of 157 metabolites were detected in both groups on day 5, day 7, and day 11. The OPLS-DA analysis indicated that there was evident separation between the CON and CAN group at different time (Figure 8a, d and g). As shown in Figure 8b, e and h, the RPT method revealed the high reliability of the OPLS-DA models. Details about the differential metabolites at specific time points are shown in Supplementary Table S1. The primary differential metabolites were adhumulone, cohumulone, desaminotyrosine, taurine, and 3,5-dihydroxybenzoic acid on day 5, desaminotyrosine, adhumulone, cohumulone, and 3,5-dihydroxybenzoic acid on day 7, and desaminotyrosine, p-hydroxyphenylacetic acid and cohumulone on day 11, respectively. To further explore the changes of metabolic processes, a KEGG pathway analysis of differential metabolites was performed. The affected metabolic pathways mainly focused on amino acid metabolism (i.e., D-glutamine and D-glutamate, taurine and hypotaurine, and arginine) and energy metabolism, such as nitrogen metabolism on day 5 (Figure 8c). On day 7, diet affected arginine biosynthesis and phenylalanine metabolism (Figure 8f). Amino acid metabolism (i.e., D-glutamine and D-glutamate metabolism, arginine biosynthesis, valine, leucine and isoleucine biosynthesis, and histidine metabolism), nucleotide (i.e., Pyrimidine) metabolism, and energy metabolism involving nitrogen were impacted on day 11 (Figure 8i).

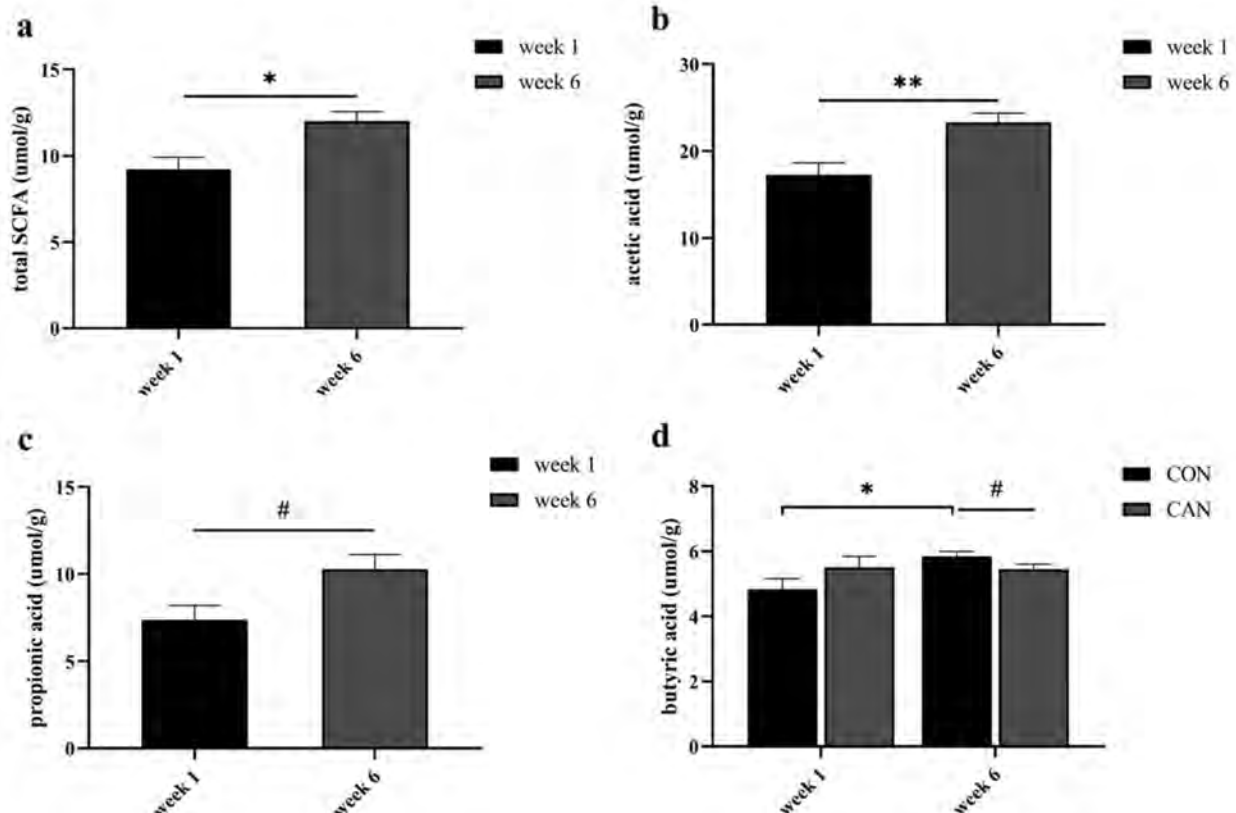


Figure 7. The effect of diets and experiment duration on the levels of SCFA and BCFA in feces at week 3 and 6: (a) SCFA, short-chain fatty acids, (b) acetic acid, (c) propionic acid, (d) butyric acid. CON, cats fed dry food; CAN, cats fed wet food. Week 1 and week 6 represent the first week and sixth week after the surgery. Data are presented as mean \pm SEM. The symbol (*) indicates statistically significant differences between two groups (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$), and the symbol (#) represents difference tendency (* $P < 0.10$).

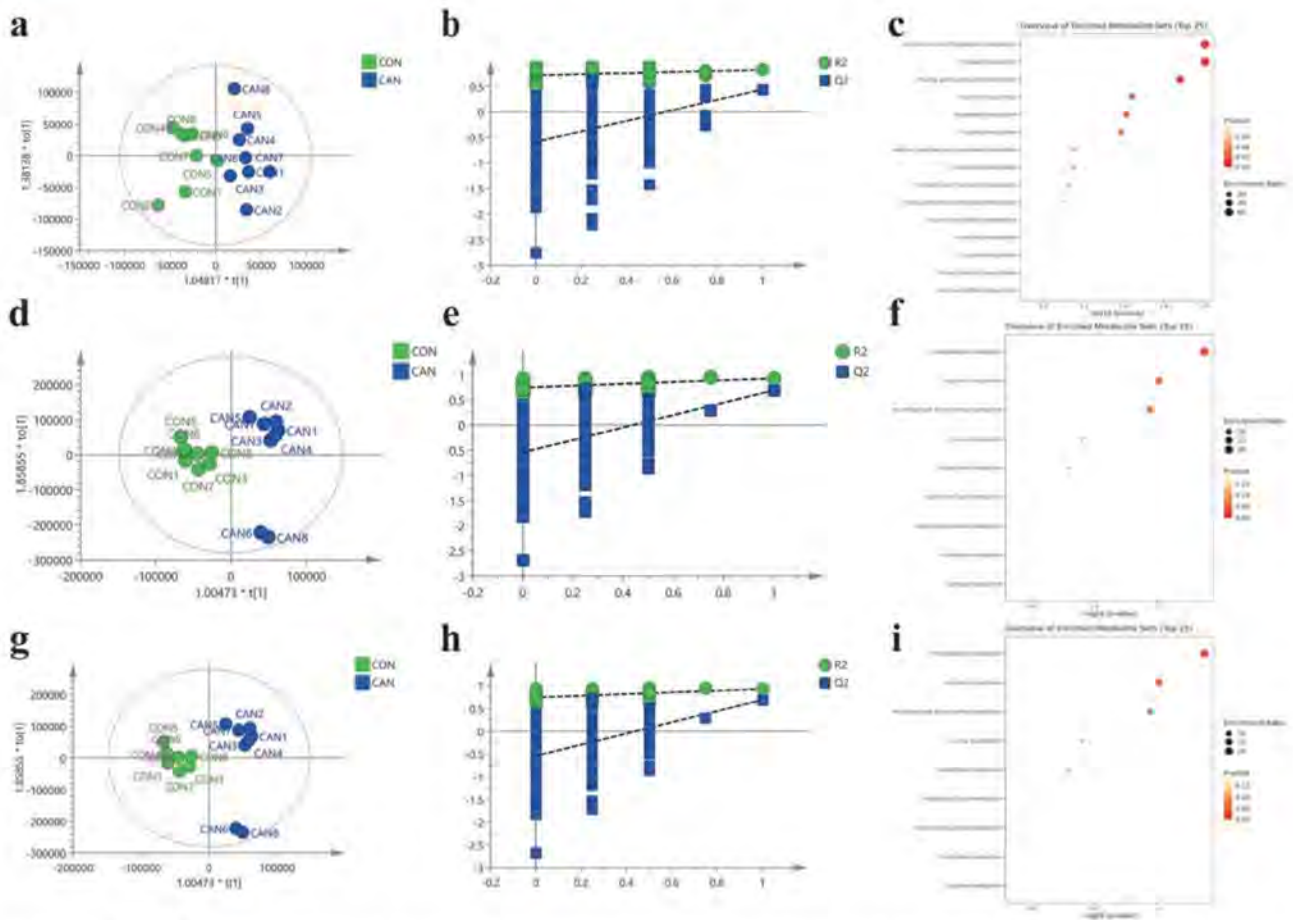


Figure 8. The effect of diets and experiment duration on the serum metabolome in cats on day 5, day 7 and day 11 and day 20 and day 48: (a, d and g) Score plots from the orthogonal partial least-squares discriminant analysis (OPLS-DA) model among two groups on day 5, 7 and 11. (b, e and h) RPT of the OPLS-DA models among two groups on day 5, 7 and 11. (c, f and i) Bubble chart of the metabolic pathway analysis of differential metabolites among two groups on day 5, 7 and 11. CON, cats fed dry food; CAN, cats fed wet food. Day 5 represents the day before the neutering surgery, and day 7 and day 11 represent the first and fifth day after the surgery.

Serum metabolome on day 20 and day 48

For the analysis of serum metabolome on day 20 and day 48, we used the same analytical process as for data from day 5, 7, and 11 (Figure 9). The results showed that 87 metabolites were detected in total and there was obvious separation between two dietary groups at the two selected time points (Figure 9a and d). As shown in Figure 9b and e, the RPT method also revealed the reliability of the OPLS-DA model. The differential metabolites at varying time points are listed in Supplementary Table S2. The major differential metabolites were 2,4-Dihydroxybenzoic acid, desaminotyrosine, cohumulone and lenticin on day 20 and 48. Metabolic pathways that were affected by diet on day 20 were mainly centered on lipid (i.e., arachidonic acid) metabolism and amino acid (i.e., tryptophan) metabolism (Figure 9c), and on day 48 focused on amino acid metabolism (i.e., biosynthesis of phenylalanine, tyrosine, and tryptophan, and tryptophan metabolism) and aminoacyl-tRNA biosynthesis (Figure 9f).

Discussion

Acute recovery from castration surgery

Previous studies have reported that during the short period after surgical castration, there is a decline in feed intake

probably due to systematic inflammation, wound pain, and reduced gastric motility according to previous studies (Kushner et al., 2006; Tewari et al., 2013). Diet with high palatability and moisture, such as canned food can contribute to the recovery of voluntary food intake (Corbee and Kerkhoven, 2014), and is beneficial to rehydration and nutrient absorption (Sachdeva et al., 2013). In contrast, significant differences between the two diet groups regarding BW, daily DMI, and BCS were not observed shortly after surgery in our study. Even though voluntary water intake was not recorded in our study, cats fed dry food generally have less total water intake and decreased urine volume compared to cats fed wet canned diet (Seefeldt and Chapman, 1979; Thomas et al., 2017). In addition, the canned food had higher fat, protein, and energy content on a dry matter basis than the dry food (Table 1). Meanwhile, digestibility of most nutrients in CAN group was at higher rate than CON group (Table 4), which is similar to what has been reported in other studies (Bermingham et al., 2013, 2018). Therefore, even with similar DMI between diet groups, the CAN food could allow for more water, nutrient, and energy intake which is important for the restoration of circulation of biofluids, and promoting the nutrient and energy utilization (Corbee and Kerkhoven, 2014).

Behavioral measures have been used to assess pain of cats in clinical research (Mollenhoff et al., 2005). Cats which are

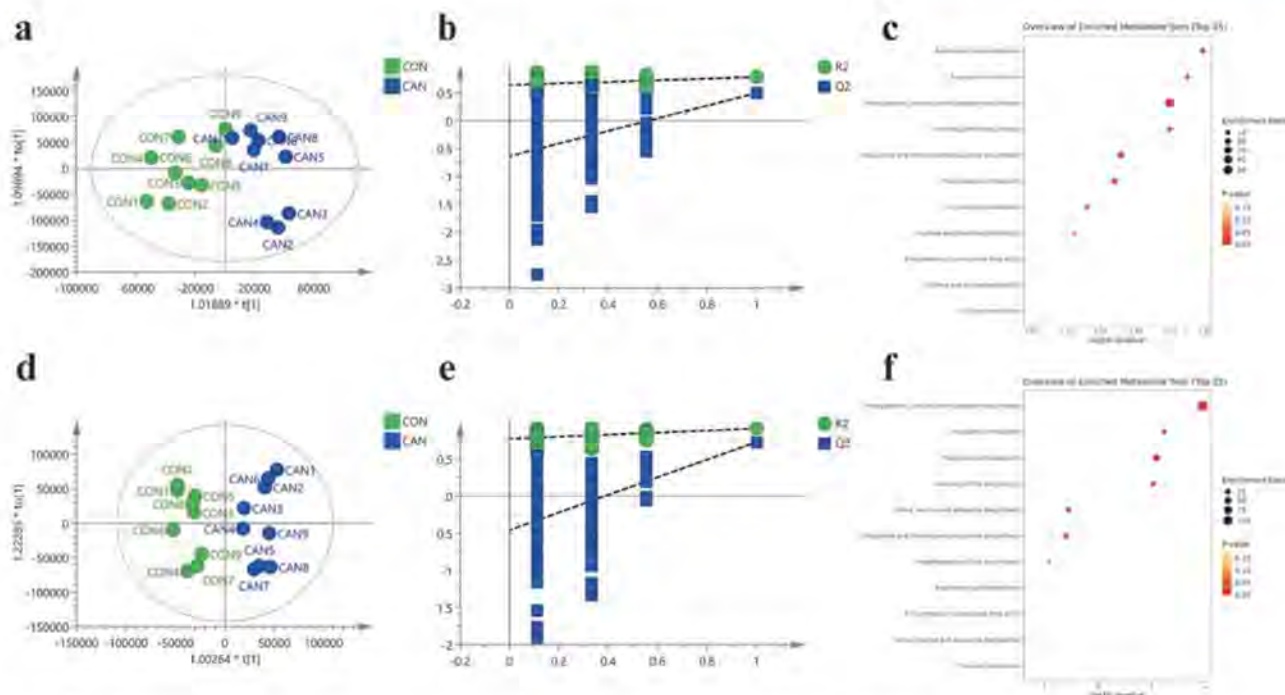


Figure 9. The effect of diets and experiment duration on the serum metabolome on day 20 and 48. (a and d) Score plots from the orthogonal partial least-squares discriminant analysis (OPLS-DA) model among two groups on day 20 and 48. (b and e) RPT of the OPLS-DA models among two groups on day 20 and 48. (c and f) Bubble chart of the metabolic pathway analysis of differential metabolites among two groups on day 20 and 48. CON, cats fed dry food; CAN, cats fed wet food. Day 20 and 48 represent the 14th and 42nd day after the surgery.

comfortable are more likely to interact with humans and their bodies appear to be more relaxed (Brondani et al., 2011). Cat-human interaction and cat's behavior observed in our study indicated that the physical pain was weaker for cats in CAN group, as reflected in the lower scores in vocalization and comfort evaluation compared to CON cats. A dry wound surface without obvious inflammation and swelling (i.e., a lower WSS) represents a better wound recovery (Drudi et al., 2018). Wet canned food tended to lower the score level of inflammation and redness of wound color in cats in week 1 after surgery compared to dry food. Therefore, feeding the wet food seems to accelerate postoperative wound recovery in our study.

Higher lipase levels in the CON group may indicate a risk of pancreatic inflammation in cats (Zavros et al., 2008). Collective evidence suggest that moisture content affect the health of animal urinary system. A previous study found that the probability of developing urethral calculi in canines provided diet of moisture (78%–82%) is much lower than subjects fed the dry diet (Lekcharoensuk et al., 2002). Higher ratio of BUN/SC was suggested to be associated with renal dysfunction in cats (Finco and Duncan, 1976), and a lower ratio in CAN group in the current study indicated that wet diet might contribute to the recovery of renal function after surgery.

Exposed wound and compromised health condition after surgery is susceptible to infection by pathogenic microorganisms in the environment. IgG as the major antibody and exclusive antitoxin could contribute to immune response by neutralizing viruses and bacteria (Woof and Kerr, 2006). The difference in IgG levels on day 5 and day 7 between cats in CON and CAN groups indicated that feeding wet diets for a certain period before surgery is conducive to improve the

immune function in cats. Higher IgG level is also associated with better surgical outcome (Chen et al., 2016). Accordingly, we estimate that the relief of postoperative pain and recovery of somatic function by wet food in cats may be achieved through the activation of humoral immunity and the alleviation of wound affection. The reason why the level of inflammatory cytokines did not differ between groups may be due to the small incision of the surgery and sufficient postoperative care in both groups of cats. Stressful events such as ovariohysterectomy can trigger oxidative stress in female dogs which could further aggravate the trauma and inflammation (Ali et al., 2020; Sakundech et al., 2020). As an antioxidative enzyme inside cells, SOD could protect cell structures and functions by eliminate free radicals (Ali et al., 2020). In our study, diet of wet food may function better in protecting cats from oxidative damage than dry food since serum SOD level was higher in cats of CAN group than CON group.

The serum metabolome composed of all the small molecules involved in the metabolic processes in serum which is influenced by diets, environment and gut microbiota (Wikoff et al., 2009). The OPLS-DA model revealed an obvious difference in serum metabolome between cats fed wet and dry diet in this experiment, suggesting that diet variation affected the serum metabolites. The differed metabolic pathway mainly focused on arginine biosynthesis in the postoperative period. Plenty of studies have reported that arginine metabolism could regulate the immune response of the body by regulating the differentiation and maturation of macrophages and B lymphocytes (de Jonge et al., 2002; Martí i Líndez and Reith, 2021). The other altered metabolic pathway, glutamine and glutamate metabolism was also shown to be involved in the oxidative stress and inflammation (Wang et al., 2022). So, the differences in the immune response and antioxidant capacity

between cats in the two diet groups might be attributed to their metabolomics changes in certain amino acids, such as arginine, glutamine, and glutamate.

Long-term change following castration surgery

Due to changes with sexual and satiety-related hormones (e.g., cholecystokinin, total peptide YY and estrogen) after castration, cats tend to have better appetite and reduced activity level which leads to a positive energy balance that could contribute to massive weight gain and the development of obesity (Schauf et al., 2016; Kutzler, 2020; Phungviwatnikul et al., 2020). Consistently, our results revealed a dramatic increment of daily DMI and BW in cats of the CON group at week 5 and 6 after castration compared to baseline, while the same measurements in cats fed wet food remained at a relatively stable level over the experimental period. The BCS of CON group also followed similar trend as DMI and BW. These differences between cats fed dry and wet food may attribute to the lower energy density and higher satiety of the wet-diet (Rowe et al., 2015). Faster filling of the stomach by the moisture in wet food can activate the stretch receptors and mechanoreceptors to prevent excessive energy intake (Havel, 2001). A relevant study showed that, in the case of ad libitum feeding, wet canned food resulted in BW loss in cats after only 3 weeks in comparison to cats fed the same food but with water being removed beforehand (Wei et al., 2011). Besides, a diet with high moisture content was shown to increase activity level in cats, which also helped to burn energy to maintain a normal body condition in cats (Cameron et al., 2011). Apparent nutrient digestibility of diet is critical for animals to gain the essential nutrition and energy (Schauf et al., 2021). Even though the digestibility of most nutrients (i.e., DM, CP, and EE) in our study was significantly higher for the wet canned food than for the dry food (Table 4), DE of wet food was significantly lower than that of dry food. The dry food of higher energy density is one of the risk factors for obesity in adult cats according to a cross-section study (Öhlund et al., 2018). Therefore, compared to dry kibbles, wet foods of high satiety and low energy density might help neutered cats to control DMI and energy intake, and as a result maintain normal body weight after surgical sterilization.

Obese cats usually have higher TG, as well as lower concentration of HDL in serum which could promote the excretion of excess cholesterol in extrahepatic tissues (Jordan et al., 2008; März et al., 2017). After being castrated, the level of TG in cats fed diet of high energy density (i.e., dry kibbles) increased substantially over time, which corresponded to their changes of BW and BCS. However, lower TG and higher HDL-C concentration was observed in the CAN group compared to CON. The changes of TG and HDL may be correlated to BW and BCS in cats. The results indicated that free feeding of dry food after castration promotes weight gain and may eventually result in the development of obesity in cats, while canned food may reduce this risk by maintaining BW and BCS relatively stable.

As the products of fermentation of polysaccharides by gut microorganism (GM), SCFA mainly include acetic acid, propionic acid, and butyric acid (Tan et al., 2014). The SCFA-producing gut microbiota, along with SCFA are involved in the regulation of many physiological processes, such as inflammation (Suchodolski, 2016) and nutrient metabolism (Wernimont et al., 2020), and were shown closely related to overweight and obesity in pet cats and dogs (Kieler

et al., 2016; Wernimont et al., 2020). The results of the current study indicate that SCFA and butyric acid production is closely related to weight gain or obesity in cats. There is evidence that the concentration of acetic acid and propionic acid in gut were negatively associated with the rate of BW loss in dogs (Kieler et al., 2017). In agreement, our study showed that total fecal SCFA, as well as acetic and propionic acid, increased over time (i.e., from week 1 to week 6) after castration, during which time period the increase of BW and BCS was also observed in cats, especially those on dry food. Meanwhile, fecal content of butyric acid exhibited an increase over time only in cats fed dry food. Consistently, concentration of fecal butyric acid in obese children was higher than that in normal-weight children (Riva et al., 2017), and the level of fecal butyric acid was positively correlated with the distribution of body fat in children (Wei et al., 2021). Therefore, increased SCFA and/or butyric acid production, which is likely due to the increased DMI and increased substrate reaching the colon for fermentation, such as in the case of cats fed dry diet, may be closely involved in promoting weight gain and/or the development of obesity in cats. Even though SCFA in colon could provide a small amount of energy for dogs and cats (Suchodolski, 2016), SCFA/butyrate production may not directly contribute to the significant weight gain observed in the cats in our study, due to their limited tolerance of intestinal fermentation.

The metabolomics data on day 20 and 48 may provide information of the mechanisms underlying the long-term effects of the two diets on BW and BCS in cats after castration. The differing metabolites and metabolic pathways between CON group and CAN group were also shown to be related to weight gain and/or obesity in humans. Examples include the differing metabolic pathways of phenylalanine and tyrosine between obese and lean people (Morris et al., 2012), the disorder of tryptophan metabolism in obese adults (Cussotto et al., 2020), and the association of higher levels of arachidonic acid with visceral fat accumulation in man, with higher levels present in adipose tissue in overweight and obese people (Savva et al., 2004; Inoue et al., 2013). Therefore, we speculate that the different changes in BW and BCS between two dietary groups might be related to the metabolic pathways of certain amino acids, such as the biosynthesis of tyrosine and tryptophan, and the metabolism of phenylalanine and arachidonic acid.

In addition to moisture content, the proximate composition (e.g., fat and protein content) of the two diets included in our study differed due to varied ingredients and manufacturing techniques. Food intake in cats was also not restricted. Therefore, a major limitation of our study is that while the current data does indicate a potential benefit of wet-canned food in cats, we could not determine which factor(s) of the diet (e.g., moisture content, nutrient composition or level of caloric intake) contributed to the observed findings. In addition, cats of different sex may respond differentially to castration and experience different severity from the surgery. We did try to balance the sex distribution in two treatment groups to reduce the gender effect. But still sample size differed largely for two sexes, which was also a limitation of this study. We originally included sex in the model for data analysis but sex showed no significant effect on various parameters. Therefore, it was later removed from the final model. Future studies may increase sample size for both sexes to better elucidate the gender effect on cat recovery from castration surgery and the potentiality of dietary regulation on their performance.

Moreover, further studies may investigate the correlation of GM and the serum metabolome to further the understanding of how different diets could affect wound recovery and the control of BW and body condition in cats.

CONCLUSION

The dry and wet canned food included in this study had varied effects on cats experiencing castration, probably due to the differences in moisture content, nutrient composition and nutritional support between the diets. The high palatability of canned food as a result of higher moisture content, and protein and fat content on DM basis, might promote wound recovery shortly after sterilizing surgery in cats by increasing their water and energy intake, nutrient digestion, and anti-oxidative and immune capacity. The wet food also helped maintain BW and BC in cats after castration in that cats in CON group gained weight significantly faster than cats in CAN group over the 4 weeks after the castration surgery. This may be primarily due to energy dilution by the high-water content in the wet food when compared to the dry food. The underlining mechanism may involve differing effects of the two diets on SCFA/butyric acid production and certain metabolic pathways (e.g., amino acid metabolism).

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

Acknowledgment

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Conflict of Interest Statement

Hongcan Huang was employed by Guangdong Munchkin Biotechnology 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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Gallic Acid Alleviates Gut Dysfunction and Boosts Immune and Antioxidant Activities in Puppies Under Environmental Stress Based on Microbiome–Metabolomics Analysis

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Early-life exposure to environmental stress disrupts the gut barrier and leads to inflammatory responses and changes in gut microbiota composition. Gallic acid (GA), a natural plant polyphenol, has received significant interest for its antioxidant, anti-inflammatory, and antimicrobial properties that support the maintenance of intestinal health. To assess whether dietary supplementation of GA alleviates environmental stress, a total of 19 puppies were randomly allocated to the following three dietary treatments for 2 weeks: 1) basal diet (control (CON)); 2) basal diet + transportation (TS); and 3) basal diet with the addition of 500 mg/kg of GA + transportation (TS+GA). After a 1-week supplementation period, puppies in the TS and TS+GA groups were transported from a stressful environment to another livable location, and puppies in the CON group were then left in the stressful environment. Results indicated that GA markedly reduced the diarrhea rate in puppies throughout the trial period and caused a moderate decline of serum cortisol and HSP-70 levels after transportation. Also, GA alleviated the oxidative stress and inflammatory response caused by multiple environmental stressors. Meanwhile, puppies fed GA had a higher abundance of fecal Firmicutes and *Lactobacillus* and lower Proteobacteria, *Escherichia-Shigella*, and *Clostridium_sensu_stricto_1* after transportation. As a result, the TS+GA group had the highest total short-chain fatty acids and acetic acid. Also, the fecal and serum metabolomics analyses revealed that GA markedly reversed the abnormalities of amino acid metabolism, lipid metabolism, carbohydrate metabolism, and nucleotide metabolism caused by stresses. Finally, Spearman's correlation analysis was carried out to explore the

comprehensive microbiota and metabolite relationships. Overall, dietary supplementation of GA alleviates oxidative stress and inflammatory response in stressed puppies by causing beneficial shifts on gut microbiota and metabolites that may support gut and host health.

Keywords: environmental stress, gallic acid, puppy, antioxidant, inflammatory response, microbiome, metabolomics

INTRODUCTION

Stress response is a ubiquitous physiological response elicited when the threat to the homeostasis is perceived by the organism due to environmental, physical, or psychological stimuli (1). Early-life exposure to a specific environment can influence the development and function of multiple organs and systems, including the central nervous, gastrointestinal, and immune systems (2–4). Current evidence suggests that the hypothalamic–pituitary–adrenal (HPA) axis is the major pathway that controls the production of the stress hormones, glucocorticoids (GC) in response to various environmental factors (e.g., oxidative stress, heat, and osmotic stress). A series of metabolic and immune-suppressive effects (5) are elicited by GC, acting through the glucocorticoid receptor. Specifically, the HPA axis is activated by the secretion of corticotropin-releasing hormone (CRH) from the hypothalamus, which induces the anterior pituitary gland to release adrenocorticotrophic hormone (ACTH), and then ACTH stimulates the adrenal cortex to release the GC, mainly cortisol (COR), which negatively regulates CRH production to terminate the stress response cascade (6–8). Moreover, heat shock proteins (HSPs), a kind of stress-induced proteins ubiquitously found in germs and mammals (9–11), are heavily involved in dealing with environmental stress (12). Particularly, HSP-70 serves as a molecular chaperone to protect cells against the stresses of various types and origins. A recent study has demonstrated that HSP-70 helps to maintain and stabilize the intestinal tight junctions, as a result generating a stronger intestinal barrier in the ileum of stressed animals (13, 14). Simultaneously, environmental stressors trigger the production of intracellular reactive oxygen species (ROS) that can disrupt the cellular antioxidant defense system (15). Stress-induced production of ROS may be mediated by the inflammatory response because inflammation is associated with high levels of ROS, and strong stressors can induce an inflammatory response (16).

Stress not only affects the physiological and stress system but also destroys gut microbiota (GM) (17–19). The human body is inhabited by trillions of microorganisms that participate in nutrient metabolism and influence the health and immune responses of the host (20–22). *Lactobacillus* and *Bifidobacterium* are the main genera of probiotic bacteria, which enhance the host immune system and favorably modulate gastrointestinal physiology (23, 24). Moreover, the producers of short-chain fatty acids (SCFAs), the phylum Firmicutes and the genera *Faecalibacterium* and *Roseburia*, may also be considered beneficial bacteria (25–28) because SCFAs are a carbon energy source for intestinal epithelial cells and

can induce the development of intestinal Treg cell with potent anti-inflammatory functions (29–31). Conversely, the pathogenic bacteria Enterobacteriaceae (belong to the phylum Proteobacteria), a family including *Escherichia*, *Shigella*, *Proteus*, and *Klebsiella*, is often associated with the development of systemic inflammation (32, 33). It is increasingly recognized that the acute and chronic stressors that activate the HPA axis can modulate GM and may be one causal factor in gut dysbiosis (1). In support, recent evidence has begun to connect GM and its metabolites to gastrointestinal diseases, inflammation, and psychological metrics in humans suffering from multiple stressors (8, 17, 18). Collectively, these studies provide preliminary evidence that GM may respond to environmental stress.

Polyphenol performs antioxidant and anti-inflammatory properties and can modulate oxidative stress and inflammatory signaling (34–36). Growing evidence indicates that polyphenol contributes to gut health *via* the modulation of colon microbiota composition (37–39). Gallic acid (GA), also known as 3,4,5-trihydroxybenzoic acid, is a natural polyphenol compound present in fruits, vegetables, and herbal medicines (40). It has been reported that GA effectively inhibited inflammation (41, 42) and oxidation (43, 44) *in vitro* and *in vivo* and altered metabolic and bacterial profiles in the colitis model (45). As far as we know, there is little discussion about whether GA can relieve the damage caused by multiple stressors. Based on previous research, we hypothesize that multiple stressors can cause inflammation and oxidative stress by promoting the growth of pathogenic bacteria species, thereby causing diarrhea; and dietary supplementation of GA may have a role in alleviating these symptoms.

Beagle dogs are considered excellent models for human microbiome research because of the high similarities in structures and functions between dog and human microbiomes (46). To determine whether changing environment and adding GA are efficacious in preventing the deleterious effects of stress on antioxidative and immune system activity, we transported puppies from a stressful environment to a livable environment. In detail, we evaluated the diarrhea rate, physiological stress, antioxidant capacity, inflammatory response, and metabolites by dietary supplementation of GA at 500 mg/kg before and after transportation. In parallel, the 16S rRNA gene sequencing was adopted to monitor microbiota alterations, and untargeted metabolomics based on ultra-performance liquid chromatography–Orbitrap–tandem mass spectrometry (UPLC–Orbitrap–MS/MS) analysis method was employed to capture changes in different metabolic pathways and potential metabolic biomarkers.

MATERIALS AND METHODS

Animals and Diet

All experimental procedures were authorized by the Experimental Animal Ethics Committee of South China Agricultural University (Approval number: 2019188) and were performed following the guidelines of the Laboratory Animal Center at the South China Agricultural University. Animal welfare was monitored by research and animal care staff daily.

A total of 19 beagle dogs (**Table 1**) were selected in this study and were housed individually in pens ($1.35 \times 0.70 \times 0.75$ m kennels) under an indoor relative humidity and temperature of $96\% \pm 3\%$ and $29^\circ\text{C} \pm 1^\circ\text{C}$, respectively (outdoor relative humidity and temperature were $99\% \pm 1\%$ and $32^\circ\text{C} \pm 2^\circ\text{C}$, respectively) at a 12-h dark-light cycle at the National Canine Laboratory Animal Resource Bank, Guangzhou General Pharmaceutical Research Institute Co., Ltd (Guangzhou, China). All dogs were dewormed and vaccinated, and no drugs (such as antibiotics) that may alter the GM were given 1 month before the experiment. The blood samples were collected for serum biochemistry and blood routine examination 1 day before the trial. All blood routine and serum biochemistry data were within the normal range except for alkaline phosphatase, creatinine, creatine kinase, mean corpuscular hemoglobin, and lymph (**Table S1**), indicating that puppies under high temperature and high humidity remained in a stressed state.

Ground corn, flour, fish fat, chicken meal, beef powder, fish meal, soybean meal, amino acid, vitamin, and mineral premixes constituted the basal extruded diets. The chemical and energy composition of the basal diet is listed in **Table 2**. The basal diet meets all the nutrient recommendations by the Association of American Feed Control Officials (AAFCO, 2017) for puppies (48). Dogs were fed 100 g of diet twice daily (08:00 and 17:00) to meet the required energy needs based on the calculated metabolizable energy content of the basal diet according to the National Research Council (NRC, 2006) (49). They had free access to fresh water *ad libitum*. GA (purity > 99%) was purchased from Wufeng Chicheng Biotech Co., Ltd (Yichang, China). The dose of GA supplemented was based on previous studies (50) with minor modifications. After the adaptation period, 500 mg/kg of GA were mixed with the basal diet and individually dosed for each dog during the trial period. The daily dose of GA was divided and added equally to each of the two planned daily meals.

Experimental Design

After 4 weeks of adaptation to a basal diet, these puppies were randomly allocated to one of the three dietary treatments: 1)

TABLE 2 | The chemical and energy composition of basal diet tested.

Items ¹	Basal diet ²
DM (%)	90.53
OM (%) DM)	92.84
CP (%) DM)	23.91
Acid-hydrolyzed fat (%) DM)	4.56
TDF (%) DM)	3.95
GE (kJ/g DM)	17.00

¹DM, dry matter; OM, organic matter; CP, crude protein; TDF, total dietary fiber; GE, gross energy.

²Extruded diet: corn flour, flour, fish fat, chicken meal, beef powder, imported fish meal, soybean meal, calcium hydrophosphate, calcium chloride, lysine, methionine, vitamin A, vitamin D, vitamin E, copper sulfate, ferrous sulfate, zinc sulfate, and manganese sulfate.

basal diet (control group, CON group), 2) basal diet (transportation stress group, TS group), and 3) basal diet with the addition of 500 mg/kg of GA (TS+GA group). The experimental period was 14 days including 7 to 1 days before transportation (BT7–BT1) and 1 to 7 days after transportation (AT1–AT7). Puppies in the TS and TS+GA groups were exposed to the road transportation for 3 h (from 14:00 to 17:00) at a speed range of 50–60 km/h on day 7 of the experiment in a thermostatic truck at 26°C with 50% in humidity, and no environmental changes we made to the CON group during the study. Thirteen puppies in the TS and TS+GA groups were transported to the Laboratory Animal Center Building at the South China Agricultural University and housed individually in pens ($1.2 \times 1.0 \times 1.1$ m kennels) under a constant temperature and humidity (23°C and 70%, respectively) with a light/dark cycle of 12 h. All dogs were continued on their respective diets for another week and given access to toys for behavioral enrichment at all times and to exercise outside of their cages and socialize with each other or humans at least once a day. The study design is depicted in **Figure 1**.

Chemical Analysis of Diet

Throughout the trial period, a 200-g basal diet was collected weekly and was kept in the refrigerator at -20°C . Feed samples were dried in the oven and were ground through a 1-mm screen for chemical composition analysis. The dry matter (DM) and organic matter (OM) were determined for the diets according to AOAC (2000; method 950.46 for water and method 942.05 for crude ash) (51). Acid-hydrolyzed fat was analyzed by a fatty analyzer (FT640, Guangzhou, Grand Analytical Instrument Co., Ltd) according to AOAC (2000; method 920.39 for ether extract) (51). The crude protein (CP) was done by using the Kjeldahl method with semi-automatic Kjeldahl apparatus (VAPODEST 200, C. Gerhardt GmbH & Co. KG, Germany) and following the Official Method of AOAC (2000; method 954.01 for crude

TABLE 1 | Detailed information of beagle dogs in this study.

Group	Sample size (male:female)	Age (month)	Initial body weight (kg)	BCS ¹
CON	6 (3:3)	3.56 ± 0.32	5.32 ± 0.91	5.42 ± 0.49
TS	6 (2:4)	3.62 ± 0.34	5.05 ± 0.52	5.17 ± 0.41
TS+GA	7 (3:4)	3.54 ± 0.30	5.23 ± 0.62	5.29 ± 0.49

CON, control; TS, transportation; GA, gallic acid.

¹BCS, body condition score; all dogs were weighed, and BCS was assessed using a 9-point scale (47) before morning feeding. Data were expressed as mean \pm SD.

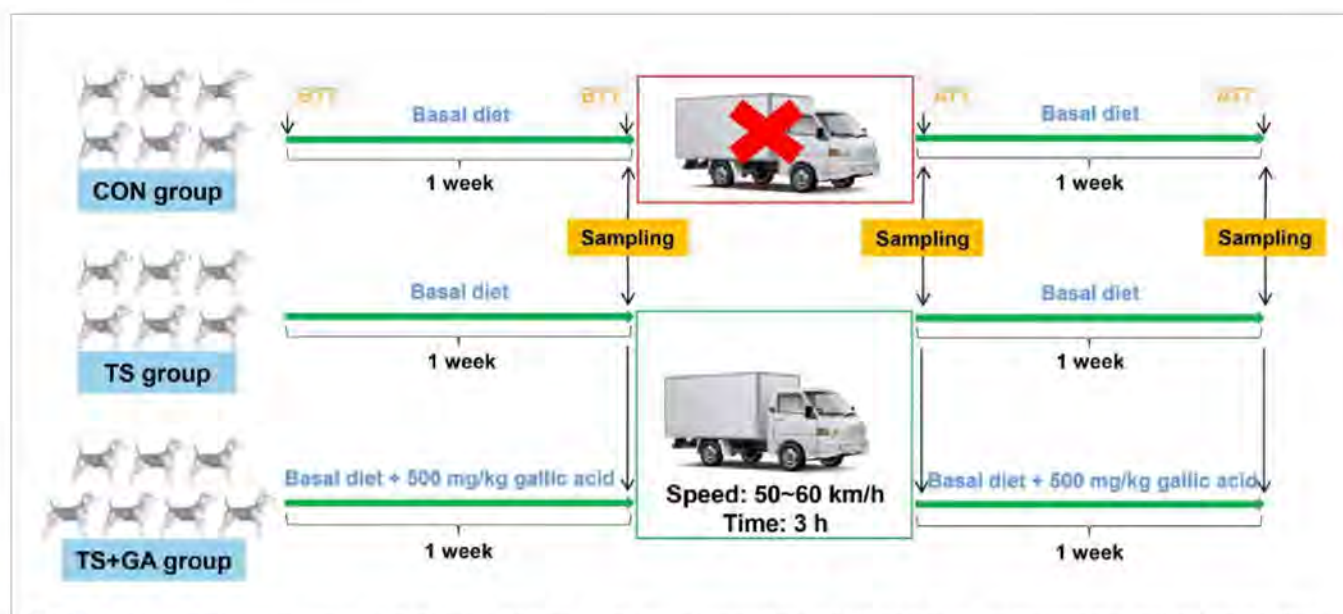


FIGURE 1 | Schematic representation of the study design. BT7, the 7th day before transportation; BT1, the 1st day before transportation; AT1, the 1st day after transportation; AT7, the 7th day after transportation. The CON group was fed basal diet with no transportation ($n = 6$), the TS group was fed basal diet with transportation ($n = 6$), and the TS+GA group was fed basal diet+500 mg/kg of gallic acid (GA) with transportation ($n = 7$).

protein) (51). The total dietary fiber (TDF) content was analyzed using an automatic fiber analyzer (FIBRE THERM FT12, C. Gerhardt GmbH & Co. KG, Germany) and AOAC (2000; method 962.09 for crude fiber) (51). Diet was analyzed for GE by oxygen bomb calorimeter (IKA C 200, IKA (Guangzhou) Instrument Equipment Co., Ltd, Guangzhou, China).

Fresh Fecal Sample Collection and Analysis

During the whole experimental period of 2 weeks, fecal scores (FS) described by Middelbos et al. (52) were assessed every day. On BT1, AT1, and AT7, fresh fecal samples were collected from the pen floor of each dog within 15 min of defecation. An aliquot for SCFAs and branched-chain fatty acids (BCFAs) measurement was stored at -80°C until analysis. An aliquot of the feces was collected and transferred to a 5-ml sterile fecal collection tube (BIORISE) for microbiota measurement, snap-frozen on liquid N_2 , and stored at -80°C until DNA extraction. Finally, an aliquot for metabolomics analysis was snap-frozen on liquid N_2 and stored at -80°C until analysis.

Blood Sample Collection and Analysis

On BT1, AT1, and AT7 after overnight fasting, a 5-ml blood sample was collected from each dog by forelimb vein and left to stand for 30 min before centrifugation at $3,500\times g$ at room temperature for 15 min. After centrifugation, the supernatants were aliquoted into microcentrifuge tubes and stored at -80°C for further analysis. Serum glutathione peroxidase (GSH-Px), malondialdehyde (MDA), total antioxidant capacity (T-AOC), and superoxide dismutase (SOD) were detected using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocol. Serum COR, GC, ACTH, HSP-70, immunoglobulin G (IgG),

tumor necrosis factor-alpha ($\text{TNF-}\alpha$), interferon- γ (IFN- γ), and interleukin 4 (IL-4) were measured using commercial ELISA kits (MEIMIAN, Jiangsu Meimian Industrial Co., Ltd., Jiangsu, China). Finally, an aliquot for serum metabolomics analysis was snap-frozen on liquid N_2 and stored at -80°C until analysis.

16S rRNA High-Throughput Sequencing DNA Extraction, Amplification, and Sequencing

On BT1, AT1, and AT7, fresh fecal samples were collected from the pen floor of each dog within 15 min of defecation. Total genome DNA from fresh fecal samples was extracted using the cetyltrimethylammonium bromide method. DNA concentration and purity were monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1 ng/ μl using sterile water. 16S rRNA genes of 16S V3-V4 were amplified using the primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') with the barcode. All PCRs were carried out with 15 μl of Phusion[®] High-Fidelity PCR Master Mix (New England Biolabs) with 2 μM of forward and reverse primers and about 10 ng of template DNA. Thermal cycling consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s, followed by 72°C for 5 min. The same volume of 1 \times loading buffer (contained SYB green) was mixed with PCR products (in equidensity ratios) and then operated with electrophoresis on 2% agarose gel for detection. Then, the mixture of PCR products was purified with Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using the TruSeq[®] DNA PCR-Free Sample Preparation Kit (Illumina, USA) following the manufacturer's recommendations, and index codes were added. The library quality was assessed on the Qubit[®] 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last,

the library was sequenced on an Illumina NovaSeq platform, and 250-bp paired-end reads were generated.

Bioinformatics Analysis

Paired-end reads were merged using FLASH (V1.2.7, <http://ccb.jhu.edu/software/FLASH/>) (53). Quality filtering on the raw tags was performed to obtain the high-quality clean tags (54) according to the QIIME (V1.9.1, http://qiime.org/scripts/split_libraries_fastq.html) (55) quality-controlled process. The tags were compared with the reference database (Silva database, <https://www.arb-silva.de/>) using the UCHIME algorithm (UCHIME, http://www.drive5.com/usearch/manual/uchime_algo.html) (56) to detect chimera sequences, and then the chimera sequences were removed (57). Then the effective tags are finally obtained.

Sequences analyses were performed by Uparse software (Uparse v7.0.1001, <http://drive5.com/uparse/>) (58). Sequences with $\geq 97\%$ similarity were assigned to the same operational taxonomic units (OTUs). For each representative sequence, the Silva Database (<http://www.arb-silva.de/>) (59) was used based on the Mothur algorithm to annotate taxonomic information. Multiple sequence alignment was conducted using the MUSCLE software (Version 3.8.31, <http://www.drive5.com/muscle/>) (60) to study the phylogenetic relationship of different OTUs. Alpha diversity indices, including Observed_species, Chao1, Shannon, Simpson, ACE, and PD_whole_tree, were calculated with QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3). Beta diversity on weighted UniFrac was calculated by QIIME software (Version 1.9.1). Principal coordinate analysis (PCoA) based on weighted UniFrac distances was displayed by WGCNA package, stat packages, and ggplot2 package in R software (Version 2.15.3). The linear discriminant analysis (LDA) effect size (LEfSe) was processed with the default setting of LDA score ≥ 4 using LEfSe software (<http://huttenhower.sph.harvard.edu/lefse/>). Correlation Network was performed using the OmicStudio tools at <https://www.omicstudio.cn/tool>. Function prediction of bacteria was conducted using PICRUSt (<http://picrust.github.com/picrust/>).

Fecal Short-Chain Fatty Acid and Branched-Chain Fatty Acid Analyses

Sample Solution Preparation

The fresh fecal samples collected on BT1, AT1, and AT7 were pretreated, and extraction of SCFAs and BCFAs was performed as follows. The frozen stool samples were placed on ice to thaw, and a 0.2-g fecal sample was added with 1 ml of ultra-pure water. After vortex for 2 min, the samples were sonicated in an ice bath for 10 min and then centrifuged at 14,000 rpm for 10 min at 4°C. The supernatant was promptly transferred to a 2-ml centrifuge tube, and then a total of 20 μ l of 25% metaphosphoric acid solution and 0.25-g anhydrous sodium sulfate were added to acidification and salting out, respectively. After vortex for 2 min, 1 ml of methyl *tert*-butyl ether was added, and the vortex was continued for 5 min, and the supernatant was further centrifuged at 14,000 rpm for another 10 min at 4°C to remove the

precipitation. Finally, the upper extraction solution was harvested and filtered through 0.22- μ m Millipore pore membrane filters to a 2-ml sample vial. Samples were stored at -20°C until gas chromatography–MS (GC–MS) analysis. All steps above were performed at 4°C or on ice.

Gas Chromatography–Mass Spectrometry Quantitative Analysis

The quantitative analysis of SCFAs and BCFAs was carried out using the GCMS-QP2020 system (Shimadzu, Tokyo, Japan). The gas chromatography was equipped with an auto-injector AOC-20i (Shimadzu) and coupled to a flame ionization detector. The chromatographic separation was performed on a DB-FFAP capillary column (30 m \times 0.25 mm \times 0.25 μ m). Sample (0.6 μ l) was injected with a 30:1 split ratio using an autosampler. The injection port was set to a temperature of 250°C. The initial temperature of the column was 80°C for 2 min and increased to 150°C at a rate of 10°C/min for 2 min, and to 180°C at a rate of 15°C/min for 5 min. The total run time was 18 min. Helium (He; 99.999%) was the carrier gas with a flow rate of 3 ml/min. The MS parameters were electron impact mode at ionization energy of 70 eV. The ion source and interface temperatures were 230°C and 250°C, respectively. The solvent delay time was 1 min, 230°C. The acquisition mode was selected at ion monitoring mode with a scan interval of 0.3 s.

Fecal and Serum Untargeted Metabolomics Analyses

Sample Processing

The fresh fecal and serum samples collected on BT1, AT1, and AT7 were processed as described previously (61) with slight modifications. Briefly, frozen stool samples stored at -80°C were thawed at 4°C. Approximately 60 mg of sample was weighed and put into 2-ml round-bottom microcentrifuge tubes. Metabolites were extracted by adding 600 μ l of methanol:water (1:1, v/v), and magnetic beads were added to the microcentrifuge tubes for homogenization using a homogenizer. Ultrasonic crushing was performed at a low temperature for 10 min, followed by -20°C for 30 min. The samples were then centrifuged at 14,500 rpm, 4°C for 15 min, and 200 μ l of supernatant was dried in a vacuum centrifuge. Immediately afterward, the samples were redissolved with 200 μ l of 50% methanol each and vortexed for 2 min. After ultrasonic crushing for 10 min at a low temperature, the microcentrifuge tube was centrifuged again at 14,500 rpm, 4°C for 15 min. Finally, the supernatant was stored in a sample injection bottle for analysis. Meanwhile, to prepare for the quality control (QC) sample, 100 μ l of supernatant from each sample was taken in a 15-ml centrifuge tube in order to examine the stability and reproducibility of the entire analysis process. Frozen serum samples collected on BT1, AT1, and AT7 were thawed at 4°C, and vortexed for 2 min. For each sample, 200 μ l of serum sample, 800 μ l of methanol, and 10 μ l of indole acetic acid ethyl ester (internal standard) were sequentially added to the 1.5-ml RNAase-free centrifuge tube and vortexed for 2 min. The samples were then centrifuged at 14,500 rpm, 4°C for 15 min; and 800 μ l of supernatant was dried in a vacuum centrifuge for

3 h, blow-dried with nitrogen, and processed immediately. The next operation processes and QC sample preparation were similar to those of fecal samples.

Multivariate Analysis

UPLC-Orbitrap-MS/MS analysis method was carried out as described previously (62), with minor modifications. The Compound Discoverer 2.1 (Thermo Fisher Scientific) data analysis tool was employed to automate complete raw data preprocessing and was applied to identify metabolites by searching the mzCloud library and mzVault library. In this study, MetaboAnalyst 5.0 (<https://www.metaboanalyst.ca>) was used to perform multivariate analysis. Principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) of metabolites were performed. Pathway enrichment analysis was performed by using the enrichment analysis module on MetaboAnalyst 5.0. The visualization results of the models were obtained with MetaboAnalyst 5.0.

Statistical Analysis

SPSS 26.0 and GraphPad Prism 8.0 software were used for statistical analysis and graphical presentation. One-way ANOVA followed by the multiple range test of least significant difference was used to determine the statistical significance of multiple comparisons. All data were expressed as the mean \pm standard error (SE). Significant differences were at $p < 0.05$, and tendencies were at $p < 0.10$. To preliminarily screen the differential metabolites, we selected the metabolites that had a p -value of less than 0.05 (calculated by Student's t -test) and a variable importance in projection (VIP) score greater than 1.0 (calculated using Orthogonal PLS-DA model). Spearman's correlation values and significance were computed with the R version 3.6.1. Clustering correlation heatmap with signs was performed using the OmicStudio tools at <https://www.omicstudio.cn>.

RESULTS

Effect of Gallic Acid on Fecal Scores, Serum Hormone, HSP-70, Antioxidant Capacity, and Inflammatory Factors in Puppies

Changes in FS are shown in **Figure 2A**. It is evident that the TS+GA group had lower FS than the CON or TS group on BT6 ($p < 0.01$), BT3 ($p < 0.05$), and BT1 ($p = 0.085$). And we found that FS increased in the TS group on AT1 ($p = 0.085$) and AT2 ($p < 0.05$). During the whole experimental period (**Figure 2B**), puppies fed GA (2.61 ± 0.05) had a normal fecal shape relative to the CON (3.17 ± 0.07) and TS groups (3.13 ± 0.07) ($p < 0.001$). Total diarrhea rate (TDR) in the CON, TS, and TS+GA groups were 26.5%, 22.6%, and 4.1%, respectively, and GA reduced TDR by as much as 84.5% and 81.9% compared with the CON and TS groups, respectively.

Puppies fed a diet containing 500 mg/kg of GA for 7 days had a trend toward lower COR compared with the CON group ($p = 0.06$,

Figure 2C). Over time, there was no significant change. The TS group displayed a higher glucocorticoid (GC) level on AT7 ($p < 0.01$; **Figure 2D**). Similarly, ACTH acts on the adrenal cortex and stimulates GC and COR secretion; thus, it had a similar change as GC and COR (**Figure 2E**). No difference in HSP-70 was observed on BT1 and AT1 (**Figure 2F**); however, over time, the TS group had a higher HSP-70 level than the CON group on AT7 ($p < 0.05$), and puppies fed GA had no significant change as compared with the TS group.

There was no different GSH-Px activity between the CON and TS groups on BT1 and AT1 (**Figure 2G**), while puppies fed GA had higher GSH-Px activity than the CON group on AT1. And a decreasing trend of GSH-Px activity was observed in the TS group over the CON group on AT7 ($p = 0.057$). Dietary GA supplementation markedly improved the GSH-Px activity after transportation ($p < 0.01$). Additionally, the TS group had a marginally higher MDA level than the CON group on AT1 ($p = 0.058$, **Figure 2H**), whereas the TS group had a decreasing trend of MDA than the CON group ($p = 0.061$), and the TS+GA group had a decreasing MDA level over the CON group on AT7 ($p < 0.05$). The T-AOC and SOD contents had no obvious change among groups (**Figures 2I, J**).

The TS group tended to decrease the serum IgG level on AT1 relative to the CON group ($p = 0.093$, **Figure 2K**), while puppies fed GA had a higher IgG level than the TS group ($p < 0.05$). Though both the CON and TS+GA groups had surprisingly higher TNF- α levels than the TS group on BT1 ($p = 0.059$, $p < 0.05$, **Figure 2L**), the TS group had a higher TNF- α level than the CON group over time on AT7 ($p < 0.05$), and no significant difference was observed between the TS+GA and TS groups. Similarly, the TS and TS+GA groups showed an unexpected increase in IFN- γ level over the CON group on BT1 ($p = 0.053$, $p < 0.05$, **Figure 2M**), but there was no difference among groups after transportation. Furthermore, IL-4 level sharply decreased in the TS group over the CON group on AT7 ($p < 0.01$, **Figure 2N**), while puppies fed GA had a significant increase of IL-4 level than the TS group ($p < 0.01$).

Effect of Gallic Acid on Gut Microbial Composition and Structure in Puppies

On BT1, puppies fed a basal diet at 500 mg/kg of GA for 7 days had more Observed_species and higher Chao1 and ACE indices than those of the CON group ($p < 0.05$, **Figure 3A**). No difference was observed among the three groups on AT1 and AT7. From the difference of beta diversity index based on weighted UniFrac distances, PCoA plots revealed distinct separation between the CON and TS+GA groups on BT1 and AT1 ($p < 0.05$, **Figure 3B**), whereas the CON group had a trend toward significant separation relative to the TS group on AT7 ($p = 0.095$), especially that puppies fed the dietary supplementation of GA had distinct separation over the TS group ($p < 0.05$).

The most abundant phyla included Firmicutes (60.24%), Actinobacteria (11.47%), Fusobacterota (6.52%), Actinobacteriota (3.52%), Proteobacteria (3.24%), and Bacteroidota (1.13%) at various time points (**Figure 3C**). Puppies fed GA had the highest Firmicutes abundance on AT1 and tended to have higher

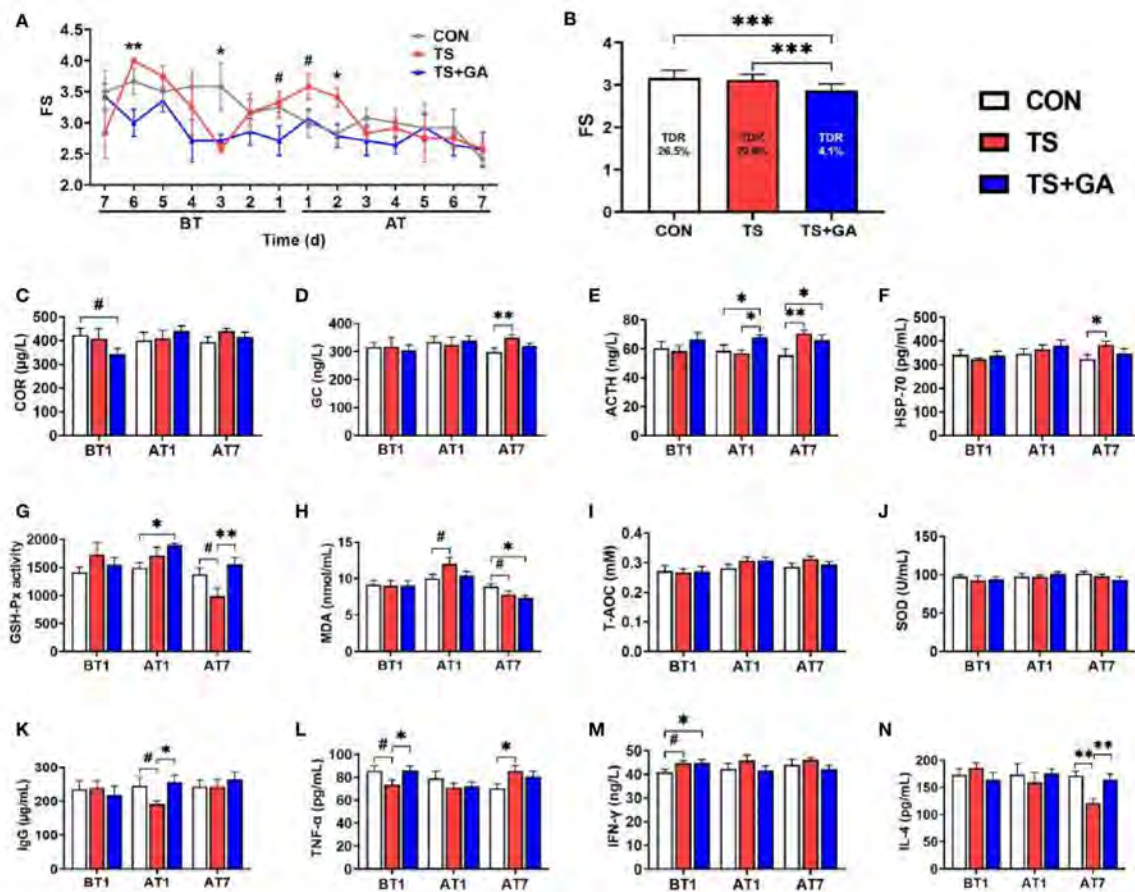


FIGURE 2 | Effect of gallic acid (GA) on fecal score (FS) (A, B), serum hormone (C–E), HSP-70 (F), antioxidant capacity (G–J), and inflammatory factors (K–N) in puppies (n = 6 or 7). The symbol (*) indicates statistically significant differences between two groups (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$), and the symbol (#) represents difference tendency (# $p < 0.10$). BT1, the 1st day before transportation; AT1, the 1st day after transportation; AT7, the 7th day after transportation. TDR (total diarrhea rate, %) = [cases of diarrhea during 14 days/(14 days \times total puppies for each group)] \times 100. CORT, cortisol; ACTH, adrenocorticotropic hormone; GC, glucocorticoid; HSP-70, heat stress protein 70; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; T-AOC, total antioxidant capacity; SOD, superoxide dismutase; IgG, immunoglobulin G; TNF- α , tumor necrosis factor- α ; IFN- γ , interferon- γ ; IL-4, interleukin 4.

Firmicutes than the CON group ($p = 0.055$). Furthermore, GA caused inhibition of Proteobacteria growth induced by transportation stress ($p < 0.05$). Also, the most abundant families included Erysipelotrichaceae (23.28%), Peptostreptococcaceae (12.53%), Lachnospiraceae (12.22%), Bifidobacteriaceae (11.36%), Peptostreptococcaceae (7.18%), Lactobacillaceae (6.60%), and Fusobacteriaceae (6.52%) at various phases. Decreasing Peptostreptococcaceae abundance was observed in the TS+GA group compared with the CON group on AT1 ($p < 0.05$). In contrast, a relative abundance of Lactobacillaceae was higher in the TS+GA group compared with the CON group on AT1 ($p < 0.05$). Relative abundance of Eggerthellaceae in the TS group significantly increased over the CON group, and the TS group had a higher Eubacteriaceae abundance than the CON and TS+GA groups ($p < 0.05$). Finally, the most abundant genera were *Allobaculum* (16.08%), *Bifidobacterium* (11.36%), *Peptoclostridium* (11.08%), *Blautia* (8.48%), *Lactobacillus* (6.60%), *Turicibacter* (5.26%), *Cetobacterium* (4.40%), *Escherichia-Shigella* (2.46%), *Streptococcus*

(2.33%), *Fusobacterium* (2.08%), *Collinsella* (1.67%), and *Faecalibacterium* (1.27%). Relative abundance of *Romboutsia* significantly decreased in the TS+GA group compared with the CON group on BT1. Relative abundances of *Lactobacillus* and *Faecalibaculum* were higher, and relative abundances of *Escherichia-Shigella* and *Clostridium_sensu_stricto_1* were lower in the TS+GA group compared with the CON or TS group on AT1 ($p < 0.05$). The TS group had a higher relative abundance of *Allobaculum* and *Dubosiella* than the CON group on AT7 ($p < 0.05$), while no difference was observed in the TS+GA group; and both the TS and TS+GA groups had lower *Turicibacter* relative to the CON group ($p < 0.05$).

Differential taxon abundances were further confirmed by LEfSe analysis. The histogram with logarithmic LDA score >4.0 and cladogram is shown in Figure 4A. On BT1, the LEfSe analysis indicated that Peptostreptococcaceae and *Streptococcus* in the CON group were the most abundant, whereas on AT1, the predominant bacterial strains in the TS group were *Escherichia-*

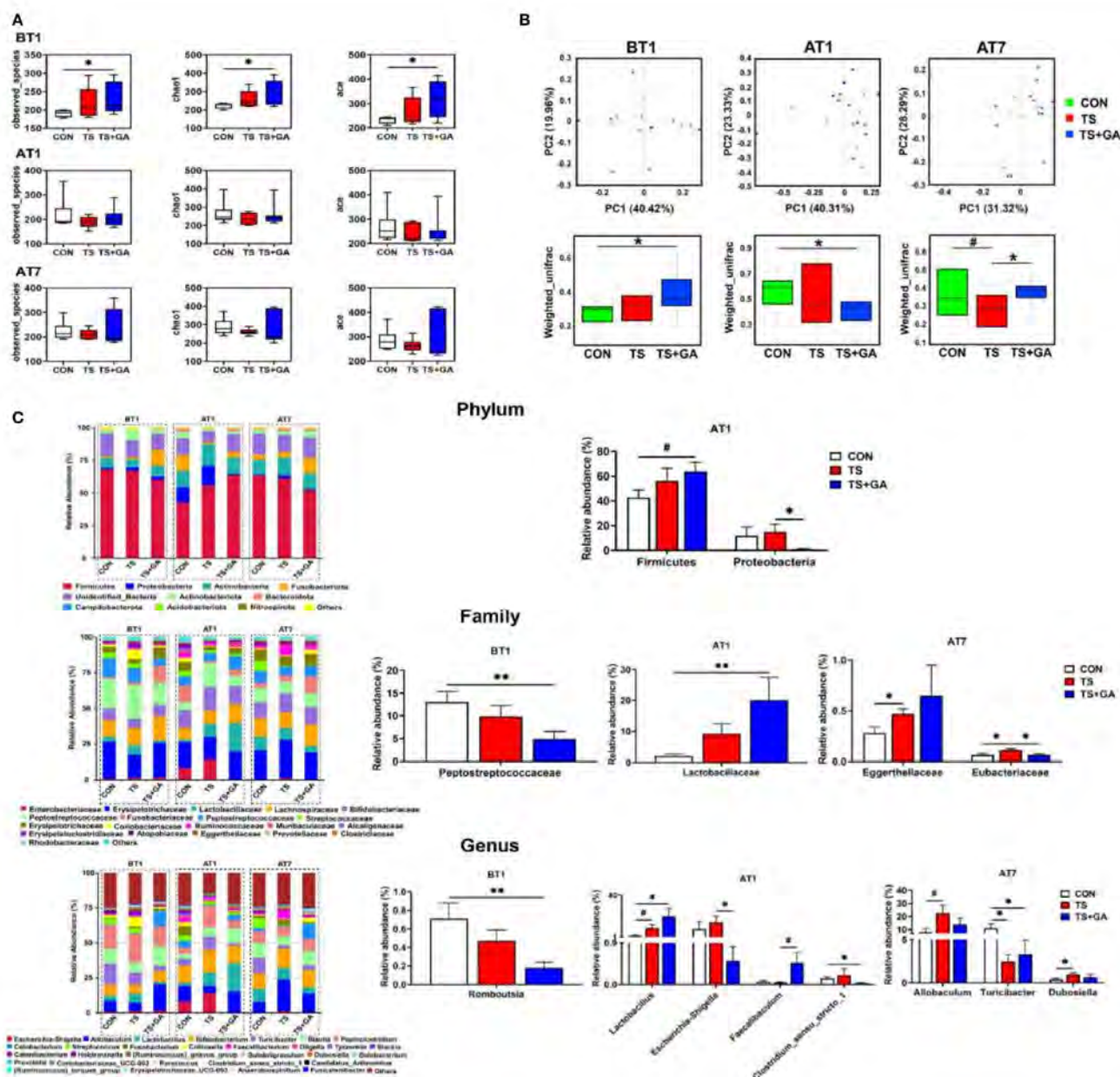


FIGURE 3 | Effect of gallic acid (GA) on gut microbial composition and structure in puppies (n = 6 or 7). Alpha diversity (**A**), principal coordinate analysis (PCoA) based on weighted UniFrac distances (**B**), predominant fecal microbial communities, and different bacteria at the phylum, family, and genus levels (**C**). The symbol (*) indicates statistically significant differences between two groups (*p < 0.05 and **p < 0.01), and the symbol (#) represents difference tendency (#p < 0.10). BT1, the 1st day before transportation; AT1, the 1st day after transportation; AT7, the 7th day after transportation.

Shigella and *Escherichia coli*. Fortunately, *Lactobacillus*, *Lactobacillus murinus*, and *Lactobacillus reuteri* were the highest in the TS+GA group, while no difference was observed on AT7. We next determined the relationship and interaction among fecal microbiota using Spearman's correlation analysis. As shown in **Figure 4B**, *Escherichia-Shigella* negatively modulated *Faecalibaculum*, *Lactobacillus*, and *Bifidobacterium* and positively modulated *Streptococcus* and *Clostridium*.

sensu_stricto_1 in the network. *Allobaculum* positively modulated *Faecalibaculum*, *Dubosiella*, *Cetobacterium*, and *Fusobacterium*. There was a positive network among *Catenibacterium*, *Prevotella*, *Collinsella*, *[Ruminococcus]_gnavus_group*, *Holdemanella*, *Blautia*, and *Peptoclostridium*. In addition, we also found a positive correlation between *Romboutsia* and *Turicibacter*. Regarding Spearman's analysis, the whole network of microbiota was divided into several parts, in which genera *Escherichia-Shigella*,

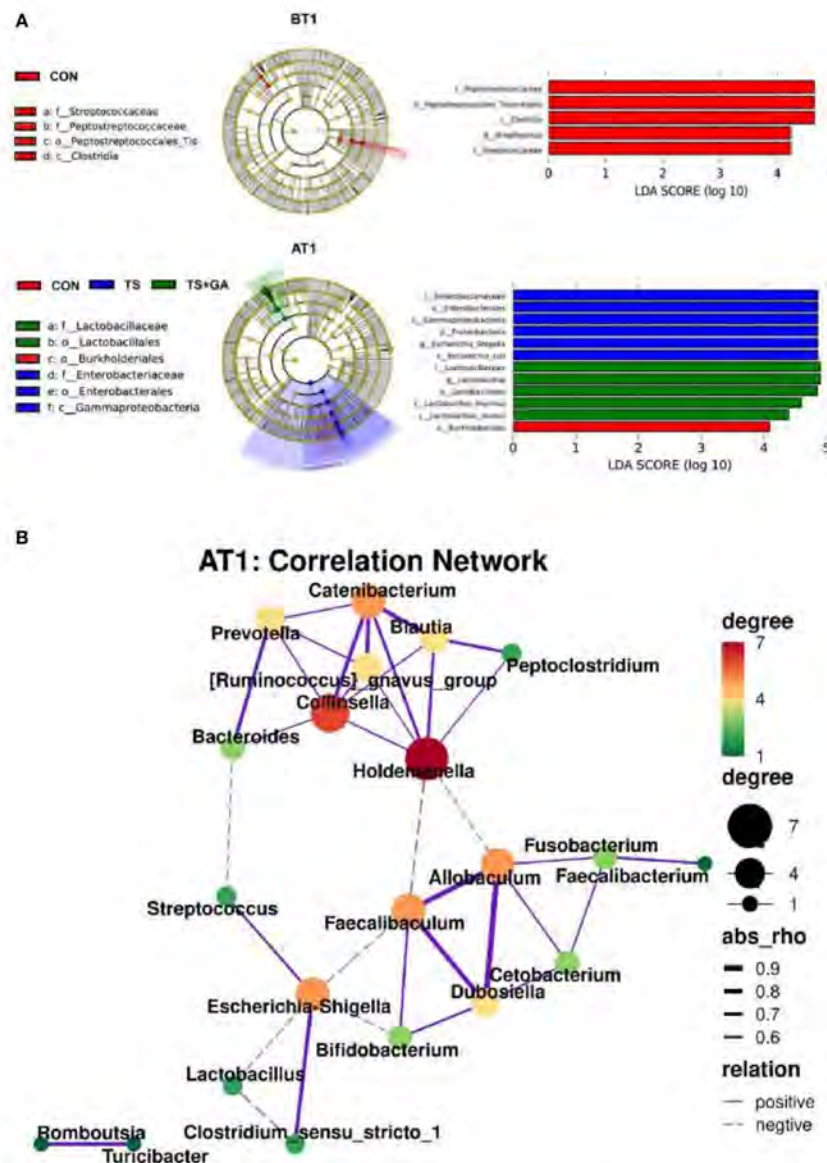


FIGURE 4 | The linear discriminant analysis effect size (LEfSe) analysis identified gut bacterial biomarkers in puppies on BT1 and AT1 **(A)**. Spearman's correlation network of fecal microbiota at genus level on AT1 (purple solid line, positive correlation; gray dotted line, negative correlation; thick line, significant correlation, $p < 0.05$) **(B)**. BT1, the 1st day before transportation; AT1, the 1st day after transportation.

Allobaculum, *Catenibacterium*, and *Holdemanella* dominated key positions and had close interactions with many bacteria in the community.

The gut bacterial function and pathways after transportation and GA treatment were predicted by PICRUSt analysis based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway using 16S rRNA data. On BT1, puppies fed GA had more abundant amino acid metabolism, energy metabolism, carbohydrate metabolism, nucleotide metabolism, metabolism of cofactors and vitamins, and metabolism of terpenoids and polyketide (**Figure S1A**), indicating that these metabolic pathways were significantly influenced by GA in the short

term. It is worth noting that the decreasing abundance of genes involved in energy metabolism and glycan biosynthesis and metabolism were found in the TS+GA group relative to the CON group on AT1 (**Figure S1B**), while more abundant carbohydrate metabolism was observed in the TS+GA group over the TS group. On AT7, puppies transported to another livable environment had weaker amino acid metabolism and xenobiotics biodegradation and metabolism than the CON group (**Figure S1C**), whereas energy metabolism, xenobiotics biodegradation and metabolism, and metabolism of cofactors and vitamins were markedly enhanced after GA treatment compared with those of the CON group.

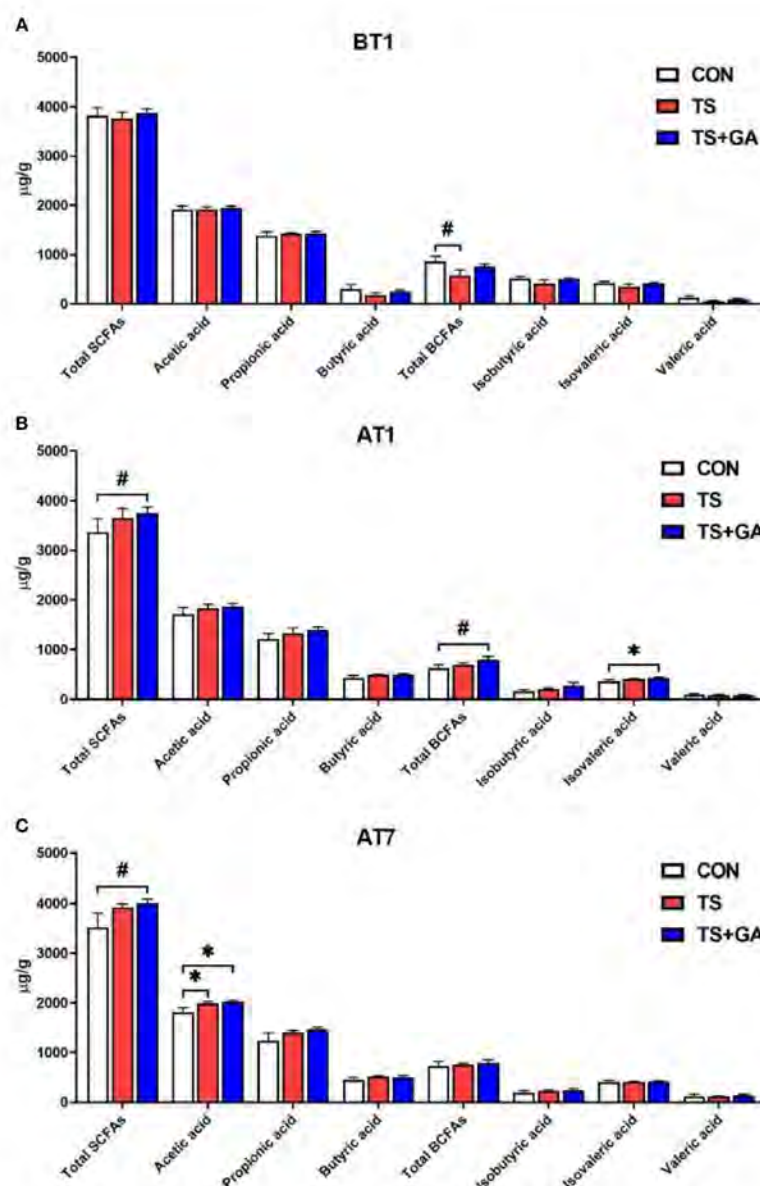


FIGURE 5 | Effect of gallic acid (GA) on fecal short-chain fatty acids (SCFAs) and branched-chain fatty acids (BCFAs) in puppies on BT1 (A), AT1 (B), and AT7 (C) ($n = 6$ or 7). The symbol (*) indicates statistically significant differences between two groups ($p < 0.05$), and the symbol (#) represents difference tendency ($\#p < 0.10$). BT1, the 1st day before transportation; AT1, the 1st day after transportation; AT7, the 7th day after transportation.

Effect of Gallic Acid on Fecal Short-Chain Fatty Acids and Branched-Chain Fatty Acids in Puppies

No significant differences in SCFAs concentrations were observed among the three groups except for total BCFAs between the CON and TS groups on BT1 ($p = 0.057$; **Figure 5A**), while puppies fed GA had a trend of increase in total SCFAs ($p = 0.083$; **Figure 5B**) and increasing total BCFAs ($p = 0.087$) and isovaleric acid ($p < 0.05$) content relative to the CON group on AT1. Similarly, the TS+GA group had a similar trend of increase in total SCFAs to the CON group ($p = 0.099$;

Figure 5C), and higher acetic acid levels were observed in the TS and TS+GA groups in comparison with the CON group on AT7 ($p < 0.05$).

Effect of Gallic Acid on Fecal Metabolites in Puppies

Multivariate statistical analysis was carried out among three groups. In this study, the PCA was used to study the differences among the CON, TS, and TS+GA groups in the fecal metabolomics by an unsupervised statistical method (**Figure 6A**). The PCA score plots showed less obvious

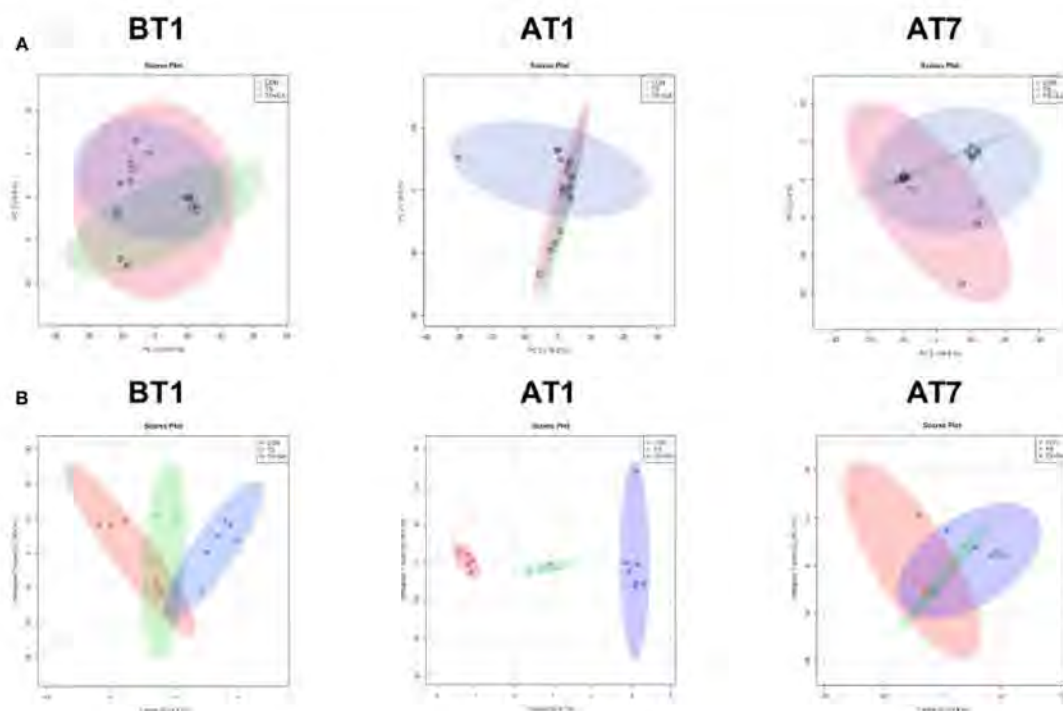


FIGURE 6 | Multivariate statistical analysis on BT1, AT1, and AT7 ($n = 6$ or 7). Score plots from the principal component analysis (PCA) model among three groups on BT1, AT1, and AT7 **(A)**. Score plots from the orthogonal partial least-squares discriminant analysis (OPLS-DA) model among three groups on BT1, AT1, and AT7 **(B)**. BT1, the 1st day before transportation; AT1, the 1st day after transportation; AT7, the 7th day after transportation.

separation at varying time points. However, the OPLS-DA model revealed a clearer difference between the three clusters on AT1 (**Figure 6B**), indicating that the difference among the three groups was the most obvious when puppies were transported from a stressful environment to another livable location.

In this study, a total of 156 metabolites were detected at all stages (**Table S2**). The differential metabolites at varying time points are shown in **Table S3**. A total of 6, 16, and 8 potential biomarkers were identified on BT1, AT1, and AT7, respectively. To gain further insight into the metabolic changes, a KEGG pathway analysis of all metabolites was performed. On BT1, the influenced pathway was mainly concentrated in glycan biosynthesis and metabolism (glycosylphosphatidylinositol (GPI)-anchor biosynthesis) (**Figure 7A**). On AT1, the most influenced metabolic pathways were amino acid metabolism (phenylalanine metabolism, tyrosine metabolism; phenylalanine, tyrosine, and tryptophan biosynthesis; valine, leucine, and isoleucine degradation; and valine, leucine, and isoleucine biosynthesis), lipid metabolism (steroid hormone biosynthesis and glycerolipid metabolism), metabolism of cofactors and vitamins (ubiquinone and other terpenoid-quinone biosynthesis, and pantothenate and CoA biosynthesis), and carbohydrate metabolism (fructose and mannose metabolism) (**Figure 7B**). On AT7, the most important metabolic pathways were carbohydrate metabolism (purine metabolism, and glyoxylate and dicarboxylate metabolism), amino acid metabolism (tryptophan metabolism), and glycan biosynthesis and

metabolism (GPI-anchor biosynthesis) (**Figure 7C**). As a result, we found that the significant differences in the metabolic pathways were mainly concentrated in AT1. The levels of predominant potential biomarkers based on the significant metabolic pathways on BT1, AT1, and AT7 are shown in **Table S4**.

Effect of Gallic Acid on Serum Metabolites in Puppies

Based on the fecal metabolomics analysis, we further detected serum metabolomics. As shown in **Figure 8A**, the PCA score plots showed distinct separation among the CON, TS, and TS+GA groups after transportation. Similarly, the score plots for the OPLS-DA model presented clear separation over time (**Figure 8B**), suggesting a difference among the three groups. From these results of multivariate statistical analysis, it is apparent that there are greater differences in serum metabolites than fecal metabolites at different stages.

In this study, a total of 147 metabolites were detected at all stages (**Table S5**). The differential metabolites at varying time points are shown in **Table S6**. A total of 13, 48, and 36 potential biomarkers were identified on BT1, AT1, and AT7, respectively. On BT1, puppies fed GA mainly influenced serum amino acid metabolism (lysine degradation, tyrosine metabolism, taurine and hypotaurine metabolism, and glutathione metabolism), carbohydrate metabolism (glycolysis/gluconeogenesis and pyruvate metabolism), and lipid metabolism (sphingolipid

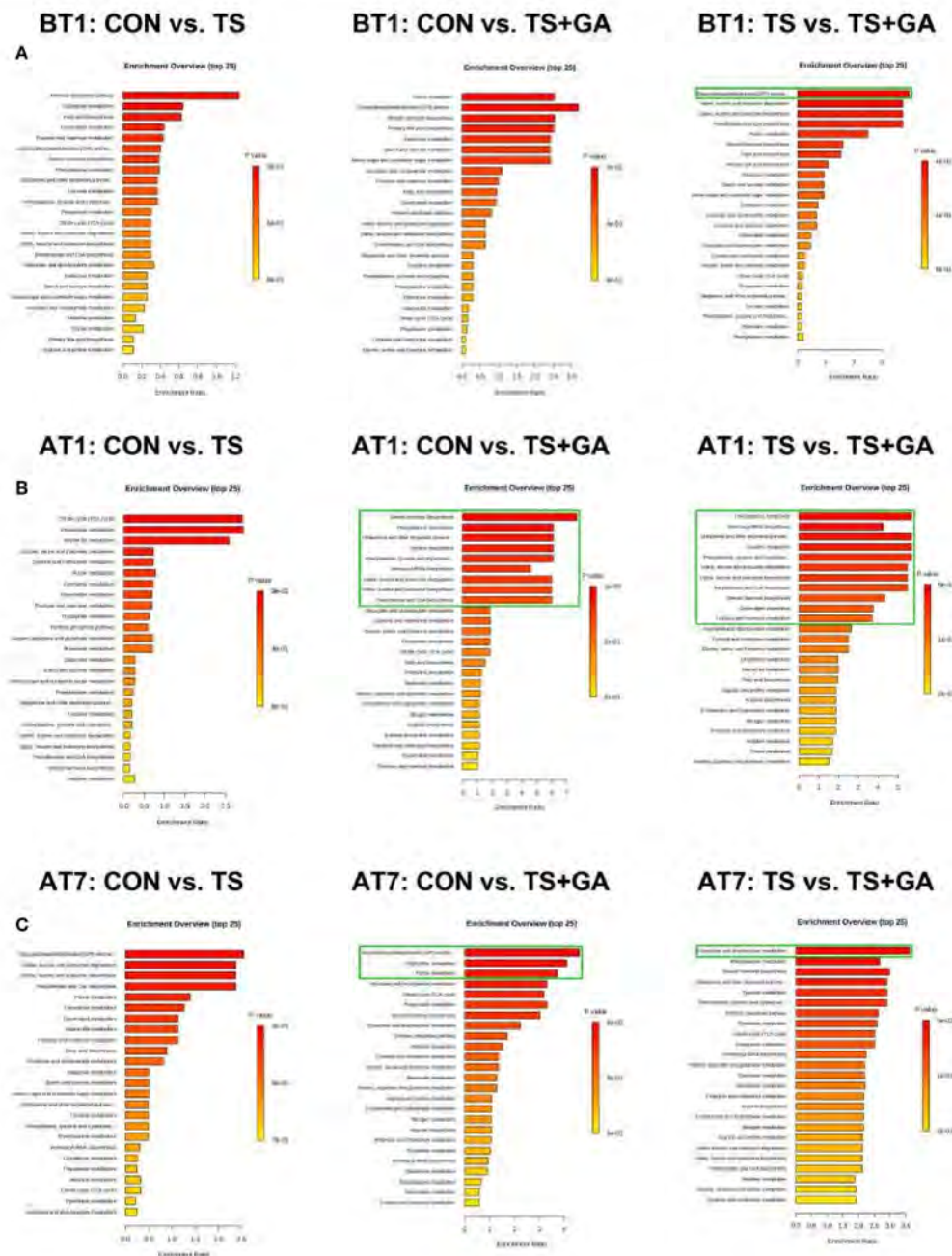


FIGURE 7 | Bar charts of the metabolic pathway analysis of differential fecal metabolites on BT1 (A), AT1 (B), and AT7 (C) ($n = 6$ or 7). The pathway enrichment analysis shows all matched pathways, and the green boxes indicate significant metabolic pathways ($p < 0.05$). BT1, the 1st day before transportation; AT1, the 1st day after transportation; AT7, the 7th day after transportation.

metabolism) (Figure 9A). On AT1, the influenced pathway was mainly concentrated in amino acid metabolism (glycine, serine, and threonine metabolism; arginine and proline metabolism; arginine biosynthesis, alanine, aspartate, and glutamate metabolism; and D-glutamine and D-glutamate metabolism), carbohydrate metabolism (glyoxylate and dicarboxylate metabolism), energy metabolism (nitrogen metabolism), and

nucleotide metabolism (pyrimidine metabolism) between the CON and TS groups (Figure 9B), whereas feeding GA was implicated in the regulation of carbohydrate metabolism (glycolysis/gluconeogenesis and pyruvate metabolism) and lipid metabolism (alpha-linolenic acid metabolism, linoleic acid metabolism, and biosynthesis of unsaturated fatty acids) compared with the other two groups. On AT7, the affected

pathways mainly involved amino acid metabolism (tyrosine metabolism and cysteine and methionine metabolism) and lipid metabolism (sphingolipid metabolism and fatty acid biosynthesis) in the TS group compared with the CON group (**Figure 9C**); notably, significant enrichment of several major metabolic pathways, such as amino acid metabolism (cysteine and methionine metabolism; tyrosine metabolism; valine, leucine, and isoleucine degradation; valine, leucine, and isoleucine biosynthesis; lysine degradation; and taurine and hypotaurine metabolism), carbohydrate metabolism (glycolysis/gluconeogenesis, pyruvate metabolism, and fructose and mannose metabolism), lipid metabolism (glycerolipid metabolism, fatty acid biosynthesis, primary bile acid biosynthesis, and alpha-linolenic acid metabolism), and nucleotide metabolism (purine metabolism), was significantly changed by GA. The levels of predominant potential biomarkers based on the significant metabolic pathways on BT1, AT1, and AT7 were presented in **Table S7**.

The Correlation Analysis of Metabolites and Microbiota

Spearman's correlation analysis was performed for the differential feces and serum metabolites and fecal microbiota obtained by high-throughput 16S rRNA sequencing. On AT1, we found that fecal L-arginine, L-valine, phenylacetaldehyde, and tetrahydrodeoxycorticosterone were positively correlated with the relative abundance of *Clostridium_sensu_stricto_1* (**Figure 10A**). And glyceraldehyde, L-glutamic acid, L-tyrosine, L-

valine, and tetrahydrodeoxycorticosterone were positively correlated with the relative abundance of *Escherichia-Shigella*. L-Glutamic acid, L-tyrosine, and L-valine were also positively correlated with Proteobacteria. Conversely, L-tyrosine and phenylacetaldehyde were negatively correlated with Lactobacillaceae and *Lactobacillus*. In addition, we also observed a weak positive association between *Faecalibaculum* with total BCFA (isobutyric acid and isovaleric acid), and the total SCFAs (acetic acid and propionic acid) had a weak positive association with Firmicutes. On AT7, uridine diphosphate-*N*-acetylglucosamine was negatively correlated with the relative abundance of *Turicibacter*. Furthermore, butyric acid and isobutyric acid had a weak positive association with the *allobaculum*.

As shown in **Figure 10B**, alpha-linolenic acid, citric acid, L-lactic acid, oleic acid, and spermidine were positively correlated with *Clostridium_sensu_stricto_1* on AT1. Likewise, alpha-linolenic acid, L-lactic acid, and oleic acid were also positively correlated with *Escherichia-Shigella*. And a significant positive association was found between L-lactic acid with Proteobacteria. In contrast, alpha-linolenic acid, oleic acid, and spermidine were negatively correlated with Lactobacillaceae and *Lactobacillus*. Additionally, strong negative and positive associations of the L-arginine with *Faecalibaculum* and the arachidonic acid with Firmicutes were observed. On AT7, serum phytosphingosine and taurochenodeoxycholic acid had a reverse association with Eggerthellaceae. Similarly, glyceraldehyde and L-carnitine had positive and negative associations with *Turicibacter*. And

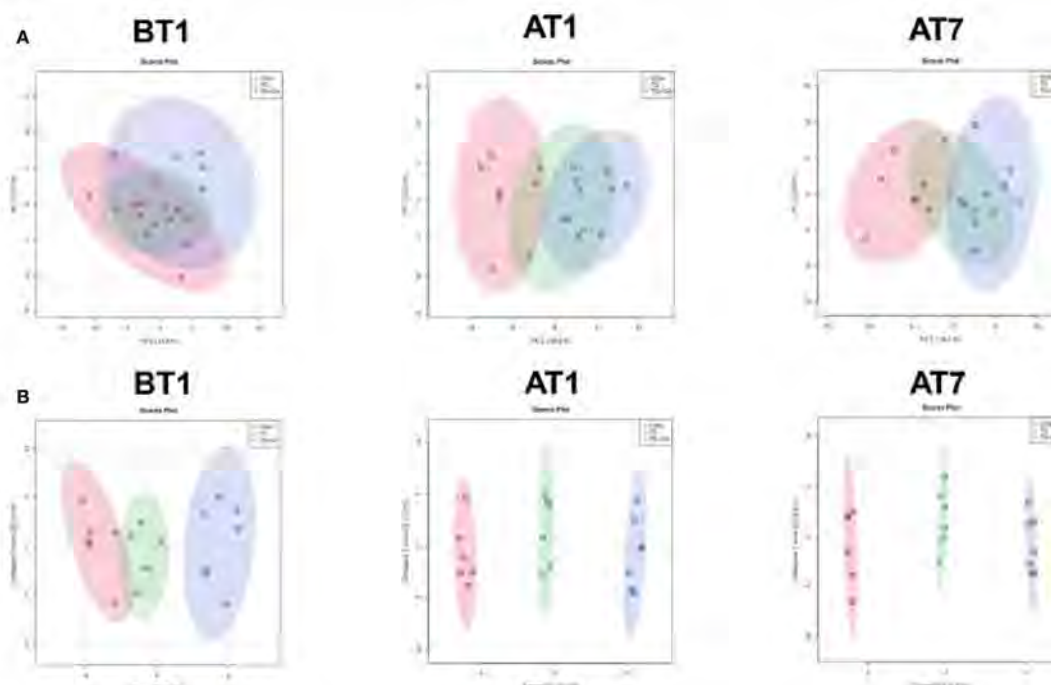


FIGURE 8 | Multivariate statistical analysis on BT1, AT1, and AT7 ($n = 6$ or 7). Score plots from the principal component analysis (PCA) model among three groups on BT1, AT1, and AT7 (**A**). Score plots from the orthogonal partial least-squares discriminant analysis (OPLS-DA) model among three groups on BT1, AT1, and AT7 (**B**). BT1, the 1st day before transportation; AT1, the 1st day after transportation; AT7, the 7th day after transportation.

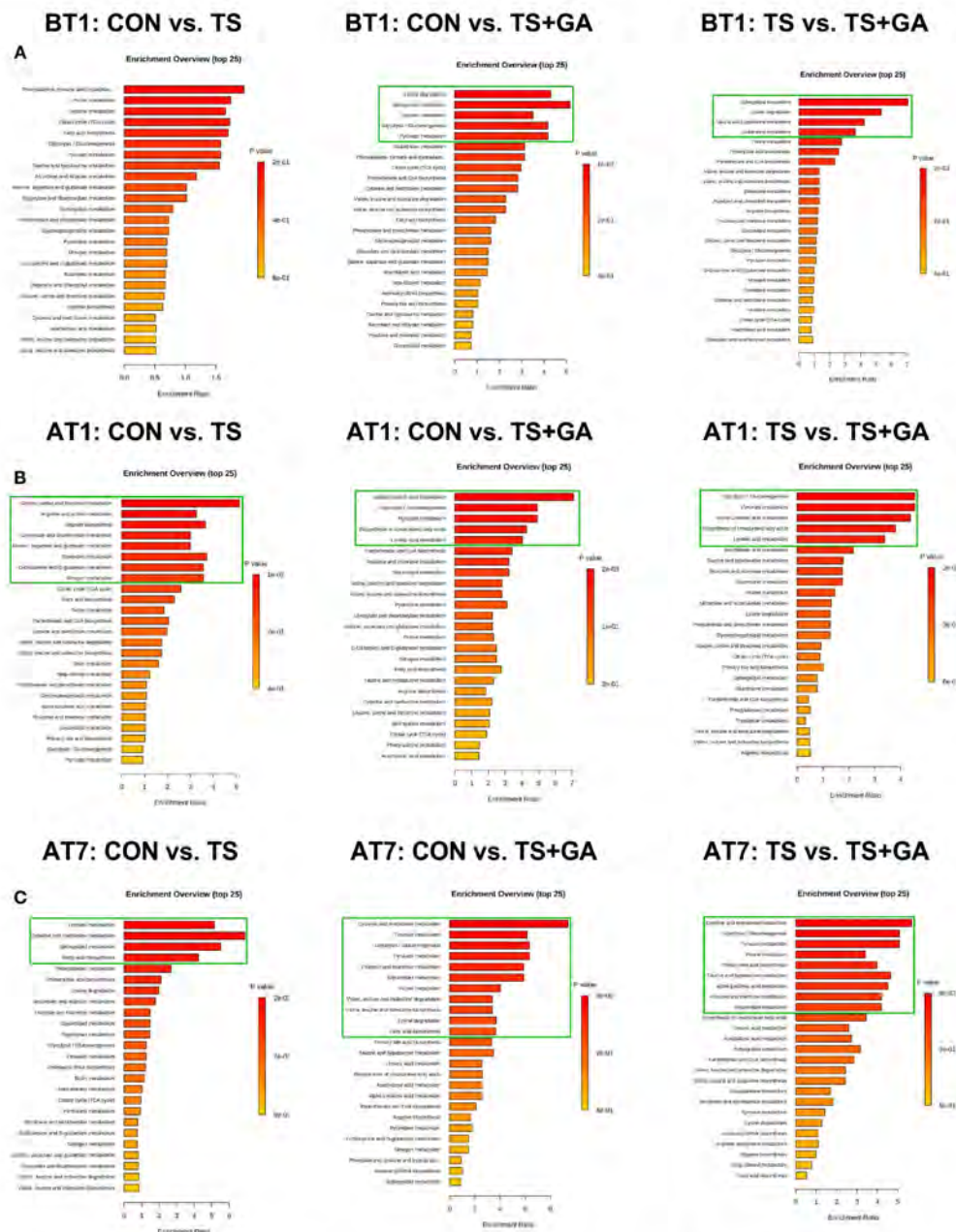


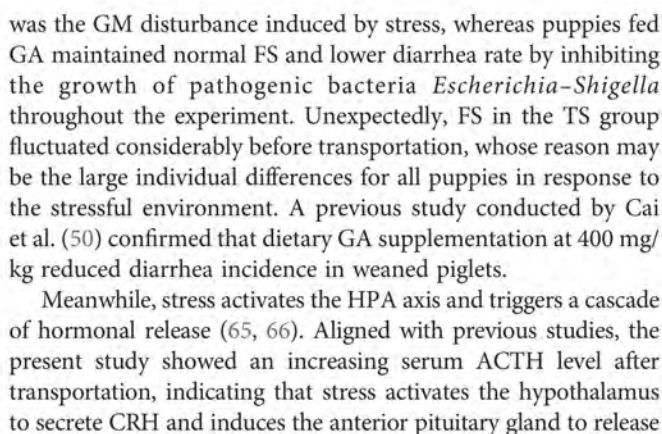
FIGURE 9 | Bar charts of the metabolic pathway analysis of differential serum metabolites on BT1 (A), AT1 (B), and AT7 (C) ($n = 6$ or 7). The pathway enrichment analysis shows all matched pathways, and the green boxes indicate significant metabolic pathways ($p < 0.05$). BT1, the 1st day before transportation; AT1, the 1st day after transportation; AT7, the 7th day after transportation.

dodecanoic acid and homovanillic acid were positively correlated with Eubacteriaceae.

DISCUSSION

To date, adequate evidence exists to support the antioxidation, anti-inflammatory, and antimicrobial activities of GA (45, 63,

64). In this study, we summarized the results of 16S rRNA gene sequencing and metabolomics analysis and discussed the effects of environmental stress and GA on the host-microbial metabolic axis from the relationship between metabolic biomarkers and gut bacteria. Our results suggested that dietary GA supplementation reduced multiple stress-induced diarrhea in puppies by enhancing systemic and intestinal defenses. The diarrhea rate still seemed to be high over a short period, and a possible reason



ACTH. The ACTH acts on the adrenal cortex to produce GC COR, which negatively regulates CRH production to terminate the stress response cascade. However, dietary supplementation with 500 mg/kg of GA resulted in lower serum COR, GC, and ACTH levels on day 7 after transportation, indicating that GA has great potential to relieve stress. High HSP-70 level is also induced by inflammatory stress and oxidative stress except for heat shock (67–69). Our results revealed that puppies in the TS group had higher HSP-70 levels after transportation, which were consistent with increased inflammatory response (TNF- α ↑, IL-4↓) and oxidative stress (GSH-Px↓) caused by transportation and changing environment, while GA suppressed upregulation of HSP-70 level. Similarly, studies on other polyphenol compounds in animals also obtained similar results (70, 71).

Previous studies revealed that the addition of dietary GA could modulate different signaling pathways through a wide range of inflammatory cytokines and enzymatic and non-enzymatic antioxidant defense systems (72). The enzymatic antioxidant defense system is generally the primary line of antioxidant defense in ROS detoxification (73). Additionally, MDA is the principal end-product of the lipid peroxidation process (74). So far, several studies reported the antioxidant action of GA (64, 75), and GA could provide the protection for various potential diseases including cancer, cardiovascular disease, and metabolic disease under oxidative stress by restoring the lipid peroxidation levels, normalizing or enhancing the levels of SOD, CAT, GSH-Px, GST, and GSH (76–78). In the present study, we found that multiple stressors resulted in a significant decrease in serum GSH-Px activities and an increase in MDA production in puppies. Nevertheless, dietary supplementation with GA at 500 mg/kg protected puppies from oxidative damage by increasing the activity of serum GSH-Px, which can efficiently eliminate free radicals and reduce the synthesis of MDA. Our results were consistent with those described in other studies.

Cytokines also play an important role in the regulation of intestinal function (79), while the overproduction of proinflammatory cytokines has a negative influence on intestinal homeostasis (80). It has been reported that the release of the pro-/anti-inflammatory and inflammatory mediators, such as IL-2, IL-4, IL-5, IL-13, IL-33, TNF- α , IFN- γ , and NF- κ B, could be downregulated by GA to prevent excessive inflammatory responses (41, 81, 82). Similarly, our results indicated that environmental stress caused a systemic inflammatory response by decreasing serum IgG content, increasing the production of proinflammatory cytokines TNF- α and IFN- γ , and reducing the secretion of anti-inflammatory cytokine IL-4 contents. However, GA effectively reversed the inflammatory responses in puppies, indicating that 500 mg/kg of dietary GA can improve anti-inflammatory function in stressed puppies. Also, a recent review concluded that GA plays an anti-inflammatory role by modulating the GM (40).

Previous studies indicated that GA was effective in a broad spectrum of antibacterial applications against pathogens including *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus mutans*, *Chromobacterium violaceum*, *Campylobacter jejuni*, and *Listeria monocytogenes* (83–85). Our study also reached a similar inhibitory effect on pathogenic bacteria. Dietary supplementation with 500 mg/kg of GA improved the bacterial diversity, inhibited the growth of *Escherichia-Shigella* and *Clostridium_sensu_stricto_1*, and enhanced *Lactobacillus* and *Faecalibaculum*, especially 1 day after transportation. Our results were further verified with LEfSe analysis, which also found that the TS group was associated with enrichment of Proteobacteria, *Escherichia-Shigella*, and *E. coli*, while *Lactobacillus*, *L. murinus*, and *L. reuteri* dominated the GA treatment (86, 87). Spearman's correlation analysis revealed the symbiotic relationship between bacteria. The high coexistence of *Escherichia-Shigella* with *Streptococcus* and *Clostridium_*

sensu_stricto_1 suggested the possibility of a syntrophic relationship among these bacteria. However, *Escherichia-Shigella* negatively correlated with *Faecalibaculum*, *Lactobacillus*, and *Bifidobacterium*. In agreement, the high relative abundance of pathogen *Escherichia-Shigella* is reported to be accompanied by the low relative abundance of *Lactobacillus* (88, 89). Our results are in general agreement with the previous studies, which found a decrease in Lactobacillaceae and Prevotellaceae and an increase in Firmicutes and Proteobacteria phyla in dextran sodium sulfate-induced colitis in mice, and GA treatment could modulate the microbiota composition toward a similar proportion to the control group (45, 90). Furthermore, Lima et al. reported that *Escherichia-Shigella* is one of the leading pathogenic causes of diarrhea, affecting approximately 80–165 million individuals (91). We can therefore infer that GA has a potential prophylactic effect on diarrhea caused by *Escherichia-Shigella*. GA also can induce changes in the microbiota toward a more favorable composition and activity, including the production of SCFAs and BCFAs in the colon (90).

The digestive tract contains an abundance of gut microbiota-derived metabolites (92). As one of the most important microbiota-derived metabolites, SCFAs are generated through colonic fermentation of dietary fibers (93, 94) and exert a beneficial effect on host health by reducing colonic pH and inflammation (95, 96), stimulating enterocyte growth, and improving mucus production and epithelial health (97). A previous study showed that increases in fecal SCFAs were found when relative abundances of Firmicutes, Lactobacillaceae, Clostridiales, *Roseburia*, Lachnospiraceae, and Erysipelotrichaceae were increased (98). These studies were in accordance with our findings that dietary GA treatment led to the increment of fecal total SCFAs and acetic acid concentrations. Further Spearman's correlation analysis revealed that fecal SCFAs have a positive association with Firmicutes (Erysipelotrichaceae, *Faecalibaculum*, *Allobaculum*, *Turicibacter*, and *Dubosiella*) and Lactobacillaceae (*Lactobacillus*) after transportation. Fecal BCFAs (e.g., isobutyric, isovaleric acid, valeric acid) are generated by microbial fermentation of branched amino acids, valine, leucine, and isoleucine (99, 100) and have effects on lipid and glucose metabolism (101). The highest total BCFAs and isovaleric acid concentrations were observed in the TS+GA group at 1 day after transportation, which had a positive association with *Faecalibaculum*, indicating that BCFAs may be produced by *Faecalibaculum*. The conclusion needs further validation. In short, these results indicate that GA protects against environmental stress-induced inflammation by improving the intestinal microbial structure and increasing the relative abundance of SCFA-producing bacteria.

Microbiota-derived metabolites, often secreted in the intestine and translocated across the intestinal barrier into the circulating system, are very important modulators for host metabolism (102, 103). In our study, metabolomics based on UPLC-Orbitrap-MS/MS analysis method was applied to investigate the changes of fecal metabolites in beagle dogs. The KEGG enrichment analysis declared that environmental stress mainly disturbed amino acid metabolism, carbohydrate metabolism, lipid metabolism, and metabolism of cofactors and vitamins in puppies, while dietary intake of GA helped to restore this imbalance. Changes in the

metabolic pathway were consistent with the PICRUSt analysis. Our findings were largely similar to the results reported by the previous study, whose metabolic data revealed that the GA-induced feces and urine metabolic changes in mice mainly focus on increasing carbohydrate metabolism (gluco-related metabolism) and lipid metabolism (bile acid metabolism) and decreasing amino acid metabolism (45). By screening differential metabolites in major differential metabolic pathways, fecal phenylacetaldehyde, L-tyrosine, L-valine, serotonin (amino acid metabolism), xanthine, adenosine, xanthosine, uric acid, phosphoglycolic acid (carbohydrate metabolism), tetrahydrodeoxycorticosterone, and glyceraldehyde (lipid metabolism) were upregulated due to the GA treatment. We considered them as the biomarkers for evaluating the influence of dietary GA treatment on fecal metabolites in puppies.

4-O-Methylgallic acid (4-OMeGA) is the primary metabolite of GA in human plasma and urine (104–106). The current study detected high levels of 4-OMeGA in the serum of puppies, indicating that GA may exert its function mainly by further transforming to 4-OMeGA. Serum metabolomics revealed that environmental stress mainly influenced amino acid metabolism, carbohydrate metabolism, lipid metabolism, energy metabolism, and nucleotide metabolism, while puppies fed GA reversed the shift. This finding is similar to that of Shi et al. who reported that metabolic changes associated with GA intake include glycogenolysis, glycolysis, tricarboxylic acid (TCA) cycle, and metabolism of nucleotides, choline, bile acids, amino acids (107). Consistent with fecal biomarkers analysis, serum metabolites of L-arginine, creatine, spermidine, 4-hydroxyproline, L-proline, L-glutamic acid, pyruvic acid, N-acetylmethionine, citrulline, L-glutamine, L-valine, L-isoleucine, L-lysine, carnitine (amino acid metabolism), citric acid, L-lactic acid, glyceraldehyde (carbohydrate metabolism), alpha-linolenic acid, oleic acid, linoleic acid, arachidonic acid, 13-L-hydroperoxylinoleic acid, chenodeoxycholic acid, taurine, cholic acid, taurochenodeoxycholic acid (lipid metabolism), ureidopropionic acid, hypoxanthine, inosine, and uric acid (nucleotide metabolism) were chosen as the biomarkers for evaluating the influence of dietary GA treatment on serum metabolites in puppies.

Spearman's correlation analysis found that fecal L-valine, L-tyrosine, L-glutamic acid, phenylacetaldehyde, and tetrahydrodeoxycorticosterone were positively correlated with the relative abundance of *Clostridium_sensu_stricto_1* (Firmicutes) and *Escherichia-Shigella* (Proteobacteria). However, interestingly, L-tyrosine and phenylacetaldehyde were oppositely correlated with and *Lactobacillus* (Lactobacillaceae). Serum L-lactic acid, alpha-linolenic acid, citric acid, oleic acid, and spermidine were positively correlated with *Clostridium_sensu_stricto_1* and *Escherichia-Shigella*, whereas alpha-linolenic acid, oleic acid, and spermidine were negatively correlated with *Lactobacillus* (Lactobacillaceae). Simultaneously, the positive correlation between serum metabolites and bacteria were L-arginine (*Faecalibaculum*), phytosphingosine and taurochenodeoxycholic acid (*Eggerthellaceae*), L-carnitine (*Turicibacter*), and dodecanoic acid and homovanillic acid (*Eubacteriaceae*); and serum arachidonic acid and glyceraldehyde had a positive association with Firmicutes and *Turicibacter*, respectively. Further research

is needed to provide a clear explanation between GM and fecal and serum metabolome in puppies supplemented with GA.

CONCLUSION

The GA markedly reduced the incidence of diarrhea and alleviated multiple environmental stressor-induced oxidative stress and inflammatory responses in puppies. The microbiome and metabolomics analyses revealed that environmental stress caused intestinal microbiota and metabolic disorders, while GA reversed the abnormalities. The comprehensive microbiota and metabolite relationships were established. In summary, we systematically elucidated the beneficial effects of GA treatment on stressed dogs from the host-microbial metabolic axis point of view. Future studies that can focus on the interactions between microbiota and metabolites may prove efficacious for understanding the precise mechanisms of the beneficial effects of polyphenol on health.

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://www.ncbi.nlm.nih.gov/bioproject/PRJNA782241>.

ETHICS STATEMENT

The animal study was reviewed and approved by the Experimental Animal Ethics Committee of South China Agricultural University.

AUTHOR CONTRIBUTIONS

KY generated the ideas, designed the study, detected the samples, and wrote the initial manuscript. LinZ and BD guided and revised the manuscript. XD and SJ participated in the data analysis and contributed to the draft of the manuscript. JD made feasible suggestions for the experimental design and manuscript. MZ analyzed the results. CW, ZQX, LimZ, AT, SY, PL, ZLX, SH, and FZ detected the samples. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2021.813890/full#supplementary-material>

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	4	生命科学学院	基于不对称PCR与G-四链体显色技术联用的鲍曼不动杆菌的便捷可视化检测方法	胡喆、方媛媛	高泽涛、侯晓阳、汪佳颖
	5	兽医学院	狂犬病毒纳米抗体的原核表达和亲和纯化	罗永文	钟凯宁、陈欣婷
二等奖 (15项)	1	材料与能源学院	水溶性姜黄素-丝氨酸/苏氨酸偶联物抑制 α -葡萄糖苷酶活性研究	熊亚红、李春远	余雪柔、许佳仪、吴晓婷
	2	动物科学学院	提取小鼠结肠癌细胞 RNA 以探究二氢杨梅素通过下调 MLKL转录水平对其坏死性凋亡的影响	邓百川	吕庆功、张振一、杨济盛
	3	动物科学学院	肌肤焕彩的奥“泌”—探究羊乳和牛乳外泌体对皮肤细胞活性影响的差异	陈婷	杜锦诗、张鑫硕、吴佳旋
	4	动物科学学院	老年萌宠降脂保健——辣木叶有效成分对脂肪细胞脂脂的作用	孙加节、张永亮	陈梓晴、李慧婷、李咏琪
	5	林学与风景园林学院	肉桂枝叶精油联合肉桂渣资源化利用—天然植物驱蚊水的制备及其功效评价	李雁群	后颖帆、田筱雅、翁锦鹏
	6	农学院	利用 HPLC 探究水稻色泽与水稻子粒黄酮类化合物含量的关系	王金陵、谢恩	麦俊泰、刘浩帆
	7	农学院	基于群体淬灭的新型植物病害防控技术研究及应用	陈少华	颜演宸、刘思绮、余虹晓
	8	生命科学学院	产GABA乳酸菌的分离鉴定及GABA产量测定	林如琴	吴思琦、贾茹、孙雨洁
	9	生命科学学院	紫锥菊地上部分多糖提取及探究其抗氧化活性研究	何韩军	吴炫智、徐一策、廖浩辰
	10	食品学院	枯草芽孢杆菌诱变亚种在新型微生物清洁剂中的应用	李雪玲	张嘉恩、陈新钰、蔡江燕
	11	兽医学院	穿心莲内酯抑制狂犬病毒的效果初探	罗均	蔡濠欣、谭海容、杨梓卉
	12	兽医学院	新型氨基糖苷类修饰酶耐药基因的克隆及抑菌活性	连新磊	李培培、陈子妍
	13	植物保护学院	感染黄龙病菌的柑橘斑驳叶片的病理生理及组织结构分析	许美容	周雪妍、肖雨馨
	14	植物保护学院	铜绿假单胞菌群体感应抑制剂的筛选	崔紫宁、陈建平	黎璇、苏筱轩、陈静怡
	15	资源环境学院	枯草芽孢杆菌绿色荧光蛋白标记及其在菜心中的定殖	吕辉雄	谢祥琳、吕高阳、刘金英
三等奖 (25项)	1	材料与能源学院	未来抗生素替代品的希望——拟黑多刺蚁中抗菌肽的提取纯化及抑菌活性测定	王磊	雷淑婷、谭韵曦、李欣然
	2	材料与能源学院	陈皮中黄酮的提取及其抗氧化性的检测	赵慧	叶景欣、梁力行、王婷
	3	材料与能源学院	五指毛桃中总氨基酸的提取与鉴定	赵慧	钟佩珊、杨婉溶、郑翊思
	4	动物科学学院	超声波辅助水酶法从桑叶中提取 β -谷甾醇及其抗菌活性的检测	杨婉莹、陈芳艳	许方正、夏万军
	5	动物科学学院	紫杉状海门冬有效成分缓解反刍动物温室气体排放——助力双碳目标	孙加节	齐佃轩、刘钰雯、李忠杰
	6	动物科学学院	探究鸡蛋壳膜酶解提取抗菌肽工艺及制备新型抗菌敷料的研究	朱勇文	龚微莎、刘芳元、于书娜
	7	动物科学学院	变废为宝——探究有机溶剂萃取柚子皮精油及柠檬烯对缓解焦虑助眠作用——助眠香薰的推广	朱勇文	蔡德豪、陈永康、朱芊芊
	8	动物科学学院	助力“无抗养殖”——姜黄素和百里香酚的提取并探究混合灭菌效果	张玲娜	陈乘慧、严苏禾、阳海丽
	9	动物科学学院	鸭支原体的快速检测——一种未知病原抗体检测靶标筛选新思路	李鸿鑫、简文成	范曼婷、曾亦晨
	10	动物科学学院	利用梯度沉降法进行动物组织不同细胞亚群的分离	杨化强	刘经宇、程黎明、赵学宇
	11	都柏林国际学院	黄精多糖的提取及其抗氧化能力的测定	黎攀、黄卫娟	孙弋航、何书伦、侯佳作
	12	海洋学院	虾青素/生姜提取物复合涂膜液应用于水果保鲜的研究	吴坤	邱佳苡、蓝雪、王馨平
	13	农学院	大米发酵脱镉制备米饮料	无	杨紫嫣、徐妍、冯毅
	14	农学院	大豆黄浆水中异黄酮的高效、安全提取	羊海军、刘自强	黄绍杭、康鸿益、李庆君
	15	生命科学学院	创面修复的“天山来客”——雪莲多糖的提取及功能分析	巫光宏、方媛媛	陈咏荷、沈皓纯
	16	生命科学学院	夏枯草提取物对禾谷镰刀菌抑菌效果的测定	母培强、何晃毓	梁艺莺、陈子玄、关森
	17	生命科学学院	高温淀粉酶制剂改造	张文彬、郭雪倩	陈杰桢、吴璨芝、谢攸德
	18	生命科学学院	农副产品“资源化”探究——橙皮维生素C的提取、分析和多功能身体乳的制备	白玫	张婉娴、陈婉仪、刘金明
	19	食品学院	果胶基荷载姜黄素复合体系的构建及稳定性评价	王凯	王璧莹、翁晓岚
	20	兽医学院	新一代“生物技术导弹”——H7亚型禽流感病毒的纯化与活性鉴定	冯赛祥	陈博昊、邓道伦、郑雍磊
	21	兽医学院	新型质粒接合转移抑制剂的筛选、评估及其机制研究	孙坚	陆心怡、郑佳敏、林奕豪
	22	兽医学院	基于代谢组学与网络药理学研究美丽崖豆藤抗疲劳分子机理	孙永学、陈建新	黄允序、付思源
	23	园艺学院	火龙果皮甜菜素的提取及彩色肥皂的制备	秦永华	胡诗欣、徐泽柳、伦影枫
	24	资源环境学院	不同波长的光对厌氧氨氧化系统的影响研究	梁瑜海	沈磊鹏、黄杏玲、卢通
	25	资源环境学院	模拟胃液中纳米塑料对牛乳铁蛋白的消化动力学影响及作用机制	陈澄宇	詹蕾卉 赖涵宇

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导出

申请单号	申请人	申请人所在单位	参与人	参与人所在单位	服务主题	申报项目	服务对象	是否补报	开始时间	结束时间	申请时间	服务工时	开展培训讲座次	培训人数	发放技术资料数量
FW202407291753225986	张玲娜	动物科学学院	张玲娜	动物科学学院	流浪猫收容技术指导	成果、报告、建议获得领导正式批示或采纳 (国家级)	广东冰冰优宠品牌管理有限公司	是	2024-07-07	2024-07-07	2024-07-29	3	0	0	0
FW20240729181252372	张玲娜	动物科学学院	张玲娜	动物科学学院	训犬基地技术指导	成果、报告、建议获得领导正式批示或采纳 (国家级)	杨堤训犬俱乐部	是	2023-07-07	2023-07-07	2024-07-29	3	0	0	0
FW20240729205850807	张玲娜	动物科学学院	张玲娜	动物科学学院	楼房猪场技术指导	成果、报告、建议获得领导正式批示或采纳 (国家级)	广西扬翔股份有限公司	是	2024-05-30	2024-05-31	2024-07-29	6	0	0	0
FW20240905172614902	张玲娜	动物科学学院	张玲娜	动物科学学院	科学养宠科普广东经济科教频道	-	广东广播电视台	是	2023-10-17	2023-10-17	2024-09-05	5	0	0	0
FW20240906112820565	张玲娜	动物科学学院	张玲娜	动物科学学院	常熟流浪动物救助中心莫城基地	成果、报告、建议获得领导正式批示或采纳 (国家级)	常熟流浪动物救助中心莫城基地	是	2024-08-22	2024-08-22	2024-09-06	1	0	0	0

证书

兹聘请 张玲娜 同志为 国家生猪产业技术创新战略联盟理事，任期四年（2024年12月—2028年12月）。
特颁此证！

This is to certify that Lingna Zhang is appointed for a term of four years (From Dec. 2024 to Dec. 2028) as Director of the National Swine Industry Technology Innovation Strategic Alliance.



聘 书

LETTER OF APPOINTMENT



兹聘请 张玲娜 同志为“广东省畜牧兽医学会宠物分会”
常务理事，聘期五年，自2022年至2026年。
此聘

广东省畜牧兽医学会

2022年6月22日

