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Looking beyond the mind: the gut microbiome as a potential new avenue for treating behavioral disorders in dogs. Current state of the art and potential future perspectives

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Abstract: The gut-microbiota-brain axis represents a complex and essential bidirectional communication system that governs physiological and mental health in dogs. Canine cognitive dysfunction, anxiety, and neurobehavioral comorbidities linked to epilepsy are progressively acknowledged as conditions potentially involving intestinal dysbiosis. Increasing evidence regarding this axis has prompted research into novel gut microbiota-based therapeutic approaches. This review examines current research investigating the modulation of gut-microbiome to treat behavioral disorders. Promising outcomes from specific probiotic strains, nutraceutical supplements, and fecal microbiota transplantation are examined, emphasizing their capacity to modulate stress markers and enhance behavioral deficits. Despite the interest surrounding these new frontiers, the existing data reveal significant heterogeneity, attributable to the diversity of the samples (companion, working, and shelter dogs) and the absence of methodological standardization. Conclusions derived from animal models (e.g., rodents) and human clinical studies, while intriguing, are not directly applicable to veterinary practice.

Key Words: gut–brain axis; behavior; canine; gut; microbiota; FTM

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Introduction

Scientific interest in the ecosystem of microorganisms that inhabit the gastrointestinal tract, commonly referred to as the gut microbiome, has grown exponentially recently (Crisante et al., 2025; Gorzelanna et al., 2024). Recognized as an essential factor for overall health and well-being, the microbiome plays a crucial role in regulating various physiological functions in the host, including digestion, energy metabolism, immune responses, and even behavior and temperament (Kim et al., 2025). For the canine species, a thorough understanding of its diversity and roles is critical to maintaining optimal health and homeostasis (Kim et al., 2025). Recent research highlighted the existence of a complex and fundamental bidirectional communication pathway between the gut and the central nervous system, known as the gut-brain axis (GBA); this axis is in constant communication and allows the gut microbiome to influence brain (MGBA), physiology and behavior (Kiełbik et al., 2024; Sacoor et al., 2024), just as it is affected by them in turn. The communication pathways seem to be diverse, encompassing neural-hormonal signals and microbial metabolite synthesis, which influence neurobiology and stress regulation in the host (Sacoor et al., 2024).

Generally, the microbiomes of healthy dogs exhibit more similarities to those of humans than to cats, perhaps attributable to a common habitat and a significant starch-rich diet. The phyla

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Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria constitute over 99% of the intestinal bacteria in dogs, whereas the gut microbiota at the genus level is predominantly represented by Fusobacterium, Bacteroides, and Prevotella, as detailed in the review by Kim et al. (2025). A well-balanced microbiome, known as eubiosis, represents an optimized microbial composition that promotes host health and metabolic functions. The disruption of microbial balance, known as dysbiosis, has been correlated to various physical conditions; moreover, a growing body of research also seems to suggest a significant link with canine behavioral disorders (Homer et al., 2023). Disturbances in the gut-brain axis caused by dysbiosis have been linked to the manifestation of disorders such as anxiety, generalized fear, phobias, and aggression, suggesting a direct impact of the microbiome on the emotional and cognitive sphere of dogs (as reported in Crisante et al., 2025). Neurological disorders (including trauma) and stress are also considered factors that alter microbiota, potentially initiating a vicious cycle that disrupts the gut-brain axis (Nicholson et al., 2019; Beurel 2023). According to present data, dogs with behavioral issues like aggression, separation anxiety, and phobias showed an increased abundance of Firmicutes. Furthermore, the family Rikenellaceae and the genus Lactobacillus were enriched in phobic dogs, whereas Bacteroides, Sutterella, Peptostreptococcus, and Oscillospira were decreased, and Catenibacterium, Megamonas, and Eubacterium were more abundant in aggressively behaved animals (Homer et al., 2023). However, while recognizing the growing interest in the field, research on the link between canine gut microbiome and behavior is still in its early stages, with relative immaturity in the field, non-standardized and non-uniform research methodologies, and little replicability of studies. Therefore, certain relationships and insights regarding the gut-microbome-brain axis (MGBA) should be approached with care at this time, particularly for their application in behavioral medicine.

With this in mind, the aim of this review is to elucidate the findings of principal studies regarding the gut-microbiome-brain axis and potential strategies in behavioral medicine, including dietary modifications, the administration of probiotics and supplements (i.e. integrative medicine), and fecal microbiome transplantation (FMT) in dogs.

Correlations between microbiome composition, anxiety and aggression

The relationship between gut microbiota composition and aggressive behavior is one of the most studied characteristics in dogs, given its relevance for animal well-being and human safety. A pilot study in 2019 (Kirchoff et al., 2019) analyzed a population of 31 rescued Pitbulls (21 with conspecific aggressive behavior, 10 controls) residing at a temporary shelter while in protective custody. The authors were the first to investigate the correlation between canine gut microbiota and aggression towards conspecifics. The results showed that aggressive dogs had a greater abundance of the phylum Firmicutes, while non-aggressive dogs had higher levels of Fusobacteria and Proteobacteria. Some taxa, such as Paraprevotellaceae and Lactobacillus, were associated with the presence of aggression. The authors suggest an association between aggression and specific intestinal bacterial populations, while noting that the lack of details on behavioral assessment procedures limits its reliability and comparability.

Similarly, Mondo et al. (2020) also profiled the composition of the canine gut microbiota associated with aggressive, phobic, and normal behaviors in shelters. The sheltered dogs were evaluated by a veterinary behaviorist and divided into two groups: aggressive vs. non-aggressive. The authors report that aggressive dogs (n = 11) showed a distinct gut microbiome composition compared to non-aggressive dogs (n = 13). In particular, aggressive dogs showed greater bacterial diversity and lower abundance of Bacteroidaceae, Alcaligenaceae, and Paraprevotellaceae, as well as an increase in the abundance of Erysipelotrichaceae. Mondo and collaborators also report that Catenibacterium and Megamonas are possible bacterial discriminants of aggressive behav-

ior (Mondo et al., 2020). Nevertheless, these results are partly consistent with those reported by Kirchoff et al. (2019).

In 2022, a larger study was conducted by Craddock and collaborators on 134 working dogs to examine aggression (Craddock et al., 2022). The main results reported by the authors highlighted a positive association between aggression and the richness of the gut microbiota. The researchers noted that a greater prevalence of Firmicutes correlated with increased aggression, aligning with the findings of Kichoff et al. (2019). Aggressive dogs had a greater abundance of Blautia, Bradyrhizobium, and Coccaceae, and a reduced abundance of the groups Lachnospiraceae and Selenomonadaceae.

Several cross-sectional studies have established significant correlations between microbiota composition and behavioral scores obtained using standardized questionnaires such as the Canine Behavioral Assessment & Research Questionnaire (C-BARQ) (Hsu & Serpell 2003). In particular, the study by Pellowe and colleagues focused on anxiety, aggression, and microbiome composition. A total of 494 dog owners completed an initial questionnaire on canine diet, lifestyle, and health; of these, 235 also completed the C-BARQ behavioral questionnaire. For fecal microbiota analysis, a selected sample of 50 dogs was included in the final study. The gut microbiota composition of the 48 dogs (of 50 selected) was sequenced from microbial DNA extracted from fecal samples and evaluated for association with the behavioral group assigned to the dog. In their cohort, the dominant phyla Bacteroidota, Firmicutes, and Fusobacteria comprised approximately 95% of the gut microbiota, a finding consistent with other studies on healthy dogs. Their analysis did not reveal any significant differences in the relative abundance of individual microbial taxa between behavioral groups (high anxiety/aggression vs. low anxiety/aggression). Despite minimal differences in relative abundance, the use of machine-learning models and compositional balance models allowed for reliable prediction of behavioral group membership (high or low) based on the overall composition of the gut microbiota. This suggests that the entire microbial profile, rather than the abundance of a single bacterium, is related to behavior. Of interest, their findings showed the microbial genus Blautia as consistently correlated with anxiety scores in companion dogs.

Correlations between microbiome composition, phobia and fear

A few studies investigating gut microbiota in connection with aggression also gathered data on fearfulness, phobic behavior, or avoidance manifestations (e.g., cowardice).

Mondo et al. (2020) found that phobic dogs displayed a higher prevalence of Lactobacillus and Rikenellaceae than the group exhibiting normal behavior. Lactobacillus species are recognized GABA makers in the gut and have been well studied for their implications in human mental health and obesity.

Afterward, the working dogs examined by Craddock et al. (2022) exhibiting elevated "cowardice scores" also showed augmented Lactobacillus abundance, corroborating the results of Mondo et al. (2020). Nevertheless, the comprehensive microbial profile exhibited substantial variation. This cohort exhibited a decreased abundance of many beneficial bacteria, such as Faecalibacterium prausnitzii, Roseburia, and Eubacterium, as well as reduced gut richness and Shannon diversity, in contrast to the findings of Mondo et al. (2020) on dogs (Craddock et al., 2022).

In 2025, we published findings from our research examining the potential association between gut microbiota and dysfunctional behaviors. This study employed a matched case-control design to investigate the relationship among gut microbiota, blood metabolites, and fear-related behaviors in domestic dogs. The evaluation of fecal microbiota revealed a differential abundance of taxa associated with the Proteobacteria and Firmicutes phyla in case (n= 8) versus control dogs (n= 8). In particular, we found a lower abundance of Gammaproteobacteria (class) and Dorea (genus) in fearful patients, who, however, showed higher levels of Erysipelatoclostridiaceae (family) and Peptostreptococcales Tissierellales (order), compared to healthy controls. Serum levels of key

neurotransmitter precursors, including glutamine and glycine, were reduced in fearful dogs, while bile acid concentrations were elevated. Correlational analyses provided support for a mechanistic link between gut microbiota and emotional behavior via gut-brain axis pathways, highlighting specific microorganisms such as Peptostreptococcales and Dorea in relation to alterations in bile acid and amino acid levels.

Correlations between microbiome composition, cognition and acute stress

To the authors' knowledge, only a single study has examined the relationship between dogs' gut microbiome and cognitive function (Kubinyi et al., 2020). This study investigated the relationship between the working memory abilities of 29 companion dogs and the composition of their gut microbiota. The dogs underwent a simple memory assessment requiring them to remember the location of a food reward among five identical open containers following a 30-second delay. Fecal samples from dogs with diminished memory capacity contained a higher proportion of Actinobacteria. The authors claim that individuals with Alzheimer's disease exhibited a similar rise in Actinobacteria (Kubinyi et al., 2020), suggesting a potential link between this taxon and short-term memory performance. The authors sought to assess the temporal changes in the composition of the gut microbiome. Following a three-month period, a second round of sampling and sequencing analysis was conducted to achieve this objective. Cognitive performance was not re-evaluated, and only the feces of six dogs were subjected to retesting. The results indicated that the microbiome composition was comparable between the initial and subsequent samplings.

Recently, researchers have investigated the impact of acute stress on the composition of gut bacteria. In 2024, Patel and colleagues enrolled 20 healthy adult mixed-breed dogs residing in a colony at the Waltham Petcare Science Institute, constituting a control/placebo group in concurrent investigations examining cannabidiol (CBD) (Patel et al., 2024). The dogs were categorized into two groups (10 dogs each) and subjected to two distinct acute stressors prevalent in the daily lives of companion dogs: a predetermined 10-minute low-speed car journey and a 45-minute separation in a test room. Fecal samples were collected three times across eight-week intervals at three time points: 24 hours prior to the event, within 24 hours following the event, and between 24- and 48-hours post-event. The study findings were predominantly negative, indicating significant stability of the gut microbiota in reaction to acute and infrequent stressors. Microbiota research revealed no significant effects on alpha diversity (including the Shannon index and species richness) or beta diversity (total composition of the bacterial community) after exposure to either stressor (car travel or separation). No notable alterations in microbiota composition were seen at the level of particular taxa. The predominant bacterial species, regardless of the stressor, comprised Blautia spp., Faecalibacterium sp., Prevotella sp., and Ruminococcus gnavus.

Impact of probiotics administration on the microbiota and behavior of dogs

In behavioral medicine, a behavior modification plan combined with pharmacological therapies is a significant therapeutic approach. Nonetheless, pharmacological interventions frequently face resistance from several caregivers, require weeks to manifest effects, and pose a risk of severe reactions, including canine serotonin syndrome (van Haaften et al. 2020). Investigations into the gut-brain axis in canines are revealing innovative strategies for managing behavioral disorders; thus, it is not unexpected that human medicine has been discussing psychobiotics for a decade. Psychobiotics are defined as probiotics that improve mental health in the host when consumed in precise amounts through interaction with intestinal commensal bacteria (Binda et al., 2024).

As far as the authors are aware, the first research on probiotic administration for behavior modification in our species of interest was conducted in 2018(McGowen et al., 2018) as a blind-

ed, placebo-controlled, crossover trial. Twenty-four anxious Labrador Retrievers participated in the study. All dogs were accustomed to a complete and balanced diet. One group of dogs was supplemented with the probiotic strain B. longum BL999 (NCC 3001) and the other group was supplemented with a placebo for six (6) weeks. After a 3-week washout period, the group that had previously received the probiotic received the placebo and vice versa for another 6 weeks. The results supported the hypothesis that B. longum BL999 has an anxiolytic effect. Treated dogs displayed improvements in general anxiety and stress responses (e.g., reduction in behaviors such as barking, excessive licking, or agitation). Caregiver ratings of anxious behaviors were significantly reduced during the probiotic treatment period compared to the period when dogs received the placebo. In addition, an improvement in the ratio of cortisol to dehydroepiandrosterone (DHEA) was observed. An altered cortisol/DHEA ratio is often associated with chronic stress. Modulation of this ratio suggests a positive effect on the hypothalamic-pituitary-adrenal (HPA) axis, which regulates the stress response. Therefore, the authors conclude that, from both a behavioral and physiological perspective, BL999 had an anxiolytic effect on anxious dogs and could be a useful tool in developing management plans aimed at improving the well-being of dogs suffering from anxiety.

Moreover, two recent clinical trials examined the efficacy of particular strains of Lactiplantibacillus plantarum, a GABA-producing strain, in alleviating anxiety and aggression. The 2022 study by Yeh et al. examined the impact of Lactiplantibacillus plantarum PS128 in mitigating canine behavioral problems. The study included 45 dogs diagnosed with aggressiveness disorder (n = 22), separation anxiety (n = 15), compulsive disorder (n = 7), and unclassifiable inappropriate behavior (n = 1, excessive barking). The behavioral assessment was conducted by a comprehensive medical history and evaluation of diagnostic questionnaires, facilitating an accurate diagnosis of behavioral issues in the physically healthy canines. The dogs were subsequently administered the probiotic Lactiplantibacillus plantarum PS128 (PS128) for a duration of two weeks. The treatment's efficacy was assessed by comparing ratings from the EDED (Evaluation of Dog's Emotional and Cognitive Disorders) and CBC (Canine Behavioral Checklist) questionnaires during visits prior to and subsequent to probiotic administration. The study's results indicated that dogs administered PS128 exhibited enhanced behavioral stability and a notable decrease in aggressiveness and separation anxiety symptoms. A notable reduction in the 5-HIAA/5-HT ratio, an indicator of serotonin turnover, was detected in dogs exhibiting separation anxiety, corroborating the theory of the involvement of the serotonergic system in the effects of the probiotic.

The recent placebo-controlled study by Bijaoui and Zimmerman (2025) examined the effects of a novel Lactiplantibacillus plantarum strain on canine behavioral issues, enrolling 40 domestic dogs with pre-existing problems, including aggression and/or anxiety. The intervention involved daily oral delivery of the probiotic Lactiplantibacillus plantarum strain (LP815™) or placebo (maltodextrin) over a duration of 4 weeks. Canines administered lactobacillus (n=28) showed a notable enhancement in aggressiveness and anxiety metrics (evaluated by the C-BARQ questionnaire). Treated dogs acclimated more rapidly following caregiver departure, showed diminished daytime activity without lethargy and exhibited more regular sleep patterns with decreased daytime napping, indicating an overall enhancement in sleep quality and a reduction in separation anxiety. The mechanism is hypothesized to be mediated by the gut-brain axis, according to the strain's capacity to create neurotransmitters, despite not being the primary target.

The strategic modulation of the gut-brain axis through probiotics has recently expanded to encompass Enterococcus faecium. Zhang et al. (2025) assessed the efficacy of a novel strain, Enterococcus faecium Kimate-X, as an alternative to traditional pharmaceutical treatments for mitigating transport stress and improving intestinal health in dogs. Sixteen male Beagle dogs, aged 4 to 5 months, were enrolled in the study. The experimental design sought to assess the effect of the probiotic on the response to transport stress following a 49-day supplementation period. The dogs were categorized by body weight into two equivalent groups (n = 8 per group): the control group

was administered a basic diet alongside a placebo (freeze-dried skimmed milk powder), whereas the treatment group received the basic diet supplemented with the probiotic Kimate-X. The stress test occurred on the 50th day, directly following the feeding period. All dogs were transported in the same vehicle for a duration of three hours. The route comprised highways and urban roads, with an average speed of 60 km/h. Metagenomic analysis indicated an increase in gut microbiota diversity in treated dogs, with significantly higher microbial diversity observed at the Shannon and Simpson indices. The bacterial taxa composition at the phylum level exhibited similarities between the control and Kimate-X groups; however, the relative abundance of predominant phyla varied. Moreover, elevated levels of short-chain fatty acids (SCFAs) were observed in the feces of treated dogs. The authors noted that dogs treated with Kimate-X exhibited significantly lower serum cortisol levels post-transport compared to controls, suggesting the need for further research to validate the use of probiotics as a novel strategy for stress management in animals.

Impact of integrative medicine on the microbiota and behavior of dogs

Integrative medicine adopts a holistic approach to addressing patients' physical and mental health by combining unconventional and traditional therapies. The aim of this medical approach is to assess and address the entire pet, encompassing both behavioral and physical clinical manifestations, while considering the environment, the illness, and the caregiver connection. Treatment modalities include standard and complementary therapies, focusing on the most effective, least invasive, cost-efficient, and least hazardous approaches to achieve beneficial outcomes (Landsberg, 2023). Increasing evidence underscores the significance of nutraceuticals such as alpha-casozepine, l-theanine, tryptophan, cannabidiol, cannabis, essential oils, and melatonin in addressing behavioral disorders in dogs, due to their anxiolytic and antistress properties, positioning them as alternatives to conventional therapies (Sechi et al., 2017; Orlando, 2018; Tynes & Landsberg, 2021; Cannas et al., 2021; Sacchettino et al., 2023; Giuliano et al., 2024; Song et al., 2025).

In 2020, Cannas et al. conducted a pilot study examining the efficacy of a dietary supplement in alleviating stress and regulating the gut bacteria in dogs. Forty dogs, aged 1 to 10 years, participated in this double-blind, placebo-controlled research trial. Ten dogs acted as control subjects, exhibiting no apparent clinical signs of anxiousness. Thirty experimental dogs displaying indications of stress and anxiety were randomly assigned to two groups: the treatment group (n = 20) and the placebo group (n = 10). Over a period of 60 days, the placebo group, consisting of 10 dogs evenly distributed by sex, was administered a placebo orally once daily, while the treatment group, including 20 dogs also balanced for sex, received a nutraceutical containing anti-inflammatory compounds (CLA, Krill), pre/probiotics, 5-HTP, and L-theanine (Relaxigen Pet Dog) supplementation. Behavior associated with stress and anxiety was assessed by questionnaires administered to owners (utilizing a 0-5 Likert scale) on days 0, 30, and 60. Fecal specimens were obtained at identical intervals for microbiota investigation. The findings indicated that dogs in the treatment group exhibited a notable enhancement in behavioral scores (anxiety and stress) relative to the placebo group, with treated dogs demonstrating over a 10% chance of improvement compared to those receiving the placebo. The study revealed that, on day 0, anxious dogs exhibited a distinct gut microbiota composition compared to healthy control dogs. Anxious dogs had elevated levels of Lactobacillus, Bifidobacteria, and Enterobacteriaceae. After treatment, dogs that received the supplement showed a significant reduction in Bacteroides spp., Prevotella spp., and Porphyromonas spp. compared to the placebo. A statistically significant decrease in Lactobacillus and Bifidobacteria was seen on both day 30 and day 60 in comparison to the placebo. Furthermore, Clostridium coccoides, Eubacterium rectale, and Enterobacteriaceae exhibited a reduction on day 60.The researchers concluded that Relaxigen supplementation may induce changes in bacterial group concentrations in anxious dogs compared to the placebo group, potentially serving as a supportive measure in stress alleviation when combined with behavioral treatment.

In 2024, Belà and colleagues investigated the effects of a nutraceutical composed of prebiotic fiber and tyndallized *Lactobacillus reuteri* DSM 32203 (Microbiotal®) on the intestinal ecology of sled dogs that are exposed to high levels of stress and athletic exercise. The study included twenty healthy adult Alaskan Husky dogs—eleven spayed females and nine neutered males. The trial lasted 30 days, which included 29 days of training and the competition day. To determine how stress and physical activity affected the intestinal microbiota of dogs, the dogs were divided into two groups: the treated group with the nutraceutical product (in addition to their diet) and the control group (dogs that continued their usual diet and exercise routine without receiving the nutraceutical). According to the study's findings, the dogs in the control group exhibited higher levels of potentially enteropathogenic bacteria, including E. coli and Streptococcus species. Meanwhile, the number of beneficial bacterial species (e.g., Faecalibacterium spp., Turicibacter spp., Blautia spp., Fusobacterium spp., and Clostridium hiranonis) decreased. Conversely, the nutraceutical-treated dogs exhibited reduced levels of enteropathogenic microorganisms (E. coli and Streptococcus spp.). The number of beneficial bacterial species (such as Turicibacter spp. and Faecalibacterium spp.) increased significantly. The Dysbiosis Index (DI) for the treated group was less than zero (normobiosis value), a result not found in untreated dogs. The authors concluded that Microbiotal® could be an excellent and valuable support for sporting dogs since it can reduce the negative effects of stress on the gut microbiota.

Impact of nutritional management, FTM on the microbiota and behavior of dogs

From prenatal until adolescence and maturity, nutrition has a significant impact on the brain's anatomical and functional development. Nutritional compounds, upon reaching the gut, are metabolized by the intestinal microbiota into various circulating metabolites that significantly impact the central nervous system. This bidirectional loop is completed by the brain's influence on gut microbiota via alterations in motility, secretion, and mucosal permeability. For example, anxiety and aggression are among the behavioral abnormalities in humans and animals that have been linked to diet, vitamin and mineral deficiencies (Tynes & Landsberg, 2021; Ephraim et al., 2022).

In 2022, 40 adult dogs were part of a nutritional study to evaluate the impact of specific ingredients on the microbiota and metabolites related to anxiety (Ephraim et al., 2022). The study adopted a crossover experimental design, controlled with a washout diet, to minimize the influence of the previous diet. Dogs were fed a washout food, then randomized to consume a control (fish oil and polyphenol blend without tomato pomace) or test (fish oil and polyphenol blend with tomato pomace) food, then the washout food, and crossed over to consume the test or control food. Each for 30 days. The results were obtained by comparing metabolites and bacterial composition during the administration of washout diets. Regarding the results on plasma metabolites related to anxiety, the most significant result was a decrease in 4-ethylphenyl sulfate (4-EPS), a metabolite known to be produced by the gut microbiota and previously associated with anxiety disorders and behaviors similar to autism spectrum disorders in mouse models. Both the control food and the test food significantly reduced 4-EPS levels compared to the washout diet. Plasma 4-EPS levels were also significantly lower after dogs ate the test food compared with the control food. Regarding the results on the intestinal microbiota, the significant increase in certain bacterial genera in the feces (particularly Blautia, Parabacteroides, and Odoribacter) was directly correlated with the decrease in plasma 4-EPS when dogs consumed the supplemented foods. The authors concluded by reporting that foods supplemented with polyphenols and omega-3 fatty acids can modulate the gut microbiota to improve the profile of anxiety-related metabolites.

Fecal microbiome transplantation is an emerging therapeutic option for a variety of diseases and is characterized as the transfer of fecal microorganisms from a healthy donor into the intestinal tract of a diseased recipient. In recent years, veterinary professionals have adapted fecal microbiota transplantation (FMT) for canine patients; however, unlike in humans, canine FMT

is predominantly oriented towards research rather than practical uses, primarily due to safety concerns (Tuniyazi et al., 2022).

A review of Dewey (2024) concentrated on research regarding the manipulation of the microbiome through FTM as an alternative treatment for canine cognitive dysfunction, analogous to human Alzheimer's disease. Dysbiosis is thought to precede cognitive decline in Alzheimer's disease and may have a causal role; restoring a compromised cognitive system recipient's microbiome with fecal microbiota from a healthy donor may facilitate the re-establishment of a healthy microbiota, potentially reversing certain impairments to the intestinal barrier, blood-brain barrier, and cerebral function. However, even if the FMT treatment has been evaluated as an innovative therapy strategy for numerous canine somatic problems, no research has examined its potential impact on alleviating symptoms of behavioral disorders in dogs (Kiełbik et al., 2024). It is noteworthy that various disciplines are also indicating the influence of bacteria on behavior.

A 2024 pilot study examined a novel therapy method, fecal microbiota transplantation, to mitigate behavioral issues frequently associated with canine epilepsy (Watanangura et al., 2024). This was a six-month, open-label, prospective pilot study with follow-up visits at three and six months after the intervention, aimed at investigating the effectiveness of fecal microbiota transplantation in alleviating behavioral comorbidities (such as anxiety and cognitive dysfunction) in dogs with drug-resistant epilepsy. The sample involved nine dogs with idiopathic epilepsy diagnosed as drug-resistant and exhibiting behavioral comorbidities (anxiety, fear, or ADHD-like symptoms). The donor was a dog with epilepsy but with normal behavior (no comorbidities) and a complete and lasting response to phenobarbital treatment. FMTs were performed three times, two weeks apart, and the dogs had follow-up visits at three and six months after FMTs. Behavioral aspects and neurotransmitter modulation were the primary findings: significant improvements were observed in the behaviors of impulsivity and hyperactivity, as well as in the reduction of fear (particularly non-social fear and fear of strangers/noises) and anxiety-related behaviors in dogs. Owners also realized an enhanced quality of life. The results showed changes indicative of diminished brain excitability, evidenced by reduced levels of aspartate and glutamate relative to baseline measurements, while concentrations of gamma-aminobutyric acid (GABA) and the GABA/glutamate ratio exhibited an increase. Concerning the gut microbiota composition, only minor taxonomic alterations were noted (such as a reduction in certain Firmicutes and an increase in Ruminococcus), while the levels of primary short-chain fatty acids and the overall diversity of the microbiota remained largely stable. None of the dogs were free after FMT. Nonetheless, a decrease in the duration of the ictal (during the seizure) and post-ictal (after the seizure) phases was noted in certain subjects. The researchers claimed that behavioral comorbidities in canine idiopathic epilepsy may be alleviated via FTM, recommending randomized controlled trials to validate the preliminary encouraging findings.

In the same year, research by Lin and colleagues investigated - for the first time- the impact of FTM on canine behavior, particularly focusing on the work performance and stress profiles of police dogs (Lin et al., 2024). Twenty Wolf Cyan dogs were randomly allocated to receive either physiological saline or fecal suspension at low, medium, or high dosages- via oral gavage for a duration of 14 days. Dogs after FMT treatment were also subjected to an hour road transportation and then were evaluated for serum stress indicators. Growth performance, police efficacy, serum biochemical analysis, and gut microbiota were assessed two weeks following FMT. The findings indicated that serum stress markers (including cortisol) following road transport significantly diminished in dogs administered FMT compared with the control group. FMT resulted in enhanced working performance in dogs, including parameters such as courage, attention, and olfactory ability, in comparison to the control group. Regarding microbiota composition, the fecal samples from FMT dogs were characterized by higher abundances of the genera Lactobacillus, Prevotella, and Fusobacterium and lower concentrations of Cetobacterium, Allobaculum, Bifidobacterium, and Streptococcus, thus appearing to effectively repopulate the gut microbiota in

Kunming police dogs. Additionally, a decrease in diarrhea incidence and a general enhancement in physical growth and performance were noted. The authors reported that the most pronounced effects were observed in the group administered the low dose of FMT.

Conclusions: advancing an integrated and multidisciplinary approach

Recent evidence convincingly supports the efficacy of MGBA as a therapeutic target for behavioral comorbidities. Nevertheless, although prior studies have shown that probiotics, supplements, FTM, and nutritional modulation can mitigate symptoms of anxiety, fear, and neurobehavioral deficits (Watanangura et al., 2024; Gorzelanna et al., 2024; Kiełbik et al., 2024), it remains essential to recognize that this area is not yet thoroughly investigated. The results are presently challenging to generalize or comprehensively compare due to the heterogeneity of the experimental models employed. Divergences in the target population constitute a significant confounding variable: it is established, for instance, that companion dogs, sled or working dogs, and dogs residing in shelters (which exhibit distinct physiological and behavioral characteristics due to environmental stress, as emphasized by Gazzano et al., 2025) are not comparable populations for microbiota research. Moreover, inconsistencies may exist in diagnostic terminology and procedures for assessing stress, anxiety, and phobia, as well as their coexistence in certain instances. This overlap can complicate treatment approaches, as practitioners must navigate these intertwined conditions to develop effective strategies. A comprehensive understanding of each disorder is essential for ensuring that patients receive tailored interventions that address their unique experiences (Gazzano&Ogi, 2020).

Consequently, the recommendation for behavioral medicine is prudent circumspection regarding the uncritical implementation of MGBA-based therapies. The management of canine behavioral pathologies requires an integrated and multidisciplinary approach: veterinary practitioners and specialists in behavior ought to adhere to the holistic concept of integrative medicine, engaging in active collaboration with nutritionists, neurologists, and other specialists. Effective treatment plans can only be developed through comprehensive global patient assessment, which encompasses medical history, environmental factors, dietary habits, and familial dynamics, integrating behavioral therapy, targeted nutritional support, and, when warranted, conventional pharmacotherapy. In the future, standardizing research protocols and conducting controlled studies with larger samples are crucial for transforming the promising potential of MGBA into validated and clinically dependable routine therapies.

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References

Beurel E. Stress in the microbiome-immune crosstalk. *Gut Microbes* 2024, *16*, 2327409.

Bijaoui EMM, Zimmerman NP. Efficacy of a Novel Lactiplantibacillus plantarum Strain Strain (LP815™) in Reducing Canine Aggression and Anxiety: A Randomized Placebo-Controlled Trial with Qualitative and Quantitative Assessment. *Animals* 2025, *15*, 2280.

Binda S, Tremblay A, Iqbal UH, Kassem O, Le Barz M, Thomas V, Bronner S, Perrot T, Ismail N, Parker JA. Psychobiotics and the Microbiota–Gut–Brain Axis: Where Do We Go from Here? *Microorganisms* 2024, *12*, 634.

Cannas S, Tonini B, Belà B, Di Prinzio R, Pignataro G, Di Simone D, Gramenzi A. Effect of a novel nutraceutical supplement (Relaxigen Pet dog) on the fecal microbiome and stress-related behaviors in dogs: A pilot study. *J. Vet. Behav.* 2021, *42*, 37-47.

Craddock HA, Godneva A, Rothschild D, Motro Y, Grinstein D, Lotem-Michaeli Y, Narkiss T, Segal E, Moran-Gilad J. Phenotypic correlates of the working dog microbiome. *npj Biofilms Microbes* 2022, *8*, 66, doi:10.1038/s41522-022-00329-5.

Crisante A, Newberry F, Clegg SR, Mitchell GL, Pike TW, Ratcliffe V, Spain A, Wilkinson A, Zulch H, Mills DS. A critical review of research concerning the gut microbiome in dogs and its relationship with behaviour. *Appl. Anim. Behav. Sci.* 2025, doi:10.1016/j.applanim.2025.106755.

Dewey CW. Poop for thought: Can fecal microbiome transplantation improve cognitive function in aging dogs? *Open Vet. J.* 2025, *15*, 556.

Ephraim E, Brockman JA, Jewell DE. A diet supplemented with polyphenols, prebiotics and omega-3 fatty acids modulates the intestinal microbiota and improves the profile of metabolites linked with anxiety in dogs. *Biology* 2022, *11*, 976.

Gazzano V, Ogi A. Canine phobia. *Dog Behav.* 2020, *6*, doi:10.4454/db.v6i2.121.

Giuliano VO, Sacchettino L, Gazzano V, Ciccarelli D, Napolitano F, d'Angelo D. Supplementation with nutraceutical Calmina®(BuonaPET) improves anxiety status in shelter dogs: a pilot study. *Dog Behav.* 2024, *10*.

Gorzelanna Z, Miszczak M. Through the Intestines to the Head? That Is, How the Gastrointestinal Microbiota Affects the Behavior of Companion Animals. *Pets* 2024, *1*, 201–215, doi:10.3390/pets1030015.

Homer B, Judd J, Mohammadi Dehcheshmeh M, Ebrahimie E, Trott DJ. Gut Microbiota and Behavioural Issues in Production, Performance, and Companion Animals: A Systematic Review. *Animals* 2023, *13*, 1458, doi:10.3390/ani13091458.

Hsu Y, Serpell JA. Development and validation of a questionnaire for measuring behavior and temperament traits in pet dogs. *J. Am. Vet. Med. Assoc.* 2003, *223*, 1293-1300, doi:10.2460/javma.2003.223.1293.

Kiełbik P, Witkowska-Piłaszewicz O. The Relationship between Canine Behavioral Disorders and Gut Microbiome and Future Therapeutic Perspectives. *Animals* 2024, *14*, 2048.

Kim H, Chae Y, Cho JH, Song M, Kwak J, Doo H, Choi Y, Kang J, Yang H, Lee S, Keum GB, Wattanaphansak S, Kim S, Kim HB. Understanding the diversity and roles of the canine gut microbiome. *J. Anim. Sci. Biotechnol.* 2025, *16*, 95, doi:10.1186/s40104-025-01235-4.

Kirchoff NS, Udell MAR, Sharpton TJ. The gut microbiome correlates with conspecific aggression in a small population of rescued dogs (*Canis familiaris*). *PeerJ* 2019, *7*, e6103, doi:10.7717/peerj.6103.

Kubinyi E, Bel Rhali S, Sándör S, Szabó A, Felföldi T. Gut microbiome composition is associated with age and memory performance in pet dogs. *Animals* 2020, *10*, 1488, doi:10.3390/ani10091488.

Landsberg G, Radosta L, Ackerman L. *Behavior Problems of the Dog and Cat*, 4th ed.; Elsevier: St. Louis, MO, USA, 2023.

Lin QY, Du JJ, Xu H, Lv MK, Xu L, Li J, Cao ZH. Effects of fecal microbial transplantation on police performance and transportation stress in Kunming police dogs. *Appl. Microbiol. Biotechnol.* 2024, *108*, 46.

McGowan RT, Barnett HR, Czarnecki-Maulden G, Si X, Perez-Camargo G, Martin F. Tapping into those 'gut feelings': Impact of BL999 (Bifidobacterium longum) on anxiety in dogs. In *Veterinary Behavior Symposium Proceedings, Denver, CO*, 2018, pp. 8-9.

Mondo E, Barone M, Soverini M, D'Amico F, Cocchi M, Petrulli C, Mattioli M, Marliani G, Candela M, Accorsi PA. Gut microbiome structure and adrenocortical activity in dogs with aggressive and phobic behavioral disorders. *Heliyon* 2020, *6*, e03311, doi:10.1016/j.heliyon.2020.e03311.

Nicholson SE, Watts LT, Burmeister DM, Merrill D, Scroggins S, Zou Y, Lai Z, Grandhi R, Lewis AM, Newton LM, Eastridge BJ, Schwacha MG. Moderate Traumatic Brain Injury Alters the Gastrointestinal Microbiome in a Time-Dependent Manner. *Shock* 2019, *52*, 240-248, doi:10.1097/SHK.0000000000001211.

Orlando JM. Behavioral Nutraceuticals and Diets. *Vet. Clin. North Am. Small Anim. Pract.* 2018, *48*, 473-495, doi:10.1016/j.cvsm.2017.12.012.

Patel KV, Hunt ABG, Castillo-Fernandez J, et al. Impact of acute stress on the canine gut microbiota. *Sci Rep* 2024, *14*, 18897, doi:10.1038/s41598-024-66652-3.

Pellowe SD, Zhang A, Bignell DRD, Barrett V, Lye A, Boudreau R, Pinder L, Johnson T, Kogan LR, Kogan L. Gut microbiota composition is related to anxiety and aggression scores in companion dogs. *Sci Rep* 2025, *15*, 24336, doi:10.1038/s41598-025-06178-4.

Sacchettino L, Costanzo M, Veneruso I, D'Argenio V, Mayer M, Napolitano F, et al. Altered microbiome and metabolome profiling in fearful companion dogs: An exploratory study. *PLoS ONE* 2025, *20*, e0315374, doi:10.1371/journal.pone.0315374.

Sacchettino L, Giuliano VO, Avallone L, Napolitano F, d'Angelo D. Combining α-s1 casozepine and fluoxetine treatment with a behavioral therapy improves symptoms in an aggressive dog: An italian case report. *Vet. Sci.* 2023, *10*, 435.

Sacoor C, Marugg JD, Lima NR, Empadinhas N, Montezinho L. Gut-Brain Axis Impact on Canine Anxiety Disorders: New Challenges for Behavioral Veterinary Medicine. *Vet. Med. Int.* 2024, *2024*, 2856759.

Sechi S, Di Cerbo A, Canello S, Guidetti G, Chiavolelli F, Fiore F, Cocco R. Effects in dogs with behavioural disorders of a commercial nutraceutical diet on stress and neuroendocrine parameters. *Vet. Rec.* 2017, *180*, 18.

Song Y, Yoon M. Melatonin effects on animal behavior: circadian rhythm, stress response, and modulation of behavioral patterns. *J. Anim. Sci. Technol.* 2025, *67*, 1–16, doi:10.5187/jast.2024.e105.

Tuniyazi M, Hu X, Fu Y, Zhang N. Canine fecal microbiota transplantation: current application and possible mechanisms. *Vet. Sci.* 2022, *9*, 396.

Tynes VV, Landsberg GM. Nutritional Management of Behavior and Brain Disorders in Dogs and Cats. *Vet. Clin. North Am. Small Anim. Pract.* 2021, *51*, 711–727, doi:10.1016/j.cvsm.2021.01.011.

van Haaften KA, Grigg EK, Kolus C, Hart L, Kogan LR. A survey of dog owners' perceptions on the use of psychoactive medications and alternatives for the treatment of canine behavior problems. *J. Vet. Behav.* 2020, *35*, 27-33.

Watanangura A, Meller S, Farhat N, Suchodolski JS, Pilla R, Khattab MR, Lopes BC, Bathen-Nöthen A, Fischer A, Busch-Hahn K, Flieshardt C, Gramer M, Richter F, Zamansky A, Volk HA. Behavioral comorbidities treatment by fecal microbiota transplantation in canine epilepsy: a pilot study of a novel therapeutic approach. *Front. Vet. Sci.* 2024, *11*, 1385469, doi:10.3389/fvets.2024.1385469.

Yeh Y, Lye X, Lin Y, Li C, Fang S, Liu Y, Wang L. Effects of Lactiplantibacillus plantarum PS128 on alleviating canine aggression and separation anxiety. *Appl. Anim. Behav. Sci.* 2022, *247*, 105569.

Zhang R, Hu W, Zhong S, Chen W, Chen M, Yu Q. Impact of Enterococcus faecium Kimate-X on Reducing Stress in Dogs Through Gut Microbiota Modulation. *Vet. Sci.* 2025, *12*, 412, doi:10.3390/vetsci12050412.

Guardando oltre la mente: il microbiota intestinale come potenziale nuova via per trattare i disturbi comportamentali nei cani. Analisi dello stato dell'arte e delle potenziali prospettive future

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Sintesi

L'Asse microbiota-intestino-cervello rappresenta un complesso ed essenziale sistema di comunicazione bidirezionale che governa la salute fisiologica e mentale nel cane. La disfunzione cognitiva canina , l'ansia e le comorbilità neuro-comportamentali correlate all'epilessia sono progressivamente riconosciute come condizioni che implicano una potenziale disbiosi intestinale. Le crescenti evidenze relative a quest' Asse hanno stimolato la ricerca di nuovi approcci terapeutici basati sulla modulazione del microbiota intestinale. Questa rassegna esamina la ricerca attuale che indaga la modulazione del microbiota intestinale per il trattamento dei disturbi comportamentali. Vengono analizzati i risultati promettenti derivati da specifici ceppi probiotici, integratori nutraceutici e dal trapianto di microbiota fecale, sottolineando la loro capacità di modulare i marcatori dello stress e migliorare i deficit comportamentali. Nonostante l'interesse che circonda queste nuove frontiere, i dati esistenti rivelano una significativa eterogeneità, attribuibile alla diversità dei campioni (cani da compagnia, da lavoro o in canile) e all'assenza di una standardizzazione metodologica. Inoltre, le conclusioni derivate dai modelli animali (ad esempio, roditori) e dagli studi clinici sull'uomo, sebbene intriganti, non sono direttamente applicabili alla pratica veterinaria.