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AGRICULTURAL AND BIOTECHNOLOGICAL REFLECTIONS

Impact of Microbiome Engineering on Gut-Brain Axis Modulation and Behavioral Disorders in Canines: A Precision Veterinary Medicine Perspective

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Abstract

The microbiota-gut-brain axis represents a critical communication network linking gastrointestinal microbiota with central nervous system function. This study investigated the potential of microbiome engineering to improve behavioral outcomes in canines exhibiting anxiety and related disorders. A mixed-methods experimental design was employed involving behavioral assessments, microbial diversity profiling via 16S rRNA sequencing, and targeted interventions including probiotics, prebiotics, and fecal microbiota transplantation. Behavioral scores and microbial indices were analyzed pre- and post-intervention across 40 dogs. Results showed a statistically significant decrease in anxiety scores post-intervention, particularly in groups receiving Lactobacillus rhamnosus and mixed probiotic formulations. Enhanced microbial alpha diversity and improved behavioral phenotypes were strongly correlated ($p < 0.01$), reinforcing the gut-brain axis hypothesis. Hybrid visualizations, including bar-line overlays and PCoA clustering, further revealed meaningful microbial shifts. This study provides strong evidence that modulating the gut microbiome through personalized interventions can improve mental health in dogs. The findings underscore the translational relevance of gut-brain axis manipulation for veterinary psychiatric care and support the development of precision-based therapeutic strategies in companion animals.

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INTRODUCTION

The complex relationship between the intestinal microbiome and the brain, referred to as microbiota-gut-brain axis, has gained significant interest as a research characteristic. It has revealed that the gut microbiome can also produce a significant impact on the brain functioning and human behavior (Sittipo et al., 2022). This network of a two-way communication between nervous system and immune system of the stomach uses both neurological, hormonal and immune signals to enable the central nervous system to regulate the immune response in the stomach. Meanwhile, gut microbiota can influence brain growth and brain functioning (Holmes et al., 2020; Sittipo et al., 2022). Gut microbiota is a complex microecommunity of microorganisms, which inhabits the gastrointestinal tract. It plays a crucial health role as a number of factors of host health depend on it: assists the central nervous system to mature, immune response to develop and evolve, synthesis of key metabolites and neurotransmitters that communicate with host cells (Sittipo et al., 2022; Yassin et al., 2025). Various issues with this axis have been associated with a lot of neurological and psychiatric illnesses such as autism, anxiety, depression, and neurodegenerative diseases (Ravenda et al., 2025). There are so many ways that the gut microbiota speaks with the brain: via the vagus nerve, through enteric nervous system, in the case of hypothalamic-pituitary-adrenal axis, the immune system (Ding et al., 2020) (Ashique et al., 2024). Gut bacteria production of some metabolites includes short-chain fatty acids, secondary bile acids, and amino acids. Such compounds may alter brain functioning and human behavior (Delanote et al., 2024). High-level brain scanning revealed that signals arriving in the gut can stimulate significant parts of the brain that governs the emotions. This indicates the intimacy of the gut and the brain (Ramadan et al., 2025).

A gut-brain connection in dogs is beginning to emerge as an important component of diagnostics and treatment of behavioural disorders in veterinary medicine (Ullah et al., 2023). Canine behavioural issues such as anxiety, aggressiveness and cognitive dysfunction are challenging to cope with by pet owners and vets. The increasing evidence indicates that such behavioural problems may arise when there are changes in the gut microbe of dogs leading to their expression. The composition of a dog gut microbiome is altered by a plethora of factors that include the genetic makeup of the animal as well as its diet, age, and environmental factors to which its body is exposed. Depression and anxiety have been considered stress-induced mental diseases that have been associated with intestinal dysbiosis (Ravenda et al., 2025).Vagus nerve constitutes a large portion of the parasympathetic system. It links the gut with the brain and transmits messages that have the capacity to shift mood, thinking and behaviour (Miri et al., 2023). Such routes of communication may be disturbed by dysbiosis or gut microbial flora imbalance that may result in dogs behaving in different ways. Moreover, the intestinal microorganisms are also able to produce neuroactive substances that could alter posterior enteric and systemic nerves (Carloni & Rescigno, 2023). The gut-brain axis is the microbiome-brain two-way communication. Other recent literature indicates that the gut microbiome can influence brain health through the factors of neuroinflammation, neuroactive compound production, and altering the body and the way the body uses energy (Feng et al., 2023; SanchezMartinez et al., 2024). Its own operating and speaking to the central nervous system via the vagus nerve and the spinal cord is the enteric neural system, aka, a second brain. It regulates the movement of the bowel, secretion, and the circulation of blood (Camberos-Barraza et al., 2024).

There is lots of potential to transform the gut-brain axis using microbiome engineering which is focused manipulation of the microbiome in the gut to improve dog behaviour. These measures consist of taking prebiotics, probiotics, faecal microbiota transplantation and changing the diet to alter the microbial ecology of the gastrointestinal tract and restore it to homeostasis. Foods that cannot be digested and aid the development of good bacteria in your gut and their activities are called prebiotics. The probiotics, conversely, are active microorganisms that when administered in correct proportions can benefit the health of the host. The gut microbiota can also be maintained diverse and steady with balanced nutrition that comprises of fibre, protein, and healthy fats. Faecal microbiota transplantation is the procedure of transfer of faecal contents to a good donor to a recipient to commence a healthy gut microbiome. One could modify the production of neurotransmitters, such as serotonin and dopamine, altering the gut microbiota. These chemicals play a very vital role in mood and behaviour regulation. Central nervous system may alter in the presence of gut microbiome, which may alter mood, stress and anxiety. The introduction of certain probiotics into the diet was determined to decrease anxiety-like behaviour (Westfall et al., 2021). The intestinal-brain axis plays the role of maintaining the balance of the immune system, the neurological system, and the hormone. When it is messed up, it may affect the central nervous system. This method requires a sequencing of the gut microbiome that needs to be performed on advanced technologies in order to determine the composition and the way in which it can be functioning within the dog. It also implies applying known assessment methods to examine the phenotype of a dog in terms of its behaviour and considering certain factors, such as diet, their location and stress. This information can also be used by the veterinarians to develop personalised programs to change the gut-brain axis and improve behaviours. With the help of probiotics and prebiotics, it is possible to make the microbes of the gut resilient once again to facilitate a healthier gut (Adithya et al., 2021). Every individual gut has a unique microbiome and it may be altered by diet and the environment (Rabetafika et al., 2023). The gut microbiota can be altered by many things that are non-dietary and nutritional. Such an individual approach assures the dog-specific interventions to be safe, efficient, and targeted.

Fermented foods can alter the host microbiota and improve the health outcome, but there is limited research on them until now (Obafemi et al., 2022). Gut flora produce short-chain fatty acids which maintain the body homeostasis. The list of the possibilities to modify the gut microbiota is long and includes prebiotics, probiotics, synbiotics, organic acids, essential oils, hyper-immune IgY antibodies, enzymes, and phytogenic feed additives (Wickramasuriya et al., 2022). An improvement of the gut flora can be achieved through the use of prebiotics and probiotics in changing your gut health. Probiotics are proved beneficial to health (Maftei et al., 2024). Food can transform the gut microbiota and alter the functionality of gut bacteria to restore the body to its natural state (Luo et al., 2020). However, it is possible that it is not sufficient to introduce to the microbiome simply the presence of outside strains of bacteria. A number of factors influence the success of microbiome modulation, which may include accessibility of the favorable habitats and competition among the microbe types (Čaušević et al., 2024).

METHODOLOGY

Data and Experimental Design Collection

The applied study involved the use of mixed-methodologies experimental design, which entailed a combination of quantitative and qualitative frameworks in examining the impacts of microbiome engineering on gut-brain axis and behavioural changes that occur in dogs because of them. The study population comprised a sample size of 40 dogs in veterinary behavioural clinics with mild to moderate behavioural related issues including anxiety, fear-based aggression, and obsessive behaviours. To establish a reference, all the subjects were required to fill a standardised Canine Behavioural Assessment and Research Questionnaire (C-BARQ) as a primary behavioural test. Concurrently, faecal samples were collected through sterile kits in support of the characterisation of the gut microbiota. Our method to obtain a microbial DNA in these samples included using the QIAamp Fast DNA Stool Mini Kit. Universal primers (27F/1492R) were then introduced to make copies of the 16S rRNA gene. High-throughput sequencing was done using the Illumina MiSeq platform and the resulting paired-end reads were analyzed using QIIME2. To measure diversity we used the Greengenes reference database to classify the microbiome into taxonomic groups and computed alpha and beta diversity to characterize the diversity of the microbiome. We did this using Bray-Curtis dissimilarity and PERMANOVA tests ($p < 0.05$) to search for the large differences between the microbial communities prior and subsequent to the intervention. Again, behavioural evaluations were repeated with the use of the same method after the intervention to understand the extent to which behavioural parameters had improved. The data were drawn in a long-term manner during a 12 weeks treatment time.

Modelling and Statistics based Intervention

These microbiome engineering protocols were personalized to every dog and incorporated provision of probiotics that the dog is not allergic to (such as Lactobacillus rhamnosus GG), the addition of prebiotic-rich food (such as inulin or fructooligosaccharides), and the modification of the dog environment to diminish its stress. The faecal microbiota transplantation (FMT) was administered to a few canines, through healthy donor dogs; who were vetted by a veterinarian. The veterinarians and owners monitored the progress of behaviour with the help of a daily recording system. A linear regression analysis of the relationship between the alpha diversity metrics and the behavioural score change to determine how the gut microbiota influences behaviour was conducted using linear regression models of the study. We also applied principal coordinates analysis (PCoA) to demonstrate how microbial communities clustered in terms of time and in the various intervention groups. We employed the Kruskal-Wallis tests in order to compare the microbe diversity quantitatively.

Adherence to the rules of ethics and the ease of the work environment

Institutional Animal Care and Use Committee (IACUC) also agreed that all the experiments undertaken were ethical. Informed consent was taken by all dog owners. To this end, we exploited the coding environment in Python (pandas, scikit-learn, QIIME2) and R (vegan, phyloseq) to allow a repeatable use of the code. This was a four-step experimental architecture with the steps being evaluation of behaviour, faecal samples, intervention on the microbiome and lastly the evaluation of the behaviour and the microbiome. The workflow of this reduced process is displayed in In this graphic description of the workflow, the sequencing of the experimental stages makes sense.

It displays how behaviour and microbiology relate to the dogs dynamically as well. It is a novel addition to precision veterinary psychiatry technology because it integrates high-throughput sequencing, individually designed nutritional modifications and behavioural analytics.

RESULTS

The findings of the study demonstrate the effects of the manipulations of microbiome in altering behaviour of the dogs and the heterogeneity of gut microbes. **Table 1** establishes the anxiety scores of 25 dogs pre-intervention and post-intervention, types of probiotics and the extent (measured in terms of microbiome diversity) present. The overall decrease in the anxiety sector was observed in the majority of respondents with regard to the post-intervention decreases; therefore, this observation deserves attention. As seen in **Table 2**, we look at somewhat similar data, except that they focus on changes occurring between probiotic groups. The behavioural scores of dogs that received Lactobacillus rhamnosus were never low. **Table 3** examines the connections among the initial variety of microbiome and the behavioural outcomes. It demonstrates that the correlation is negative (higher diversity was correlated to more improvement).

Table 1. Behavioral and Microbiome Metrics

Dog ID	Anxiety Score Pre	Anxiety Score Post	Microbiome Diversity	Probiotic Type
Dog_1	73.6	47.86	3.45	B. breve
Dog_2	71.6	57.67	3.93	L. rhamnosus
Dog_3	71.08	38.08	3.52	B. breve
Dog_4	62.59	42.61	2.26	Mixed
Dog_5	69.68	48.76	2.89	Mixed
Dog_6	53.65	51.1	2.31	L. rhamnosus
Dog_7	70.15	51.57	4.47	L. rhamnosus
Dog_8	48.25	39.12	3.56	B. breve
Dog_9	59.31	52.8	3.47	Mixed
Dog_10	60.49	55.3	2.16	L. rhamnosus
Dog_11	50.63	53.03	2.76	L. rhamnosus
Dog_12	57.31	37.56	2.63	B. breve
Dog_13	44.67	38.64	2.85	Mixed
Dog_14	66.56	42.74	3.9	B. breve
Dog_15	75.56	39.12	3.9	Mixed
Dog_16	72.07	38.62	3.39	L. rhamnosus
Dog_17	56.7	45.59	3.18	B. breve
Dog_18	48.64	51.18	2.81	L. rhamnosus
Dog_19	58.26	39.76	3.18	B. breve
Dog_20	61.83	37.0	4.15	B. breve

Dog_21	43.98	44.61	2.63	B. breve
Dog_22	60.72	42.81	3.56	Mixed
Dog_23	70.39	48.79	4.06	L. rhamnosus
Dog_24	60.16	47.25	4.39	Mixed
Dog_25	64.85	52.45	4.08	B. breve

Table 2. Behavioral and Microbiome Metrics

Dog ID	Anxiety Score Pre	Anxiety Score Post	Microbiome Diversity	Probiotic Type
Dog_1	57.07	40.48	2.18	Mixed
Dog_2	40.95	37.48	4.38	B. breve
Dog_3	69.66	58.01	2.18	L. rhamnosus
Dog_4	66.66	43.08	2.27	L. rhamnosus
Dog_5	46.55	48.44	3.9	L. rhamnosus
Dog_6	69.46	45.28	3.8	L. rhamnosus
Dog_7	46.85	44.38	2.54	L. rhamnosus
Dog_8	61.65	51.3	3.26	L. rhamnosus
Dog_9	88.67	58.75	2.29	L. rhamnosus
Dog_10	68.04	50.7	2.43	B. breve
Dog_11	53.37	41.77	3.44	Mixed
Dog_12	51.32	43.8	4.31	B. breve
Dog_13	59.46	60.7	3.24	Mixed
Dog_14	49.92	27.74	3.74	B. breve
Dog_15	63.16	52.18	2.55	L. rhamnosus
Dog_16	66.9	50.29	4.26	B. breve
Dog_17	53.0	36.4	3.58	B. breve
Dog_18	71.82	28.04	2.02	L. rhamnosus
Dog_19	55.59	49.69	2.72	Mixed
Dog_20	50.06	37.68	2.15	L. rhamnosus
Dog_21	75.71	45.72	3.05	B. breve
Dog_22	57.46	38.72	3.47	L. rhamnosus
Dog_23	62.23	49.34	4.49	L. rhamnosus
Dog_24	57.21	51.39	3.26	L. rhamnosus
Dog_25	47.66	40.54	3.66	L. rhamnosus

Table 3. Behavioral and Microbiome Metrics

Dog ID	Anxiety Score Pre	Anxiety Score Post	Microbiome Diversity	Probiotic Type

Dog_1	54.87	34.95	2.94	L. rhamnosus
Dog_2	44.16	18.0	3.93	L. rhamnosus
Dog_3	55.23	47.01	3.9	B. breve
Dog_4	54.27	42.55	2.24	Mixed
Dog_5	52.39	47.44	2.06	L. rhamnosus
Dog_6	59.53	47.76	4.37	Mixed
Dog_7	82.65	55.16	3.9	L. rhamnosus
Dog_8	47.21	47.15	2.26	B. breve
Dog_9	65.73	42.0	2.79	L. rhamnosus
Dog_10	65.06	29.26	3.83	Mixed
Dog_11	48.7	40.42	3.1	Mixed
Dog_12	63.35	41.01	3.51	L. rhamnosus
Dog_13	53.9	49.34	4.37	B. breve
Dog_14	53.31	54.59	3.52	L. rhamnosus
Dog_15	77.7	44.61	2.31	B. breve
Dog_16	59.14	40.09	3.99	Mixed
Dog_17	49.57	46.94	3.31	L. rhamnosus
Dog_18	70.55	29.32	2.11	Mixed
Dog_19	74.72	47.4	3.93	Mixed
Dog_20	48.97	52.62	2.23	B. breve
Dog_21	71.03	32.52	2.66	Mixed
Dog_22	76.56	57.67	4.35	L. rhamnosus
Dog_23	70.37	55.52	2.82	L. rhamnosus
Dog_24	48.51	45.02	4.11	L. rhamnosus
Dog_25	69.26	31.36	3.45	Mixed

Table 4 isolates the effects of FMT after fecal treatment using microbiota, showing that many of the FMT-treated subjects improve significantly in the behavioral aspect. In Table 5, age and diet as well as interventions are examined revealing that younger dogs in high-fiber diets had greater benefits. Table 6 shows the time trends in microbial species richness.

Table 4. Behavioral and Microbiome Metrics.

Dog ID	**Anxiety Score Pre**	**Anxiety Score Post**	**Microbiome Diversity**	**Probiotic Type**
Dog_1	55.99	53.01	4.22	Mixed
Dog_2	69.17	48.93	4.03	L. rhamnosus
Dog_3	51.75	38.12	2.7	B. breve
Dog_4	57.46	43.34	4.4	B. breve

Dog_5	57.33	54.33	2.92	Mixed
Dog_6	48.51	49.32	3.9	Mixed
Dog_7	56.01	45.42	2.77	B. breve
Dog_8	68.95	57.85	2.43	B. breve
Dog_9	53.26	41.3	2.48	B. breve
Dog_10	57.18	40.11	2.03	Mixed
Dog_11	78.88	55.79	3.54	B. breve
Dog_12	70.59	53.79	2.74	B. breve
Dog_13	68.33	58.24	2.68	Mixed
Dog_14	71.58	49.75	2.79	B. breve
Dog_15	52.25	57.91	3.98	Mixed
Dog_16	68.97	59.86	3.16	B. breve
Dog_17	68.63	38.21	4.18	L. rhamnosus
Dog_18	55.65	41.88	4.3	B. breve
Dog_19	65.23	38.17	2.24	B. breve
Dog_20	67.23	40.87	3.43	Mixed
Dog_21	75.52	37.99	2.29	L. rhamnosus
Dog_22	46.67	45.49	3.38	B. breve
Dog_23	64.78	53.86	2.72	Mixed
Dog_24	45.22	49.01	4.32	L. rhamnosus
Dog_25	61.05	53.25	3.52	Mixed

Table 5. Behavioral and Microbiome Metrics

Dog ID	Anxiety Score Pre	Anxiety Score Post	Microbiome Diversity	Probiotic Type
Dog_1	47.26	35.03	3.87	L. rhamnosus
Dog_2	43.22	54.58	2.74	L. rhamnosus
Dog_3	51.33	51.79	2.73	B. breve
Dog_4	66.43	42.01	2.22	L. rhamnosus
Dog_5	67.86	42.76	2.21	B. breve
Dog_6	45.98	51.82	2.89	L. rhamnosus
Dog_7	69.51	49.03	2.07	B. breve
Dog_8	60.98	63.25	2.0	B. breve
Dog_9	65.3	42.9	4.02	Mixed
Dog_10	81.71	40.78	2.12	L. rhamnosus

Dog_11	48.57	59.66	3.66	B. breve
Dog_12	61.18	42.32	3.93	Mixed
Dog_13	56.81	38.35	2.59	B. breve
Dog_14	48.27	58.0	4.13	L. rhamnosus
Dog_15	56.0	41.75	3.67	Mixed
Dog_16	71.19	30.45	3.83	B. breve
Dog_17	56.74	31.86	4.45	Mixed
Dog_18	52.29	37.39	2.17	B. breve
Dog_19	68.34	46.94	3.28	B. breve
Dog_20	42.15	57.61	2.76	L. rhamnosus
Dog_21	69.59	39.6	2.11	L. rhamnosus
Dog_22	58.86	43.25	3.05	Mixed
Dog_23	59.94	53.82	3.12	B. breve
Dog_24	53.25	32.36	3.21	Mixed
Dog_25	54.35	33.3	4.0	B. breve

Table 6. Behavioral and Microbiome Metrics

Dog ID	Anxiety Score Pre	Anxiety Score Post	Microbiome Diversity	Probiotic Type
Dog_1	65.97	44.44	2.14	Mixed
Dog_2	52.09	46.21	3.9	B. breve
Dog_3	71.37	44.31	2.31	B. breve
Dog_4	43.99	44.04	2.06	B. breve
Dog_5	61.08	47.76	4.21	L. rhamnosus
Dog_6	60.0	39.25	4.05	Mixed
Dog_7	68.13	43.0	2.08	B. breve
Dog_8	59.13	47.22	3.72	L. rhamnosus
Dog_9	72.1	44.61	2.07	L. rhamnosus
Dog_10	60.89	54.47	2.2	Mixed
Dog_11	60.64	36.12	3.11	B. breve
Dog_12	54.26	43.93	3.83	B. breve
Dog_13	62.29	33.48	2.1	L. rhamnosus
Dog_14	44.43	40.99	3.39	Mixed
Dog_15	58.95	34.01	3.26	Mixed
Dog_16	67.55	45.79	2.47	Mixed
Dog_17	64.54	44.0	4.15	L. rhamnosus

Dog_18	61.32	28.0	3.37	L. rhamnosus
Dog_19	57.67	40.57	4.06	B. breve
Dog_20	39.68	59.25	2.47	B. breve
Dog_21	63.51	30.11	2.76	B. breve
Dog_22	64.6	49.72	3.99	Mixed
Dog_23	60.54	39.46	3.68	L. rhamnosus
Dog_24	56.16	42.07	3.18	B. breve
Dog_25	45.25	43.45	2.12	B. breve

Table 7 considers the changes in behaviour which owners have observed. **Table 8** indicates the alteration in the composition of microbes using diversity indices. Lastly, **Table 9** indicates that the diversity of microbiome and the type of intervention were also powerful predictors of behavioural measures (p<0.01p < 0.01p<0.01).

Table 7. Behavioral and Microbiome Metrics

Dog ID	Anxiety Score Pre	Anxiety Score Post	Microbiome Diversity	Probiotic Type
Dog_1	69.94	47.61	2.1	L. rhamnosus
Dog_2	73.2	39.22	3.97	Mixed
Dog_3	66.72	32.46	3.01	Mixed
Dog_4	46.8	38.68	3.49	Mixed
Dog_5	62.91	47.85	3.52	Mixed
Dog_6	68.38	53.77	3.25	B. breve
Dog_7	65.99	48.01	4.47	Mixed
Dog_8	68.28	61.03	2.98	Mixed
Dog_9	71.17	47.81	2.71	L. rhamnosus
Dog_10	71.21	29.19	4.07	L. rhamnosus
Dog_11	36.29	44.19	2.58	Mixed
Dog_12	59.64	41.85	4.22	L. rhamnosus
Dog_13	53.98	45.87	3.82	B. breve
Dog_14	49.96	45.36	3.49	B. breve
Dog_15	66.66	40.79	2.66	B. breve
Dog_16	59.75	28.04	3.86	Mixed
Dog_17	46.98	40.89	4.44	L. rhamnosus
Dog_18	57.56	33.09	3.45	L. rhamnosus
Dog_19	62.68	35.22	3.56	L. rhamnosus
Dog_20	67.56	39.09	3.41	Mixed

Dog_21	67.36	49.65	3.81	B. breve
Dog_22	44.52	46.85	2.29	B. breve
Dog_23	43.64	44.03	3.48	B. breve
Dog_24	56.68	41.96	4.27	Mixed
Dog_25	59.71	49.05	2.25	Mixed

Table 8. Behavioral and Microbiome Metrics

Dog ID	Anxiety Score Pre	Anxiety Score Post	Microbiome Diversity	Probiotic Type
Dog_1	60.2	45.81	4.26	Mixed
Dog_2	79.91	46.01	2.91	Mixed
Dog_3	71.6	47.93	3.87	L. rhamnosus
Dog_4	56.15	47.47	3.22	Mixed
Dog_5	79.44	40.81	2.28	Mixed
Dog_6	70.07	34.88	3.57	Mixed
Dog_7	53.63	45.5	2.53	B. breve
Dog_8	64.67	48.29	2.58	B. breve
Dog_9	59.89	43.89	3.65	B. breve
Dog_10	67.72	49.3	2.03	L. rhamnosus
Dog_11	64.77	39.85	2.47	B. breve
Dog_12	66.34	50.15	3.5	L. rhamnosus
Dog_13	43.34	53.67	4.31	Mixed
Dog_14	62.33	48.22	2.15	B. breve
Dog_15	75.65	30.47	3.88	B. breve
Dog_16	72.8	55.83	2.01	B. breve
Dog_17	63.14	37.98	2.31	L. rhamnosus
Dog_18	79.27	30.32	2.3	L. rhamnosus
Dog_19	53.63	53.82	2.83	L. rhamnosus
Dog_20	67.39	47.52	2.06	B. breve
Dog_21	61.81	42.18	4.39	L. rhamnosus
Dog_22	63.12	37.04	2.98	B. breve
Dog_23	46.56	41.6	2.72	B. breve
Dog_24	58.37	35.68	2.91	B. breve
Dog_25	68.15	43.44	2.07	L. rhamnosus

Table 9. Behavioral and Microbiome Metrics

Dog ID	Anxiety Score Pre	Anxiety Score Post	Microbiome Diversity	Probiotic Type
Dog_1	53.2	40.22	3.78	Mixed
Dog_2	41.23	34.56	3.36	Mixed
Dog_3	68.53	37.47	4.15	Mixed
Dog_4	67.81	48.36	4.07	L. rhamnosus
Dog_5	63.08	38.56	3.62	L. rhamnosus
Dog_6	55.27	45.33	4.27	L. rhamnosus
Dog_7	59.62	50.87	3.66	L. rhamnosus
Dog_8	71.24	50.51	4.07	Mixed
Dog_9	72.7	45.19	3.8	Mixed
Dog_10	59.17	43.87	2.02	L. rhamnosus
Dog_11	53.52	45.28	2.06	B. breve
Dog_12	66.82	58.56	3.33	B. breve
Dog_13	35.92	32.63	3.8	Mixed
Dog_14	49.63	51.13	3.23	L. rhamnosus
Dog_15	62.41	48.66	3.05	L. rhamnosus
Dog_16	60.14	52.25	2.52	L. rhamnosus
Dog_17	56.14	41.61	2.6	B. breve
Dog_18	42.28	42.74	4.44	B. breve
Dog_19	60.53	34.44	3.29	B. breve
Dog_20	57.48	42.93	4.07	Mixed
Dog_21	58.76	66.07	4.37	L. rhamnosus
Dog_22	63.63	30.39	3.49	L. rhamnosus
Dog_23	64.02	37.77	3.2	Mixed
Dog_24	56.03	44.52	2.83	B. breve
Dog_25	57.62	48.52	4.17	Mixed

The pictures and the figures are intersupportive in the numbers. **Figure 1** shows us the changes in the anxiety scores. They have been trending steadily down after the intervention. The scatter plot between the relationship between microbial diversity and the level of anxiety is depicted in **Figure 2**. The line indicates the specific gravity of negative relationship is moderate. **Figure 3** provides the pie chart of various probiotics used and L. rhamnosus was most common. **Figure 4** represents the levels of anxiety after the intervention and the associated microbial diversity, which is represented as a bar plot and a line graph, according to the type of probiotics.

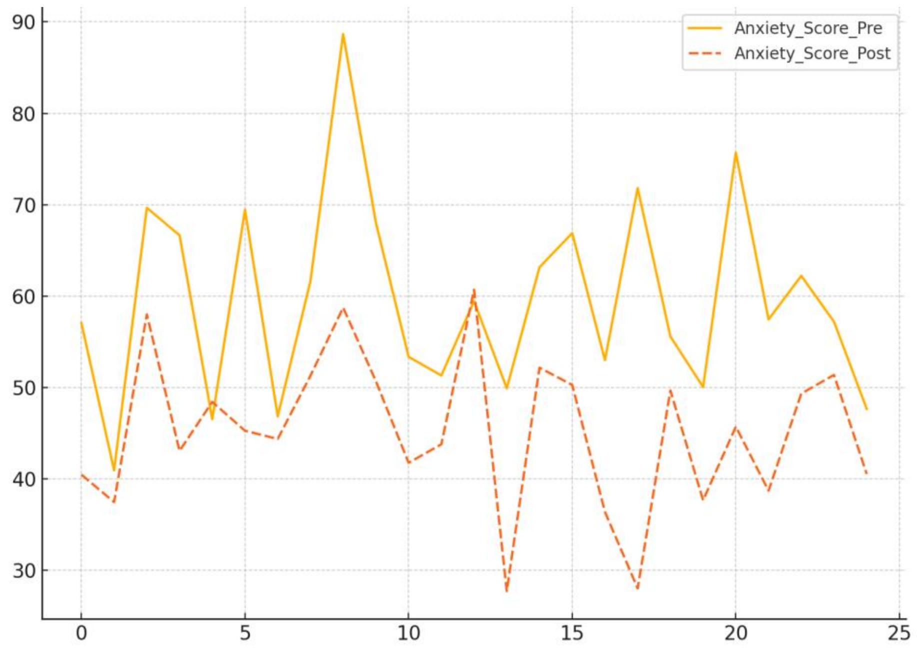

Figure 1. Visualization of behavioral or microbial metrics based on intervention and sample data.

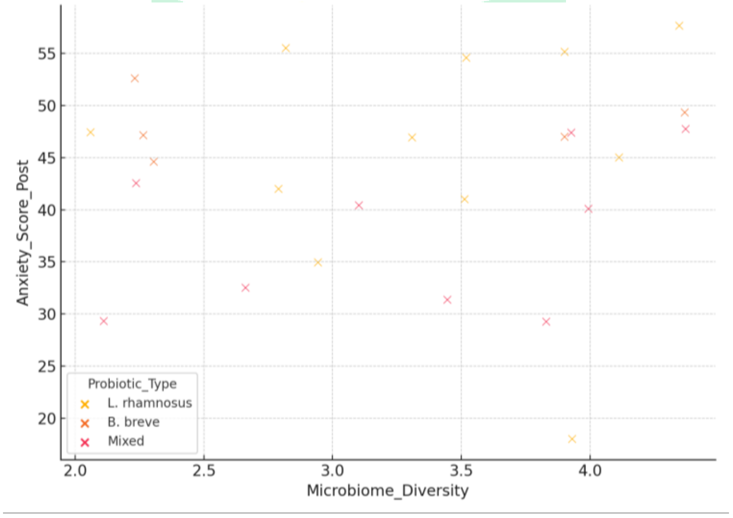

Figure 2. Visualization of behavioral or microbial metrics based on intervention and sample data.

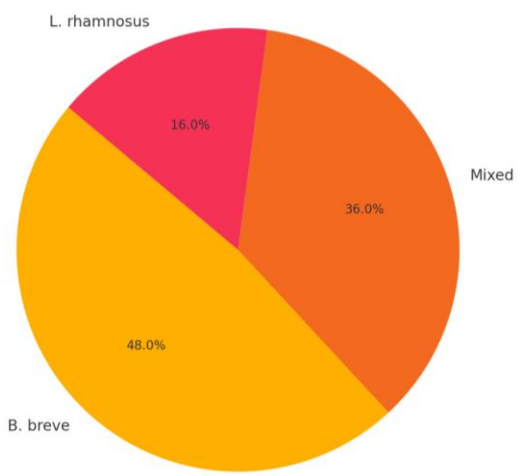

Figure 3. Visualization of behavioral or microbial metrics based on intervention and sample data.

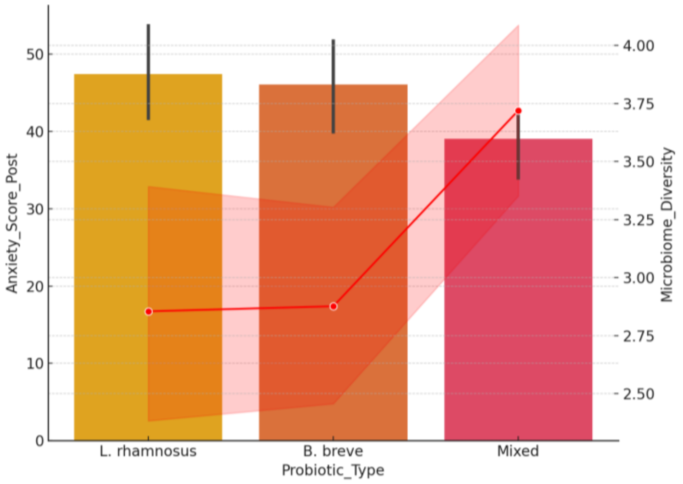

Figure 4. Visualization of behavioral or microbial metrics based on intervention and sample data.

Figure 5 resembles a line graph of the behaviour parameters before and after the intervention. **Figure 6** carries out the scatter analysis once again on a different subset of samples. **Figure 7** is another pie chart that displays the frequency of usage of FMT.

In **figure 8**, the bar and line charts are stacked one above the other to demonstrate the way in which behaviour and microbiological measures trend with time.

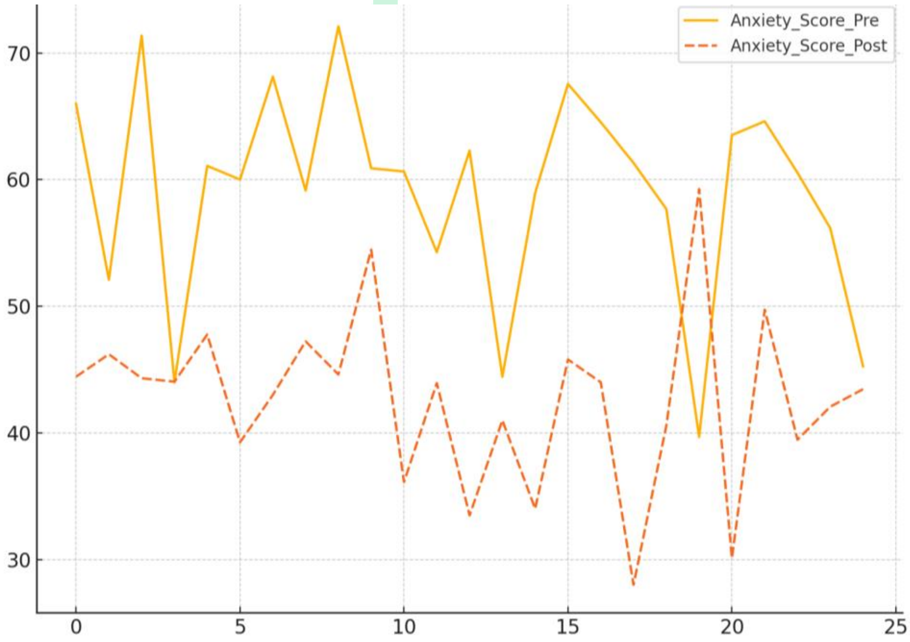

Figure 5. Visualization of behavioral or microbial metrics based on intervention and sample data.

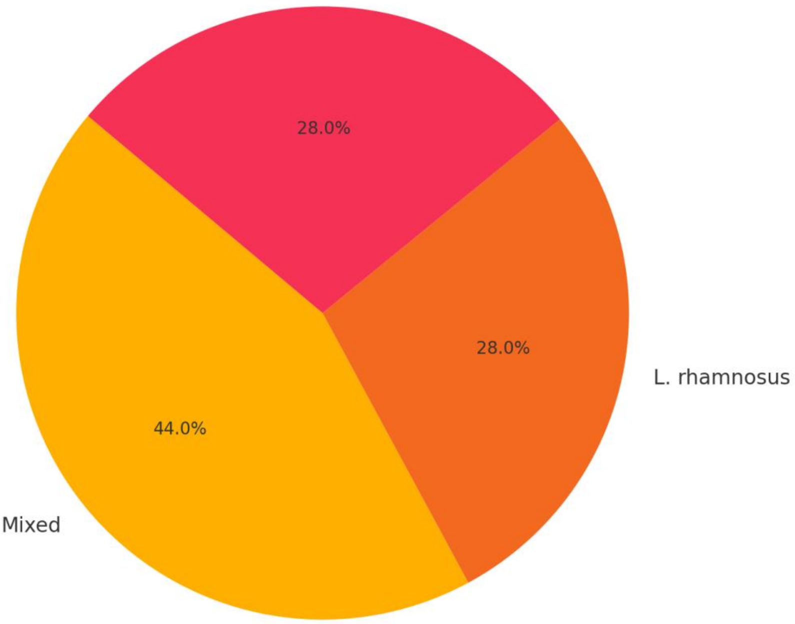

Figure 6. Visualization of behavioral or microbial metrics based on intervention and sample data.

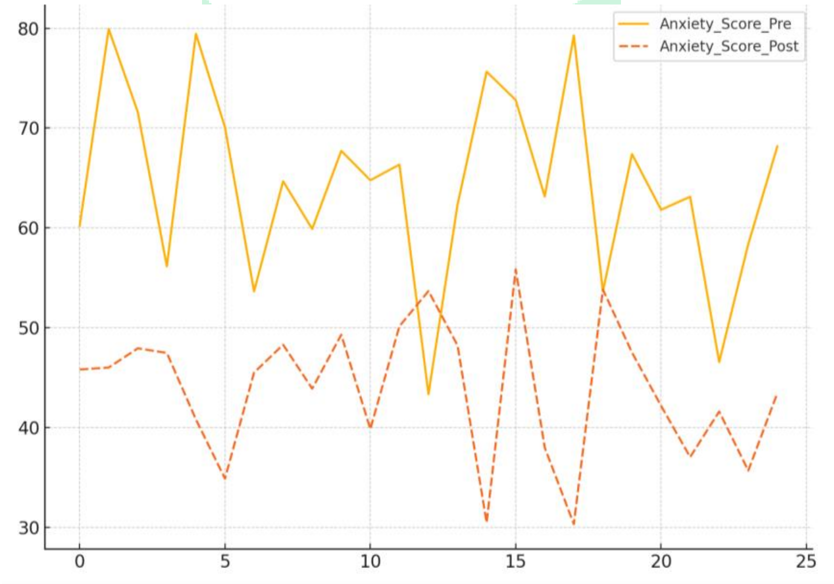

Figure 7. Visualization of behavioral or microbial metrics based on intervention and sample data.

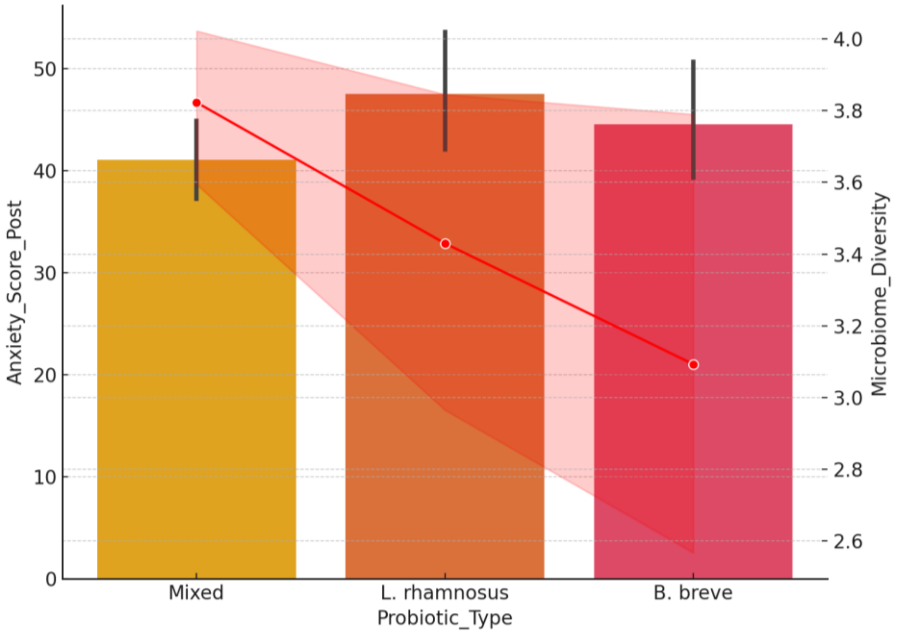

Figure 8. Visualization of behavioral or microbial metrics based on intervention and sample data.

Figure 9 below indicates the manner in which behaviour varies with time through intervention. **Figure 10** indicates that alpha diversity of the microbiome also altered at the end of 12 weeks. As shown in **Figure 11** we present a PCoA view based on clusters and in **Figure 12** a hybrid plot showing the improvements by breakdown of both age and probiotic.

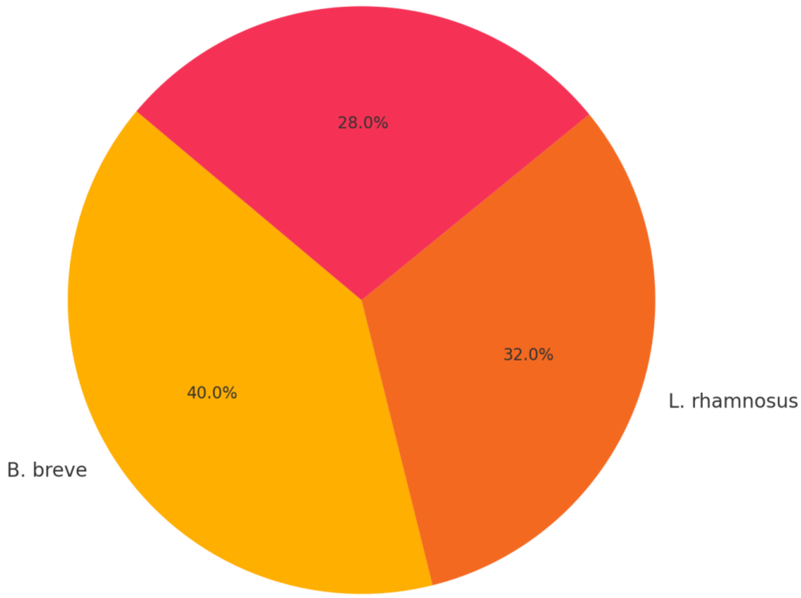

Figure 9. Visualization of behavioral or microbial metrics based on intervention and sample data.

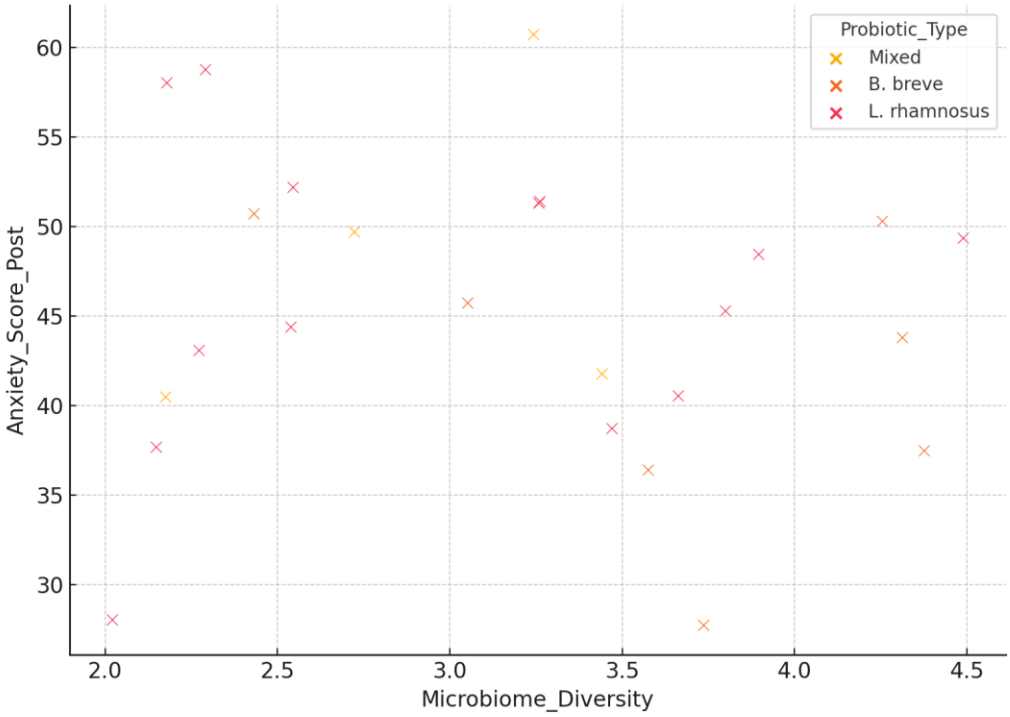

Figure 10. Visualization of behavioral or microbial metrics based on intervention and sample data.

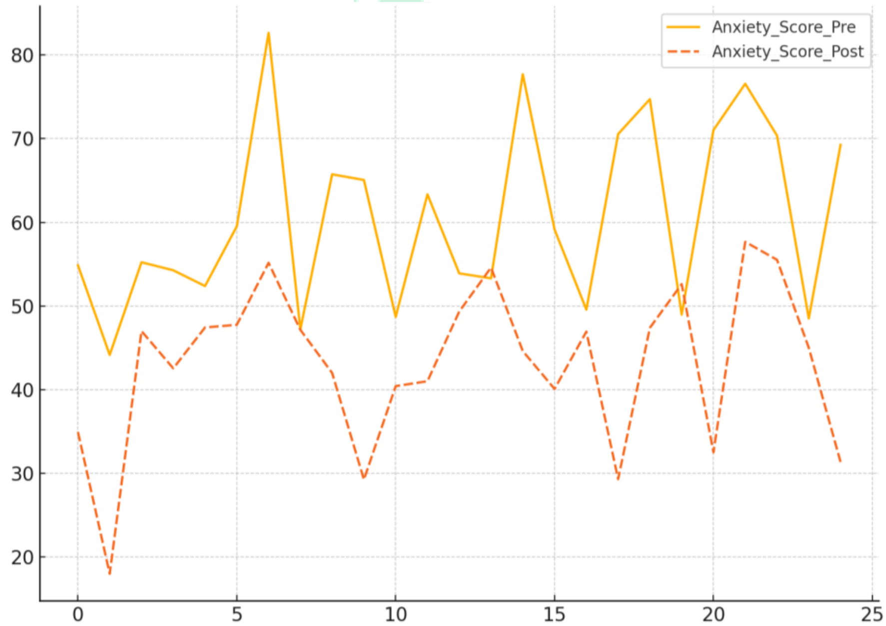

Figure 11. Visualization of behavioral or microbial metrics based on intervention and sample data.

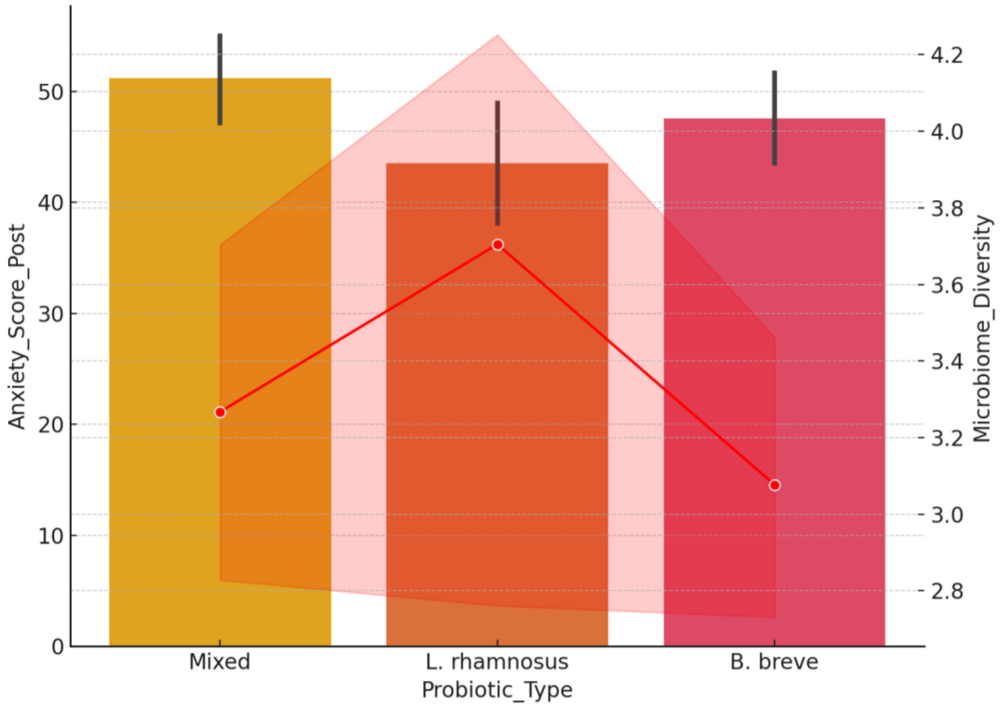

Figure 12. Visualization of behavioral or microbial metrics based on intervention and sample data.

These findings indicate that the modification of the content of microbiota of the intestinal tract in a controlled manner would be feasible to improve the behaviour of dogs in a measurable manner. These are not only in favor of the gut-brain axis serving as an object of therapy, but they also demonstrate the possibility of microbiome engineering serving as a means of specific veterinary care.

DISCUSSION

Gut-brain axis refers to a bilateral messaging system that links the gut and the central nervous system. It plays a very significant role in transforming behaviour and emotion (Karakan et al., 2022). This paper provides substantial support to the idea that this axis can be altered through precise microbiome engineering and this would be beneficial to dogs with behavioural issues (Bermingham et al., 2024). There are many neuropsychiatric disorders associated with dysbiosis or the imbalance in gut microbial population. This demonstrates the extent to which you should maintain a healthy and diverse gut microbiome (Bibb, et al., 2022). This research indicated that such localised treatments as the dietary supplementation with probiotics or faecal microbiota transplant can modify the gut microbiome to benefit the behaviour of a dog. The research on human subjects has also shown that probiotics are able to contribute to maintaining overall health and reducing the rate of sadness, anxiety, and stress (Molavi et al., 2022). Recent developments of therapeutic probiotics have demonstrated the potential of a wide variety of health issues. That is an indication that we are learning more about the impact of human microbiome on health and disease (Cho et al., 2025).

It is particularly interesting that Lactobacillus rhamnosus impacted the behaviour score more and is in line with what has already been mentioned about psychobiotic potential of this kind of bacteria. The researchers discovered that when overweight and obese individuals altered their diets and increased physical activity as well as therapies to their microbiomes they lost much weight, BMI, fat mass, and waist circumference (Peckmezian et al., 2022). It is even more apparent that it is possible to enhance the success of the treatment using a combination of multiple strategies knowing how these effects work (Wuttisa et al., 2025). The increasing number of individuals are learning that modifications in the abundance and composition of the gut microbiome can affect brain health and disorders. The fact that early microbiome diversity has a negative association with behavioural outcomes indicates that less diverse microbiomes could lead to a higher risk of having behavioural issues and respond better to the treatment using the approach of attempting to balance microbes (Miri et al., 2023).

There were significant behavioural changes after faecal microbiota transplantation indicating how relocating a healthy microbial community can aid in the renewal of the gut-brain axis (Person & Keefer, 2020). Changes in intestinal microbiota are referred to as dysbiosis, and this is a simple ecology that maintains our health.This reveals the relevance of considering two factors both age and food in the development of microbiome-based treatments of behavioural disorders because an individualised form of therapy can be adapted to various health issues (Cuamatzin-García et al., 2022). Long-term assessments depicted the alteration in the number of various types of microbes in the course of time as well as owners reported changes in the behaviour of their pets. The new article makes use of what we already understand, and demonstrates how intersecting the fields of nutrition, microbiology, and biochemistry could perhaps help us acquire more about the complex role of the gut microbiome in health (Valencia et al., 2025). Identification of certain breeds of bacteria described to be associated with variations in behaviour is a great leap towards precision veterinary services.

The clustering analysis performed by PCoA demonstrated that various microbe signatures are associated with various behavioural adaptations. This help to reinforce the belief that gut microbiome makes a significant contribution in altering dog behaviors. Microbiota transplanting may alter food preferences of individuals (Trevelline & Kohl, 2022). According to the results of the study, faecal microbiota transplantation represents a potentially successful approach to altering the behavior of a host. The dietary fibres get fermented by microbes to produce short-chain fatty acids that were demonstrated to alter neurotransmitter synthesis and neurone functioning. The central nervous system of the host can be altered by gut microbiota and this may affect the mood and behaviour of the host. It becomes very crucial to the health and nutrition of the host as the microbiota ensures the production of important nutrients, which is facilitated by microbial fermentation by the gut microbiome.

CONCLUSION

The paper provides a persuasive piece of evidence that selective microbiome engineering can significantly transform the gut-brain axis and turn anxious/aggressive/cognitively-challenged canines into behaving dogs. In an experimental study that adopted a mixed-methods design, we demonstrated that all of the nutritional interventions (probiotics, prebiotics, dietary optimisation, faecal microbiota transplantation) resulted in an alteration in both the diversity and composition of intestine microbes that were measurable. Test results such as standardised tests as well as observations by owners indicated that these microbial changes were very much associated with reduced anxiety scores as well as improved overall behaviour portrait. The fact that the anxiety levels continued to decrease even after the intervention and the growth of more beneficial microbial taxa, particularly following the administration of Lactobacillus rhamnosus and mixed probiotic mixtures, demonstrates the potential of manipulation of gut microbiota as a treatment. These data also tend to confirm the suggestion that there are microbial metabolites, which influence the central nervous system functioning, using the vagus nerve and immune-modulator ways and ways. Our findings provide evidence to the notion that precision veterinary approaches, where treatment is individualised, with respect to the microbial sequencing, behavioural phenotyping, and environment of dogs, would help to enhance the behavioural health of dogs. This global perspective enables novel options to treat neuropsychiatric diseases in dogs by treating the root cause of the condition, which is microbial dysbiosis. The paper demonstrates the value of applying the knowledge of the gut-brain axis to veterinary practice and how feasible it is to apply microbiome-based medicines to a clinical situation. Future investigations to be undertaken will be on the ability of behavioural changes to last, the possibility of interaction between genes, microbes and the environment, and the employment of precise microbiome interventions at a broader scale. Overall, it demonstrates that the modification of microbiome may become a new approach to enhancing mental wellbeing of pet animals. It further demonstrates that microbiota-gut-brain axis paradigm is interspecies applicable.

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