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How Does *Saccharomyces cerevisiae* DSM 34246 var. *boulardii* Supplementation Impact the Well-Being of Healthy Adult Dogs?

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Simple Summary: A study tested *Saccharomyces cerevisiae* DSM 34246 var. boulardii on adult dogs (West Highland White Terrier (WT) and German Shepherd (GS) to evaluate its impact on gut health. A total of 53 healthy adult dogs, were randomly assigned to a control (CTR: WT 14/28, GS 12/25) and treated (SACC: WT 14/28, GS 13/25) group. Both were fed a dry diet twice daily, with the additive given to the SACC group (5 x 10^9 CFU/kg) and a placebo to the CTR. Over 35 days, body weight, condition scores, fecal parameters, and water intake were measured. Statistical analysis showed significant improvements in body condition, fecal parameters, and IgA, indicating *S. boulardii* positively affected gut health in dogs.

Abstract: Background: a supplement containing *Saccharomyces cerevisiae DSM 34246 (Canobios-BL) var. boulardii* was tested to demonstrate its efficacy as a gut flora stabilizer in two breed of dogs, West Highland White Terrier (WT) and German Shepherd (GS). Methods: a total of 53 healthy adult dogs, 28 WT and 25 GS, were included in the study. The animals were randomly assigned to a control group (CTR: WT 14/28, GS 12/25) and to a treated group (SACC; WT 14/28, GS 13/25). The CTR Group was fed with dry commercial food, while the SACC one was fed with the same food but supplemented with the feed additive *S. boulardii* at the concentration of 5 X 10^9 CFU/kg. The study lasted 35 days and included six evaluation time points (T_0-T_5). Body Weight (BW), Body Condition Score (BCS), Fecal Score (FS), FS measured with a penetrometer (FSp), Fecal Dry matter (DM), Fecal Humidity (UM) and Fecal IgA (IgA) were measured at each time point. Results: a significant improvement ($p<0.05$) in BCS, FS, FSp, MCS, DM, UM and IgA of the treated group with respect to the control one for both WT and GS were reported. Conclusions: the positive effect of *S. boulardii* supplementation on the improvement of the fecal parameters and on the maintenance of good physiological as well as biological conditions in healthy adult dogs in breeding conditions.

Keywords: pet; probiotic; feed supplements; yeast product

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1. Introduction

The gastrointestinal (GI) tract is populated by a variety of microbes, which were recently demonstrated to play a major role in both human and animal health [1]. In fact, "the healthy gut" is overall linked to the well-being of the host not only because it is a vital organ for digestion and absorption of nutrients, but also as it is involved in the defence against pathogens and in the maintenance of the immune system [2,3].

The gut microbiota is essential for maintaining the homeostasis of the host especially when there is a microbes' unbalance in the gastrointestinal (GI) tract, possibly leading to dysbiosis [1,3]. Dysbiosis has been found associated with intestinal and non-intestinal diseases [1], but was also observed in healthy adult dogs living in stressed conditions [3]. In this respect, the use of supplements for maintaining the gut health is increasing also in veterinary medicine, as their administration resulted effective and associated with limited side effects [4]. For example, probiotics - intended as live organisms able to provide health benefits to the host [3,4] are largely used to maintain GI health and mitigate stress-dependent dysbiosis in animals [5]. Specifically, they support the barrier function of intestinal tissues and its regeneration [3,6].

Several bacteria have been recently identified as probiotics [7] and, in particular, the beneficial effects related to the supplementation of *Saccharomyces cerevisiae var. boulardii* in both animals [3,8–10] and humans [11,12] have been preliminary reported. Indeed, administration of *S. boulardii* in humans affected by GI diseases, such as chronic diarrhoea and inflammatory bowel disease (IBD), has demonstrated clinical effectiveness, indicating that such a probiotic might positively interfere into inflammatory conditions [4,11]. In this respect, the *S. boulardii* probiotic activity in humans has been linked with multiple pathways, such as improvement of gut barrier function, pathogen competitive exclusion, production of antimicrobial peptides, immune modulation and trophic effects on the gut [4,13]. Similarly, *S. boulardii* started to be preliminarily used as probiotic also in pets [2,3,14]. In particular, one study showed an improvement in the intestinal status and general health conditions of dogs suffering from chronic enteropathies when treated for 60 days with *S. boulardii* [14]. In addition, a different study involving healthy dogs in breeding conditions reported promising outcomes regarding the intestinal microbiota and the nutritional as well as the stress status resulting from the supplementation of *S. boulardii* for 42 days [3].

Based on the above-mentioned considerations, the aim of this work was to demonstrate the efficacy of the feed additive *S. cerevisiae DSM 34246 (Canobios-BL) var. boulardii* as "gut flora stabilizer" under eubiosis conditions in healthy dogs. Nutritional parameters, *i.e.* Body Weight (BW) and Body Condition Score (BCS), and fecal quality parameters, *i.e.* Fecal Score (FS), Fecal Score measured with a penetrometer (FSp), Fecal Dry matter (DM), Fecal Humidity (UM) and Fecal IgA (IgA), were selected as indicators of the gut health and used to assess effectiveness in stabilizing gut flora.

2. Materials and Methods

2.1. Animals and Study Design

This randomized controlled trial included a group of 53 adult dogs (age from 1 to 5 yr) were included in the study. In details, 28 dogs were West Highland White Terrier (WT) (10 males, 18 females), and the other 25 were German Shepherd (GS) (13 males and 12 females), selected from two ENCI-registered breeders in Italy.

Animals were randomly assigned to Control (CTR: WT 14/28, GS 12/25) and Treated (SACC: WT 14/28, GS 13/25) groups.

Both groups were fed with a commercial complete pet food for adult dogs (Monge Natural Superpremium Grain Free all breeds Adult Anchovies with Potatoes and Peas) two times a day, from at least 7 days before the beginning of the study.

The amount of daily food was calculated based on the following equation (NRC, 2006): ME (kcal/day) = 110 × (kg BW)$^{0.75}$

A placebo (Maltodextrin powder) or a supplement containing *S. cerevisiae DSM 34246 (Canobios-BL) var. boulardii* (5,0 x 10^9 CFU/kg) [15] was added to the pet food given to the CTR or to the SACC

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groups, respectively, once a day for the whole duration of the study (35 consecutive days). The study was divided in five experimental times (from T_0 to T_5), having each time point separated from the previous and/or the following one by a seven days interval.

At the beginning, the veterinarian checked the health status of the animals through a general physical examination and a copromicroscopic evaluation of their feces. All the recruited animals were healthy with no underlined conditions. In case of change of health status, pregnancy, pharmacological treatments, diet modification, pathological symptoms and/or death, the dogs were excluded from the study. All dogs included in the trial were kept in boxes (2/3 per box). The box area was 6 (±2) square meters in size, with an open space of the same size, considering the principles of animal welfare, thus avoiding any social stress due to manipulation. The breeders were informed of the design of the study and signed a written informed consent form. The experimental procedures used were approved by the Bioethics Committee of the University of Turin, Italy (approval 156895 ,14th April 2020).

2.2. Data Collection

All the tests aimed at evaluating the nutritional status of the dogs were performed by the same veterinarian, following standard guidelines [16]. The BW (kg) was recorded at T_0 (day 0), T_1 (day 7), T_2 (day 14) and T_3 (day 21), T_4 (day 28) and T_5 (day 35). The BCS [17] was recorded in scores, ranging between 1 and 9, having the points assigned after visual examination and palpation of the animal at T_0 and T_5. A score of 4 or 5 represents the ideal one [17] (WSAVA 2013). Four faecal parameters FS (1-7), FSp (1-7), DM (%), UM (%) and IgA (mg/g), were evaluated at each time point considered.

In particular, FS was determined by direct examination of fresh faeces using a 7 points scale [18,19].

The measurement of fecal hardness in kg/cm^2, was performed on fresh stool with a Penetrometer 53220 FTA (GUSS Manufacturing, PTY Ltd, South Africa) following a technique already described by Davies and colleagues (Davies et al. 1986). The amount of stool used for each analysis was at least 40-50 grams depending on the type and shape of stool itself. The fecal hardness was defined as the mean of three measurements. Fecal hardness was converted using a validated scale to obtain the FSp.

For the DM evaluation, a 1-20 g stool sample was weighted. The sample was dried in an oven at 108 ± 2°C for 24 hours and then immediately re-weighted to determine the percentage of dry matter.

For the assessment of the UM, a 5-10 g stool sample was weighed, dried in an oven at 105-110° C for 20-24 h and cooled down into a desiccator for 20-24 hours. Then, the dried sample was weighed again. The final UM result was defined as the mean of two different measurements and expressed as percentage.

Lastly, the IgA level was measured on fecal samples using a specific ELISA kit (Dog IgA ELISA Quantitation Set, Bethyl Laboratories Inc., Montgomery, TX, USA).

2.3. Statistical Analysis

The statistical analysis conducted in the study varied depending on the type of parameter considered (*e.g.* categorical or numerical). More into detail, BW, FSp, IgA, DM, and UM were assessed using the analysis of variance (ANOVA) based on a repeated measures model, implemented through the mixed procedure (PROC MIXED MODEL SAS 9.4, 2013).

The statistical model was structured as follows:

$$y = \mu+Si+Gj+Tk+GTjk+ejkn \quad (1)$$

where: y = dependent variable; μ = overall mean; Si = fixed effect of the the sex (i= F; M); G = fixed effect of the treatment (j= 0,1); Tk = fixed effect of the kth time (k= 1,6). GTjk = fixed effect of the interaction between the jth treatment and kth time; ejkn = error.

Time was treated as a repeated measurement and replicated with groups as repeated subjects. The autoregressive covariance method was used for the covariance structure. Least square means were separated using the Student's t-test.

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BCS and FS of the CTR and SACC groups were compared using the Kruskal-Wallis test (UCLA) for both the overall experimental period and the supplementation period, using PROC NPAR1WAY (SAS 9.4). If significant results were found, multiple comparison analysis based on pairwise two-sample Wilcoxon comparisons was conducted. Test statistics from two-tailed tests with P-values < 0.10 were considered significant.

3. Results

All the dogs involved in the trial were healthy during the study and no death was recorded. Moreover, they did not receive any pharmacological treatment in the 15-day before the start of the study, and no change in feed administration or in relevant consumption was highlighted.

In Table 1, the effects of the supplementation of *S. boulardii* on BW and BCS by breed are summarized. More into detail, at the beginning of the evaluation (T_0) least square means (±SE) for the BW in the WT group were 8.2 ± 0.3 (CTR) and 7.50 ± 0.3 (SACC), while in the GS group resulted 32.1 ± 1.6 (CTR) and 31.6 ±0.9 (SACC). The differences registered in BW between SACC and CTR groups were not significant.

Table 1. BW and BCS parameters measured in treated versus untreated GS and WT at different times during the study.

		West Highland White Terrier (WT n= 28)			German Shepherd (GS n= 25)		
		CTR	**SACC**	**P-value**	**CTR**	**SACC**	**P-value**
BW (kg)	**T_0**	8.2 ± 0.3	7.5 ± 0.3	0.1	32.1 ± 1.6	31.6 ± 0.9	0.6
	T_1	8.0 ± 0.3	7.6 ± 0.3	0.2	31.8 ± 1.6	32.0 ± 0.9	1.0
	T_2	8.0 ± 0.3	7.4 ± 0.3	0.2	31.7 ± 1.6	31.8 ± 1.0	0.9
	T_3	8.1 ± 0.3	7.5 ± 0.3	0.2	31.8 ± 1.6	31.9 ± 0.9	0.9
	T_4	8.3 ± 0.3	7.6 ± 0.3	0.1	31.7 ± 1.5	32.1 ± 0.9	0.9
	T_5	8.3 ± 0.3	7.7 ± 0.3	0.1	31.6 ± 1.5	32.1 ± 0.9	0.8
BCS (1-9)	**T_0**	5.6 ± 0.1	5.5 ± 0.1	0.7	5.5 ± 0.2	5.6 ± 0.1	0.5
	T_1	5.5 ± 0.1	5.0 ± 0.1	<0.1	5.4 ± 0.1	5.2 ± 0.1	0.5
	T_2	5.5 ± 0.1	4.9 ± 0.1	<0.1	5.4 ± 0.1	4.6 ± 0.1	<0.1
	T_3	5.6 ± 0.1	4.9 ± 0.1	<0.1	5.3 ± 0.1	4.4 ± 0.1	<0.1
	T_4	5.7 ± 0.1	4.8 ± 0.1	<0.1	5.3 ± 0.2	4.4 ± 0.1	<0.1
	T_5	5.6 ± 0.1	4.6 ± 0.1	<0.1	5.2 ± 0.2	4.4 ± 0.1	<0.1

Focusing on the BCS, the mean values were 5.6 ± 0.1 (CTR) and 5.5 ± 0.1 (SACC) in the WT group, while 5.5 ± 0.2 (CTR) and 5.6 ± 0.2 (SACC) in the GS group (Table 2). Interestingly, for both dog breeds, the BCS of SACC group reduced significantly overtime (i.e. from T0 to T5), but such a decrease was not evident in the CTR group (Table 1). Overall, significant differences were observed in WT and CS between CTR and SACC at T_2, T_3, T_4 and T_5

Table 2. Fs, FSp, DM, UM and IgA parameters measured in treated versus untreated GS and WT at different times during the study.

		West Highland White Terrier (WT n= 28)			**German Shepherd (GS n= 25)**		
		CTR	SACC	P-value	CTR	SACC	P-value
Fs (1-7)	T_0	4.3 ± 0.2	4.3 ± 0.2	0.9	4.3 ± 0.4	4.2 ± 0.3	0.9
	T_1	4.3 ± 0.2	4.1 ± 0.2	0.4	4.4 ± 0.2	3.8 ± 0.2	<0.1
	T_2	4.3 ± 0.2	3.6 ± 0.2	<0.1	4.0 ± 0.2	3.4 ± 0.1	<0.1
	T_3	4.1 ± 0.3	3.8 ± 0.2	0.4	4.6 ± 0.4	3.3 ± 0.2	<0.1
	T_4	4.3 ± 0.3	3.6 ± 0.2	<0.1	4.8 ± 0.3	3.5 ± 0.1	<0.1
	T_5	4.1 ± 0.2	3.4 ± 0.1	<0.1	4.3 ± 0.2	3.2 ± 0.1	<0.1
FSp (1-7)	T_0	4.5 ± 0.2	4.3 ± 0.3	0.7	4.4 ± 0.3	4.3 ± 0.3	0.7
	T_1	4.5 ± 0.1	3.9 ± 0.2	<0.1	4.6 ± 0.2	3.9 ± 0.2	<0.1

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	T_2	4.6 ± 0.3	3.2 ± 0.2	<0.1	4.3 ± 0.2	3.3 ± 0.1	<0.1
	T_3	4.6 ± 0.3	3.5 ± 0.2	<0.1	4.6 ± 0.2	3.3 ± 0.1	<0.1
	T_4	4.6 ± 0.3	3.2 ± 0.1	<0.1	4.8 ± 0.3	3.3 ± 0.1	<0.1
	T_5	4.3 ± 0.3	3.0 ± 0.2	<0.1	4.3 ± 0.2	3.1 ± 0.1	<0.1
DM (%)	T_0	30.4 ± 2.2	32.6 ± 2.8	0.5	29.2 ± 4.3	31.7 ± 2.8	0.6
	T_1	32.3 ± 2.0	34.2 ± 1.5	0.6	32.5 ± 1.9	37.3 ± 1.3	<0.1
	T_2	31.9 ± 2.1	42.6 ± 1.5	<0.1	36.1 ± 1.1	41.8 ± 1.4	<0.1
	T_3	33.3 ± 2.9	39.8 ± 2.4	<0.1	36.1 ± 3.1	41.4 ± 2.1	<0.1
	T_4	31.4 ± 2.7	42.5 ± 1.8	<0.1	34.6 ± 3.0	39.8 ± 1.7	<0.1
	T_5	33.1 ± 2.0	44.5 ± 1.2	<0.1	37.9 ± 1.7	44.0 ± 1.2	<0.1
UM (%)	T_0	68.6 ± 1.1	65.9± 2.8	0.4	66.0 ± 4.3	67.1 ± 2.8	0.7
	T_1	66.8 ± 1.0	64.5 ± 1.5	0.5	68.1 ± 2.0	61.5 ± 1.2	<0.1
	T_2	67.1 ± 1.0	56.8 ± 1.5	<0.1	67.8 ± 1.2	57.5 ± 1.4	<0.1
	T_3	65.8 ± 1.4	59.6 ± 2.4	<0.1	70.1 ± 3.1	57.9 ± 2.1	<0.1
	T_4	67.8 ± 1.4	57.0 ± 1.8	<0.1	72.6. ± 2.9	59.3 ± 1.7	<0.1
	T_5	65.9 ± 1.0	55.0 ± 1.2	<0.1	66.5 ± 1.7	55.7 ± 1.2	<0.1
IgA (mg/g)	T_0	0.6 ± 0.1	0.69 ± 0.1	0.3	0.57 ± 0.08	0.58 ± 0.04	0.8
	T_1	0.6 ± 0.1	0.68 ± 0.1	0.2	0.51 ± 0.07	0.62 ± 0.04	0.3
	T_2	0.6 ± 0.1	0.74 ± 0.1	0.2	0.51 ± 0.08	0.71 ± 0.06	<0.1
	T_3	0.6 ± 0.1	0.8 ± 0.1	<0.1	0.50 ± 0.08	0.78 ± 0.07	<0.1
	T_4	0.6 ± 0.1	0.9 ± 0.1	<0.1	0.52 ± 0.08	0.85 ± 0.07	<0.1
	T_5	0.5 ± 0.1	1.0± 0.1	<0.1	0.50 ± 0.07	0.92 ± 0.08	<0.1

The FSp relevant to both breeds belonging to the SACC group showed a statistically significant decrease overtime, with a mean value next to 3 at the end of the study. The CTR group did not point out a significant reduction in the values measured. The FSp was statistically significantly different between two groups (CTR vs SACC) at each time point (T_1-T_5) in both breed, and in the SACC groups significantly decreased moving from T_0 to T_5 (Table 2).

In the same way, the FS observed by the veterinarian showed a statistically significant decrease in the SACC groups overtime with the progression of the trial, with a value next to 3 for most of the animals at the end of the study period (T_5). In particular, in the WT group the FS was statistically significant between CTR and SACC group at T_2, T_4 and T_5 (Table 2), with the SACC group showing a decrease.

The DM and the UM measured for both breeds in the SACC group, showed a statistically significant increase compared to the CTR group, for which no significant increase in these values was registered. The increase within SACC and CTR was significant from T_1 to T_5 (Table 2).

The fecal IgA level in both breeds showed a significant increase from T_1 to T_5 in the SACC groups, while all the CTR groups did not show any significant increase. The IgA measurements pointed out statistically significant differences between groups (SACC and CTR) at T_3, T_4 and T_5 (Table 2).

Finally, the WI in CTR and SACC groups in both breeds did not show any statistically significant variation during the whole study period (Table 3).

Table 3. Water intake (WI) expressed in liter measured in treated versus untreated dogs West Highland White Terrier (WT) and German Shepherd (GS) at different time points during the study.

		West Highland White Terrier (WT n= 28)		**German Shepherd (GS n= 25)**	
		CTR	SACC	CTR	SACC
WI (liter)	T_0	0.38 ± 0.02	0.37 ± 0.02	1.58 ± 0.06	1.58 ± 0.05
	T_1	0.38 ± 0.02	0.38 ± 0.02	1.60 ± 0.05	1.59 ± 0.08
	T_2	0.39 ± 0.01	0.38 ± 0.02	1.57 ± 0.06	1.59 ± 0.08
	T_3	0.37 ± 0.01	0.38 ± 0.02	1.57 ± 0.05	1.57 ± 0.04

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	T_4	0.38 ± 0.02	0.39 ± 0.01	1.60 ± 0.06	1.57 ± 0.04
	T_5	0.38 ± 0.02	0.38 ± 0.02	1.58 ± 0.05	1.59 ± 0.05

4. Discussion

The novelest research on pets emphasizes the benefits of yeast products on the modulation of the intestinal microbiota (*i.e.* with potential increases in *Bifidobacterium* or *Lactobacillus*), being these compounds able to enhance the immune function, to reduce potentially pathogenic microorganisms, and to improve the antioxidant status [7]. For example, S. *boulardii*, *i.e.* the probiotic yeast used in this study can inhibit the colonization of pathogenic microorganisms, improve the intestinal barrier function, and regulate immunity [13]. Very recently, its efficacy in promoting intestinal health and microbiome composition has also been demonstrated in kittens [2].

In the present study, *S. boulardii* was tested as a probiotic feed additive in healthy dogs for a period of 35 days. It did not cause any short-term adverse effects, as already reported by other authors [3,14]. At the beginning of the trial, all the dogs involved were healthy and no significant differences in the parameters selected as the outputs of the evaluation were highlighted.

In recent years, an increased use of probiotics in animal diets as supplements to maintain the optimal gastro-intestinal health in both healthy pets and pets with disorders, have been reported [1].

Moreover, yeast and yeast-based products have been shown to potentially promote host's gut health in both humans and animals [7]. However, there are still limited scientific literature discussing the impact of yeast products in the dogs and cats, although the number of publications in this respect is slowly increasing [7].

Saccharomyces cerevisiae is one of the predominant yeast product used in various applications [7]. Various research works have demonstrated its beneficial effects on the intestinal health and microbiota of dogs, both in healthy animals [3] and in those having various gastrointestinal disorders [14]. In particular, its administration improved dogs' fecal consistency (higher FS), resulted in higher DM [20] and stool frequency when compared to control groups, although the values remained within the ideal score range in some of the studies [7,14,21].

In this work, at the end of the treatment period, no differences in BW were reported in the SACC groups compared to the CTR groups, while the BCS showed a significant variation in the treated versus untreated dogs. In particular, at T_0 the two groups were similar in values (5,5 - 6), then the BCS gradually decreased in the treated groups, reaching a medium value between 4,5 -5 at T_5 [17]. This finding highlights the positive effect of the supplement on BCS, reflecting a good maintenance of the nutritional condition in the two dog breeds selected, which are known to have the tendency to become overweight, as also previously reported [7].

In addition, the data collected demonstrated the positive impact of *S. boulardii* on all fecal parameters under evaluation (FS, Fsp, DM, UM, IgA) for both dog breeds.

Indeed, FS as well as FSp presented significant differences in the two groups, specifically they decreased in the SACC one, which was characterized by harder feces compared to the CTR groups, whose values were between 3 and 4 (Table 2).

A significant improvement in other two fecal parameters (DM and UM) was reported, further strengthening the positive effect of *S. boulardii* supplementation on fecal consistency due to a minor water content in the samples.

In this trial, water intake (WI) should not have modified the fecal consistency parameters, as all animals, regardless of their group, had similar values at each time point and consumed a very similar volume of water. Moreover, there was no statistically significant variation in WI between the CTR and SACC groups during the study. This result supports the positive effect of S. *boulardii* on the previously discussed fecal parameters.

Interestingly, the adminsitration of *S. boulardii* led to a significant increase in fecal IgA in the SACC group (Table 2). Fecal IgA is a biomarker for of intestinal immunity or inflammation in dogs and puppies [22] and has been recently proposed as a non-invasive marker of canine intestinal health [3,7,22]. Indeed, secretory IgA is the most important humoral protective immune factor in the intestine. It inhibits the adhesion, colonization, and penetration of microorganisms, as well as the

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absorption of food antigens [23]. The data collected in this work, consistent with findings from other studies, confirmed that the use of supplementation with yeast products in dogs increases ileal and fecal IgA, indicating enhanced mucosal immunity and immunomodulatory properties [7,20,24].

5. Conclusions

The supplementation of *S. boulardii* for 35 days, at the recommended dietary dosage of 5.0 x 10^9 CFU/kg of feed in healthy dogs of different breeds (WH and GS), significantly improved the BCS, FS, FSp, DM, UM and Fecal IgA parameters. Based on the results obtained, feed supplementation with the above-mentioned yeast product could be recommended in clinical settings for an improvement of fecal parameters also in healthy dogs, also promoting enhanced mucosal immunity. Further studies are recommended in dogs and cats suffering from gastrointestinal diseases.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data that supports the findings of this study are available on request from the corresponding author (FP).

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Conflicts of Interest: One of the authors is an employee of the Candioli Pharma S.r.l. Three of the authors are scientific consultants for the Candioli Pharma S.r.l. Candioli Pharma S.r.l is a company that may be affected by the research reported.

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