

Effect of *Enterococcus faecium* SF68® (FortiFlora®) administration in dogs with antibiotic responsive or small intestinal bacterial overgrowth diarrhoea



> Abstract

The objective of this study was to assess whether dogs suffering from small intestinal diarrhoea due to small intestinal bacterial overgrowth or antibiotic responsive diarrhoea would benefit from a combination of Purina Veterinary Diets® EN Gastroenteric® Canine Formula and Purina Veterinary Diets® FortiFlora® Canine Probiotic Nutritional Supplement (*Enterococcus faecium* SF68®). The study involved twenty-six adult dogs presenting with symptoms compatible with chronic small intestinal diarrhoea that could not be attributed to any specific cause. The dogs were randomly divided into two groups: A (EN Gastroenteric plus FortiFlora sachet) and B (EN Gastroenteric plus placebo sachet). Reassessment of symptoms was scheduled every seven days over a 20-day period. Faeces were characterised based on their consistency. Biochemical and haematological parameters of all dogs participating in the study were within normal limits throughout the entire study period. During the first week of the study, no statistically significant differences were found between groups A and B in the characteristics of the diarrhoea. After a 14-day period, comparisons between groups showed that there was a statistically significant difference ($p = 0.0002$) regarding resolution of diarrhoea in group A. During the third week of administration, group A maintained the positive outcome ($p = 0.0001$).

> Introduction

The aetiology of small intestine diarrheal syndromes in dogs may remain unclear, leading the clinician to provide symptomatic treatment. A common clinical entity in dogs is small intestinal bacterial overgrowth (SIBO), with chronic diarrhoea being the sole symptom in some cases.¹ A syndrome with analogous clinical manifestation is antibiotic responsive diarrhoea (ARD). To date, there are no diagnostic techniques that can provide an aetiological diagnosis for either of the above clinical entities.²

SIBO is defined as the abnormal increase of bacteria in the lumen of the duodenum and especially in the jejunum.^{3,4} A number of 10^4 - 10^5 bacteria and 10^8 per ml of normal small intestinal contents are found in the dog and cat respectively. Bacterial overgrowth is characterised by both an increase in the number of normal microflora and a similar increase of bacteria not found in healthy subjects. The latter has been associated with other diseases.¹ Conversely, an increase in the number of bacteria with no implication of pathogenic disease is characterised as idiopathic, primary or ARD. The latter occurs mostly in large breed dogs, especially in German Shepherds.² Affected dogs present diarrhoea, weight loss, flatulence and anorexia as a result of Vitamin B₁₂ deficiency. Diarrhoea is usually characterised by pasty texture, bad odour and the presence of excess fat in the faeces (steatorrhea). Dogs may periodically vomit.¹ The appetite remains normal or increased, while some animals may present signs of pica or coprophagy.

In cases where clinical examination reveals no other indications as to the origin of diarrhoea (such as weakness or very marked weight loss, pica and/or coprophagy, poor hair

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Keywords



- Antibiotic responsive diarrhoea
- Diarrhoea
- Small intestinal bacterial overgrowth



coat condition), and the results of biochemical and haematological examination shows no evidence of a serious disorder, the clinician often prescribes symptomatic treatment and dietary modification.

> Materials and methods

The current study included all suspected cases of chronic diarrhoea due to SIBO (particularly common in German Shepherds and other large breed dogs)² or ARD to participate in a nutritional double-blind placebo-controlled clinical trial investigating the outcome of the combination of a highly digestible diet formulated for intestinal disorders (Purina Veterinary Diets® EN Gastroenteric® Canine Formula, Purina) and the probiotic *Enterococcus faecium* SF68® (Purina Veterinary Diets® FortiFlora® Canine Probiotic Nutritional Supplement, Purina) proven efficacious in dogs suffering from diarrhoea. All dogs were enrolled after obtaining their owners' written consent.

Dogs with diarrhoea attributed to infectious diseases

(parvovirus, infectious hepatitis, distemper), protozoa, bacteria or parasites (*Giardia*, *Cryptosporidium*), neoplasms of the small bowel, food allergy or intolerance to food ingredients were excluded from the study, as well as dogs with abnormal biochemical or haematological (PCV, WBCs, RBCs, PLTs, white cells type) profile analysis, faecal examination (plain and after flotation technique) and urinalysis.

Twenty-six adult dogs were included in the study all of whom were presented to the Companion Animal Clinic of the School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki displaying symp-

toms compatible with chronic small intestinal diarrhoea at least 15 days prior to admission. Owners had habitually fed their dogs with either a commercial dry dog food or a combination of dry food and homemade meals. Signalment of all dogs enrolled in the study is presented in Table 1.

All dogs with clinical findings compatible with SIBO and ARD were included in the study, and were allocated to group A or B. Group allocation was random, and the clinician responsible for the clinical evaluation was unaware of the dog's group throughout the whole study period.

Dogs in group A were fed Purina Veterinary Diets® EN Gastroenteric® Canine Formula (dry) according to their daily needs, divided into two meals of equal quantity, plus a Purina Veterinary Diets® FortiFlora® Canine Probiotic Nutritional Supplement (*Enterococcus faecium* SF68®) sachet for a 20-day period. FortiFlora® ingredients include meat and animal derivatives, yeasts, and minerals plus gut flora stabilisers: *Enterococcus faecium* SF68® (E1705): 1x10¹² CFU/kg and trace elements (Fe, I, Cu, Mn, Zn, and Se); its key nutrient values are 50% protein, 11% fat, 9% crude ash, and 1% crude fibres.

Dogs in group B were fed with the same clinical diet as group A and a placebo sachet for a 20-day period. The placebo sachet contained the same ingredients as FortiFlora® mix without *Enterococcus faecium* SF68®.

Reassessment of symptoms was scheduled for every seven days over a 20-day period and included a complete history, physical examination, body weight assessment, faecal scoring, biochemical (albumin, glucose, BUN, Crea, ALP, ALT, Ca, P, K, Na) and haematological (PCV, WBCs, RBCs, PLTs, white cells type) profile analysis, faecal examination (plain and after flotation technique) and urinalysis.

Prior to enrolment, all dogs underwent a serology test (Snap Giardia test, IDEXX Laboratories) and faecal examination for *Giardia spp.* and cytology smears prepared by swab samples taken from the rectal mucosal surface for the detection of *Cryptosporidium*.

Faeces were characterised based on their consistency according to the Nestlé Purina Faecal Scoring System, modified by the authors for the purposes of the study.⁵

- Normal: (fully-formed, segmented appearance, firm)
 - Paste +: (moderate water content, mild and pasty with log shape, loses shape when picked up)
 - Paste + +: (high water content, pasty appearance, has texture but no defined shape)
 - Watery: (very high water content, no texture, flat)
- Any dog that presented with faecal scoring paste + or more was considered to have diarrhoea.

Table 1. Signalement of the 26 dogs that participated in the study

α/α	Group	Breed	Gender	Age (years)	Body weight (kg)
1	A	Irish setter	♂	1	12
2		Mongrel	♂	1	5
3		Mongrel	♂	3	5,4
4		Malinois	♂	2	23,7
5		Yorkshire terrier	♀	6,5	3,9
6		Siberian husky	♀	15	26,5
7		Great Dane	♀	1	29
8		German shepherd dog	♂	3,5	27,5
9		Poodle	♀	4	10
10		Mongrel	♀	5	17,5
11		English bulldog	♀	2	19
12		Mongrel	♂	3	21
13		Hellenic shepherd dog	♀	1	39
14		German shepherd dog	♂	2	21
15		German shepherd dog	♀	3	19
16		Maltese	♂	1	7
17	B	Mongrel	♂	1	12,5
18		English bulldog	♂	3	19
19		Mongrel	♀	2	8
20		Epagneul Breton	♀	7	21
21		English pointer	♀	5	27
22		Mongrel	♀	4	22
23		Siberian husky	♀	2	17
24		Mongrel	♂	1	12
25		Mongrel	♂	3	13
26		Poodle	♀	2	9

**Table 2.** Faecal scoring from the 26 dogs of groups A and B that participated in the 20-day period study

	Day 0		Day 7		Day 14		Day 20	
	Group A (n=16)	Group B (n=10)	Group A (n=16)	Group B (n=10)	Group A (n=16)	Group B (n=10)	Group A (n=16)	Group B (n=10)
Normal	0	0	6	0	12	0	16	1
Paste +	2	4	8	4	4	4	0	3
Paste ++	10	4	2	6	0	6	0	6
Watery	4	2	0	0	0	0	0	0

Comparisons of the clinical parameters between groups A and B were performed with Fisher's exact test using the statistical package SPSS 17.0. The level of significance was set at 5% ($p < 0.05$).

> Results

Biochemical and haematological parameters of all dogs participating in the study were within normal limits, throughout the entire study period. Serology, faecal examination and cytology from the rectum for *Giardia spp.* and *Cryptosporidium*, respectively, were negative in all dogs.

Dogs in both groups were adults of various breeds (Table 1) that presented with small intestinal diarrhoea (frequency, quantity per voiding, odour, and consistency). None of the animals in both groups presented with vomiting, fever or melena. The duration of diarrhoea ranged from 7 to 20 days. At admission, the owners of all dogs were willing to proceed with symptomatic treatment and dietary measures before moving on to a more sophisticated laboratory investigation of the chronic diarrhoea. This further investigation is necessary in cases of recurrence. The dogs were randomly divided into two groups (clinical symptoms and laboratory evaluations were identical for all dogs participating in the study).

During the first week of the study, no statistically significant differences were found between groups A and B in terms of the characteristics of the diarrhoea. However, in 6/16 dogs in group A, the diarrhoea completely resolved, whereas none of the animals in group B showed any improvement in this regard. Comparisons between groups A and B showed that there was a statistically significant difference ($p = 0.0002$) in the resolution of diarrhoea (after a 14-day period) in group A whose diet was supplemented with FortiFlora® in contrast to group B who received the placebo sachet. During the third week of administration, group A maintained the positive outcome achieved during the second week of the study ($p = 0.0001$). Faecal scores over time for all dogs in both groups are presented in Table 2.

The cause of small intestinal chronic diarrhoea in the dog, despite a thorough clinical and laboratory examination, may remain unclear. In this study, dogs

with chronic small intestinal diarrhoea and primary aetiology suspected as SIBO or ARD were entered into a placebo-controlled clinical trial to determine whether symptomatic relief could be achieved by feeding them a highly digestible diet indicated for intestinal disorders (Purina Veterinary Diets® EN Gastroenteric® Canine Formula) in combination with the probiotic *Enterococcus faecium* SF68® (FortiFlora®).

The results of biochemical and haematological evaluation of both groups of animals were within normal limits.

> Discussion

Regarding the aetiology of diarrhoea, only assumptions can be made. It is known that large breed dogs, and especially German Shepherds, may suffer from chronic diarrhoea as a consequence of a clinical entity characterised as ARD.^{6,7} In this clinical study, there were 6/16 large breed dogs (3 German Shepherds, 1 Hellenic shepherd, 1 Malinois, and 1 Great Dane) in group A. For the rest of the dogs in groups A and B, it can be alleged that although the primary cause was not detected at this stage, they suffered from SIBO. Progress of the relevant cases showed that bacterial overgrowth did not seem to be associated with severe intestinal diseases (e.g. inflammatory bowel disease, villous atrophy, intestinal lymphoma, lymphangiectasia, etc.) or exocrine pancreatic insufficiency.

The combined administration of a clinical diet specifically designed for intestinal disorders (Purina Veterinary Diets® EN Gastroenteric®) offers high digestibility, which promotes high nutrient absorption and helps minimise the dietary load on the compromised gut; it also contains the prebiotic inulin, which improves microbial balance by stimulating the growth of beneficial bacteria, and the probiotic *Enterococcus faecium* SF68® (FortiFlora®, Purina). This diet is proven to promote intestinal health and balance, and was effective as symptomatic treatment in helping to relieve chronic diarrhoea in the dogs in group A.

Purina Veterinary Diets® EN Gastroenteric® is a highly digestible diet which contains the proven prebiotic inulin (shown to improve microbial balance by stimulating the growth of beneficial bacteria).



Probiotics (like SF68®) have been proven to be safe and effective in regulating microbiota balance, gastrointestinal and systemic immune functioning in healthy cats and dogs.^{7,8,9,10} *Enterococcus faecium* SF68® (NCIMB10415) is a probiotic with proven effectiveness against diarrhoea.^{11,12,13} SF68® has a shorter replication time, is more stable at acidic pH, and has a more inhibitory action against pathogenic *E.coli* growth *in vitro* compared to the probiotic *Lactobacillus acidophilus*. In addition, SF68® was found to be safe and well-tolerated at 500 times its therapeutic dose in pigs. Although the composition of Purina Veterinary Diets® EN Gastroenteric® is appropriate for the nutritional management of disorders of the gastrointestinal tract, our results suggest it may act complementary to FortiFlora® with excellent results. According to our results, and taking into account that 6/16 dogs in group A were large breed dogs that are more prone to microflora imbalances, FortiFlora® could help to resolve diarrhoea quicker. As mentioned, inulin is the prebiotic source contained in EN Gastroenteric®. Previous studies have demonstrated that inulin and *Enterococcus faecium* SF® display complementary characteristics¹⁴ and the combination of inulin with *Enterococcus faecium* SF® improves the survival of the latter in the intestinal tract.¹⁵

The immune system plays an important role in preventing the adherence of inappropriate bacteria to enterocytes, with secretory IgA playing a par-

ticularly significant role.¹⁶ In a previous study with dogs supplemented with the probiotic *Enterococcus faecium* (SF68®), faecal IgA levels were shown to increase.¹⁷ Given that the dogs entered in this study were likely to have been suffering from SIBO, the positive effects of FortiFlora® on the resolution of diarrhoea may have been mediated by a modulation of gut immunological responses. The lack of effect of Purina Veterinary Diets® EN Gastroenteric® alone in this particular study may relate to the specific condition of SIBO; and our results indicate that a highly digestible gastrointestinal diet in combination with a proven probiotic such as FortiFlora® (SF68®) could be considered for nutritional management of this disorder.

FortiFlora® contains guaranteed levels of viable microorganisms (minimum 1x10⁸ CFU/g SF68®), a level proven to promote intestinal health and balance.¹⁵ According to our experience and that of other authors,¹⁸ the minimum daily per os dose should be at least 1x10⁸ CFU/unit.

Duration of usage may run into months for chronic diarrhea due to the presence inflammatory bowel disease.¹⁰

According to the results of our study, probiotics may act synergistically to a clinical diet specific for gastrointestinal disorders and help alleviate diarrhoea which is not associated with severe enteropathy or exocrine pancreatic insufficiency.

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