



Clinical Evidence Review

Understanding the link between the gastrointestinal microbiome and stress

KEY POINTS

- **A prebiotic blend of beet pulp and flaxseed reached the same beneficial faecal bacteria concentrations that are seen with commonly prescribed probiotic supplements.**
- **Milk protein hydrolysate, also known as alpha-casozepine, has been shown to decrease anxiety-related behavioural signs in dogs.**

INTRODUCTION

The gastrointestinal (GI) microbiome is the ecosystem consisting of microbiota (living bacteria, protozoa, viruses and fungi) that live symbiotically with the host's GI tract. This relationship has a direct effect on GI cells, the intestinal mucosal barrier, the host's immune system, and neurons.^{1,2} Probiotics are live microorganisms that provide health benefits to the host when administered in adequate amounts, whereas prebiotics are fermentable ingredients (e.g., soluble fibres) that provide health benefits to the host by changing the composition and/or activity of the GI microbiota.³ Both probiotics and prebiotics have been shown to have positive effects on GI health by altering the microbiome.³

The brain-gut-microbiome axis is the communication link between the central nervous system (CNS) and the GI tract.² Accumulating evidence suggests that exposure to stress is a risk factor in the pathogenesis of some GI diseases.² An inappropriate or abnormal stress

response, as seen in anxiety disorders, leads to disturbances in the hypothalamo-pituitary-adrenal axis (HPA). Changes in the GI tract include decreased gastric emptying, altered microbiome, and increased intestinal motility, permeability, and visceral sensitivity.² Resulting clinical signs may include vomiting, diarrhoea, and abdominal discomfort.

Milk protein hydrolysate, also called hydrolysed casein (which is formed by trypsin hydrolysis of milk protein) ingestion has been associated with significant alleviation of signs of stress in models of anxiety in dogs, cats, rodents, and humans.⁴⁻⁶ The exact mechanism of these anxiolytic effects is unknown but may be mediated through the gamma amino butyric acid (GABA)/benzodiazepine receptor complex.⁴ Since stress is postulated to play an important role in what has been described as the brain-gut-microbiome axis, reducing stress may help decrease adverse changes in the GI tract.²

STUDIES

The effects of commercially available probiotics and a prebiotic fibre blend on the gastrointestinal microflora of healthy dogs

STUDY OBJECTIVE

The purpose of this study was to determine if a prebiotic fibre blend containing beet pulp and flaxseed increases concentration of beneficial bacteria in the faeces of healthy dogs comparable to what is seen when probiotics FortiFlora™ and Prostora™ Max are administered.

STUDY DESIGN

Twenty adult Beagle dogs (10 females, 10 males, average age: 4 years) were fed a control food (Hill's™ Science Diet™ Adult Canine) for 4 weeks. Dogs were fed to maintain current body weight. At the beginning of this period, fresh faeces were collected for 4 days and a baseline composite sample was frozen for bacterial polymerase chain reaction (PCR) analysis. After the 4-week washout period, dogs were randomly assigned to one of 4 groups for a 2-week period in a Latin square crossover design. The groups consisted of control food, control food+FortiFlora™, control food+Prostora™ Max, and control

food+prebiotic fibre blend added to achieve a concentration of 2.5% beet pulp and 0.6% flaxseed on a dry matter basis (DMB). FortiFlora™ and Prostora™ Max were administered orally at the dose and frequency recommended by the manufacturers. Fresh faeces were collected during the last 4 days of the 2-week period for each group, and a composite sample was frozen for bacterial PCR. Faeces were analysed using bacterial PCR by the University of Illinois Animal Science Laboratory. Results were considered significant when $P < 0.05$.

RESULTS

The prebiotic fibre blend group had a significant increase in faecal *Bifidobacterium* and *Lactobacillus* concentrations, measured in colony-forming unit (CFU) log 10/grams of faeces, at 2 weeks compared to baseline ($P = 0.03$ and 0.04 , respectively) (Figure 1). Results of mean faecal *Bifidobacterium* and *Lactobacillus* concentrations for each group are shown in (Table 1).

CONCLUSION & CLINICAL IMPORTANCE

The tested prebiotic fibre blend of beet pulp and flaxseed resulted in comparable amounts of beneficial faecal bacteria concentrations to that seen with commonly used probiotic supplements.

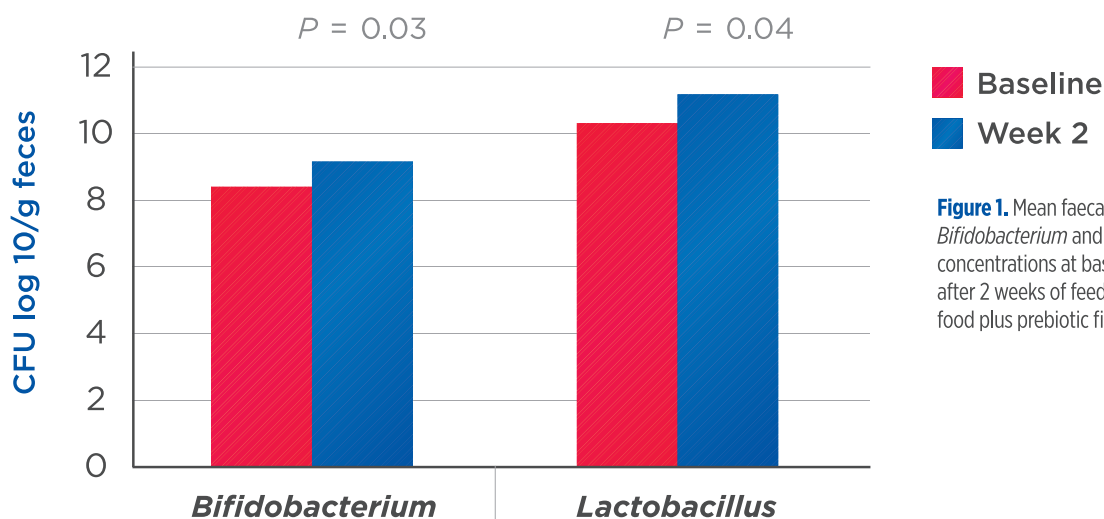


Figure 1. Mean faecal *Bifidobacterium* and *Lactobacillus* concentrations at baseline and after 2 weeks of feeding the control food plus prebiotic fibre blend.

Group	Faecal <i>Bifidobacterium</i> CFU log 10/g faeces			Fecal <i>Lactobacillus</i> CFU log 10/g faeces		
	Baseline	2 Weeks	P-Value	Baseline	2 Weeks	P-Value
Prebiotic fibre blend	8.3	9.1	0.03	10.3	11.1	0.04
FortiFlora™	8.3	9.3	< 0.01	10.3	11.3	0.01
Prostora™ Max	8.3	9.6	< 0.01	10.3	11.5	< 0.01
Control	8.4	8.5	0.58	10.4	10.5	0.67

Table 1. Mean faecal *Bifidobacterium* and *Lactobacillus* concentrations for control food+prebiotic fibre blend, control food+FortiFlora™, control food+Prostora™ Max, and control food groups at baseline and after 2 weeks of treatment. P-values represent the difference between baseline and 2 weeks.

Effects of alpha-casozepine (Zylkene) versus selegiline hydrochloride (Selgian, Anipryl) on anxiety disorders in dogs⁵

STUDY OBJECTIVE

The purpose of this study was to determine if the natural biological agent alpha-casozepine (milk protein hydrolysate) has the same anxiolytic effect as selegiline, a monoamine oxidase inhibitor (MAOI) used for the treatment of anxiety disorders and cognitive dysfunction in dogs.

STUDY DESIGN

This study was a multicentre, randomised, blinded, comparative trial. Dogs were evaluated for anxiety by using a validated dog emotional scale (Emotional Disorder Evaluation for Dogs or EDED). Dogs diagnosed with an anxiety-related behaviour that had persisted for > 4 weeks and which had a total score of > 19 out of 45 points on the EDED scale were included in the study. Owners subjectively evaluated and scored their dog's behaviour throughout the study on a scale of -10 to 10.

Dogs were randomly assigned to either the alpha-casozepine (test) or selegiline group. The test group received 15mg/kg body weight (BW) of alpha-casozepine orally once a day, while the selegiline group received 0.5mg/kg BW orally once a day. Dogs in both groups were also provided behavioral modification. Each dog was evaluated 5 times during the 56-day study. Evaluations were performed initially and at weeks 4 and 8 in the hospital; telephone evaluations occurred at weeks 2 and 6. Two separate categories were tracked at each evaluation: EDED score and owner's subjective evaluation of change. Positive results were defined as an EDED score < 20 and owner's subjective evaluation score ≥ 6/10. Successful treatment had to meet both criteria.

RESULTS

Thirty-eight dogs (19 female, 19 male) completed the study. Overall, there were 19 successes: 10 dogs in the alpha-casozepine group and 9 dogs in the selegiline group. No significant difference existed between the two groups; alpha-casozepine & selegiline were equally effective in treating anxiety based on both EDED scores and owners' evaluations. There was a significant ($P < 0.0001$) decrease in average EDED score between the initial visit and week 8 for both treatment groups (**Figure 2**). Evaluators were unable to determine treatment success until at least the 6-week evaluation; however, a trend did show improvement in EDED score starting as early as day 15.

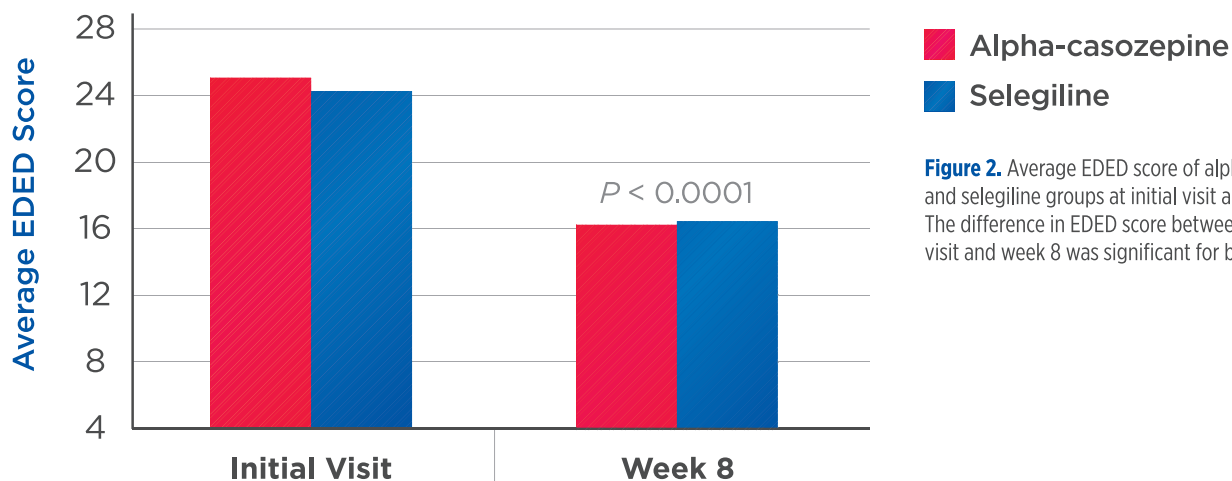


Figure 2. Average EDED score of alpha-casozepine and selegiline groups at initial visit and week 8. The difference in EDED score between initial visit and week 8 was significant for both groups.

CONCLUSION & CLINICAL IMPORTANCE

Alpha-casozepine was equally effective in controlling anxiety as selegiline, an MAOI marketed for the treatment of anxiety disorders and cognitive dysfunction in dogs. This study provides evidence for the efficacy of alpha-casozepine in the management of dogs exhibiting behaviour attributable to anxiety. Further, there were no reported side effects in dogs receiving alpha-casozepine.

SCIENTIFIC INSIGHTS SUMMARY

Stress is postulated to play an important role in the brain-gut-microbiome axis, and stress reduction is recommended as a key component of multimodal management for dogs that are experiencing stress or anxiety. Highly digestible foods containing prebiotic fibre and a stress-alleviating ingredient, such as Hill's™ Prescription Diet™ i/d™ Stress, may improve abnormalities in the brain-gut-microbiome axis and help manage dogs with stress-related gastrointestinal disorders.

REFERENCES

- ¹ Honneffer JB, Minamoto Y, Suchodolski JS. Microbiota alterations in acute and chronic gastrointestinal inflammation of cats and dogs. *World Journal of Gastroenterology* 2014;20(44):16489-16497.
- ² Konturek PC, Brzozowski T, Konturek SJ. Stress and the gut: pathophysiology, clinical consequences, diagnostic approach and treatment options. *J Physiology and Pharmacology* 2011;62(6):591-599.
- ³ Gibson GR and Roberfroid M, editors. *Handbook of Prebiotics*. Boca Raton, FL: Taylor & Francis Group, 2008;1-22.
- ⁴ Miclo L, Perrin E, Driou A, *et al*. Characterization of alpha-casozepine, a tryptic peptide from bovine alpha-s1-casein with benzodiazepine-like activity. *FASEB J*. 2001;15(10):1780-1782.
- ⁵ Beata C, Beaumont-Graff E, Diaz C, *et al*. Effects of alpha-casozepine (Zylkene) versus selegiline hydrochloride (Selgian, Anipryl) on anxiety disorders in dogs. *J Vet Behavior*. 2007;2(5):175-183.
- ⁶ Beata C, Beaumont-Graff E, Coll V, *et al*. Effect of alpha-casozepine (Zylkene) on anxiety in cats. *J Vet Behavior*. 2007;2(2):40-46.

