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PAPER

Study of faecal parameters and body condition in dogs with a diet supplemented with *Lactobacillus acidophilus* D2/CSL (CECT 4529)

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ABSTRACT

The aim of our case-control study is to evaluate the effects of a diet integrated with the probiotic *Lactobacillus acidophilus* D2/CSL (CECT 4529) on the nutritional status and faecal consistency of healthy dogs belonging to the English Cocker Spaniel (ECS) and Labrador Retriever (LR) breeds. A total of 30 dogs were enrolled in this study, and they were randomly assigned to a Control (CTR, $n = 14$) and a Treated group (LACTO, $n = 16$). The trial consisted in a 7-days adaptation period where all the animals received the same commercial food, followed by a 35-days of data collection period where the LACTO group received the food supplemented with *Lactobacillus acidophilus* D2/CSL. We evaluated Body weight (BW), Body Condition Score (BCS) and Skinfold thickness, Faecal Score (FS) and Faecal Moisture (FM). All dogs in the LACTO group maintained an ideal BCS score during the whole experimental period compared to the CTR group. A significant decrease in skin thickness was found throughout the trial in the LACTO group. A significant improvement of the FM was recorded in the LACTO compared to the CTR group in the overall period for both dog breeds, and the FS significantly decreased in the LACTO group. Our results showed good maintenance of the nutritional conditions in dogs that are prone to overweight and a significant improvement of faecal parameters, meaning that even in healthy dogs with no gastrointestinal disorder the addition of this supplement to the diet helps to maintain the optimal balance of their intestinal microbiota.

HIGHLIGHTS

- Probiotics help to maintain and promote the optimal gastro-intestinal health and well-being.
- *Lactobacillus acidophilus* D2/CSL (CECT 4529) improve faecal parameters and nutritional status of dogs.

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Skin thickness; probiotic; dog; faecal consistency; overweight

Introduction

The gastrointestinal (GI) tract is populated by a complex community of microbes, and it plays a significant role in human and animal health as well as in disease development.

When qualitative and quantitative changes of the gut microbiota happen, dysbiosis is present. Here, the reduction of positive bacteria increases the presence of pro-inflammatory and pathogenic ones in the gut. As a consequence, dysbiosis has been found associated with the development of both intestinal and non-intestinal diseases (Carding et al. 2015; Brusaferro et al. 2018). Each animal species has specie-specific microbiota, and both genetic and environmental factors result in havinge significant impacts on the structure and composition of the gut microbiota. Diet can rapidly alter the gut microbiome. For example, in the recent years, unbalanced diets and erroneous behavioural habits had led to an increase in overweight worldwide (Conlon and Bird 2014). As in humans, dysbiosis in intestinal microbiota has been associated with overweight in dogs (Grześkowiak et al. 2015; Kieler et al. 2017). It has been shown that dysbiosis can affect the animal wellbeing promoting both the development of adipose tissues and the alteration of faecal parameters (Weese et al. 2001; Marks et al. 2011).

Given these premises, to maintain the correct equilibrium in the GI microbiota, human and animal

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medicine have increased the use of prebiotic, probiotic or synbiotic (the combination of the two) and faecal microbiota transplantation (FMT) (Rose et al. 2017; Pilla and Suchodolski 2019; Whittemore et al. 2019). The higher short chain fatty acids production due to an increasing fermentation activity in large intestine of dogs (Musco et al. 2018) and cats (Calabrò et al. 2020), affects energy balance (Deng and Swanson 2015; Fluitman et al. 2017).

The concept of probiotic is based on the fact that the gut ecosystem strongly contributes to human and animal physiology and, as a consequence, its modulation helps to maintain health and reduce disease risk. Indeed, possible benefits of the probiotic use in pet animals include: modulation of the immune system, help in stress maintenance, protection from infections caused by enteropathogens, increased growth and development, control of allergic disorders and recently reduction of overweight (Grześkowiak et al. 2015). *Lactobacillus* and *Bifidobacterium* species are the most studied and used probiotics in Veterinary Medicine. Dysbiosis promotes the excretion of softer and watery or even diarrheal stools, therefore positive effects of diets supplemented with probiotics resulted in an improvement of faecal parameters (i.e. Faecal score -FS and Faecal moisture -FM) in dogs and cats (Weese et al. 2001; Herstad et al. 2010; Bybee et al. 2011; Marks et al. 2011; Gagne et al. 2013; Fenimore et al. 2017; Rose et al. 2017; Fusi et al. 2019).

Dysbiosis is common in overweight human populations, and the results of a recent review have shown that probiotics are essential tools in the treatment of obesity and lead to significant reductions in Body Mass Index (BMI), body weight and fat mass compared to placebo (John et al. 2018).

Studies on experimental animals also provide evidence of probiotic species-specific effects on body weight, fat mass, glucose metabolism, inflammatory markers, plasma and hepatic lipids, plasma cholesterol levels and energy metabolism (Brusaferro et al. 2018).

In literature, the effects of *Lactobacillus acidophilus* have been already observed in healthy cats improving faecal quality and increasing faecal lactobacilli while decreasing total coliform counts (Fusi et al. 2019). In addition, studies on laying hens and broilers showed an improvement of the productive performances, gut morphology and modification of the GI microbioma and metabolome after the administration of the same probiotic (Gallazzi et al. 2008; Forte et al. 2016; De Cesare et al. 2017; Forte et al. 2018; De Cesare et al. 2020).

The aim of our case-control study is to evaluate the effects of a diet integrated with the probiotic *Lactobacillus acidophilus* D2/CSL (CECT 4529) on the nutritional status and faecal consistency of healthy dogs belonging to the English Cocker Spaniel (ECS) and Labrador Retriever. (LR); these two breeds tend to be overweight.

Materials & methods

Animals and study design

A group of 30 adult dogs (age >1 yr) were included in the study. Half of the dogs belong to the Labrador Retriever (LR) breed ($n = 15$, 5 males, 10 females), and the other half to the English Cocker Spaniel (ECS) breed ($n = 15$, 7 males and 8 females), selected from two ENCI-registered breeders located in the Northern part of Italy. At the beginning of the study (T0) least square means (±SE) for the body weight in the LR group were 30.41 ± 0.84 (CTR) and 28.70 ± 0.69 (LACTO), while in the ECS group 12.62 ± 0.99 (CTR) and 13.19 ± 0.82 (LACTO) (Table 1). Regarding the BCS, median (25th; 75th) were 5.0 (5.0;5.0) (CTR and LACTO) in the LR group, while 5.5 (5.0;6.0) and 5.0 (5.0;6.0) in the ECS group (Table 2). Animals were kennelled in groups (2 or 3 dogs/box) considering animal welfare principles avoiding any stress. Animals were randomly assigned to Control (CTR; LR = 9, ECS = 7) and Treated group (LACTO; LR = 6, ECS = 8). In the first group animals were given a balanced commercial diet, while in the second one *Lactobacillus acidophilus* D2/CSL (CECT 4529) was added to the food, as later specified. The trial consisted in a 7-days adaptation period followed by a 35-days of data collection period. Standard cleaning and disinfection of the kennel were carried out according to regulations. Before the beginning of the study, an antiparasitic treatment was carried out using commercial molecule drugs with no antibacterial effect (Frontline® Combo, Boehringer Ingelheim, spot on, 1 administration/dog; Drontal® Plus Flavour, Bayer Animal Health, tablet, 1 administration/dog) Dogs were daily checked by the same veterinarian during the whole study period.

Feed supplement and Diet

During the entire duration of the trial, including the 7 days of adaptation period, both CTR and LACTO dogs received a complete dry commercial diet (Hill's Science Plan Adult Advanced Fitness Medium-Chicken) twice a day. We modified the standard maintenance energy requirements for adult dogs recommended by FEDIAF guidelines (FEDIAF 2019) according to NRC guidelines (NRC 2006) with a caloric restriction of 10%

Table 1. Effect of the addition of *Lactobacillus acidophilus* D2/CSL to diet on body weight (BW), skinfold thickness measured at the 4th cervical vertebra (NECK) and at the 7th/8th rib (THORAX), and faecal moisture (FM).

	Labrador Retriever (n = 15)			English Cocker Spaniel (*n* = 15)		
	CTR	*LACTO*	*p-value*	*CTR*	*LACTO*	*p-value*
BW (Kg)						
Overall period	29.90 ± 0.68	28.83 ± 0.56	0.2416	12.57 ± 0.96	12.61 ± 0.80	.9780
T0	30.41 ± 0.84	28.70 ± 0.69	0.1154	12.62 ± 0.99	13.19 ± 0.82	.6567
T1	30.41 ± 0.84	28.80 ± 0.69	0.1376	12.52 ± 0.99	12.46 ± 0.82	.9646
T2	29.40 ± 0.84	28.91 ± 0.69	0.6518	12.52 ± 0.99	12.46 ± 0.82	.9646
T3	29.40 ± 0.84	28.91 ± 0.69	0.6518	12.63 ± 0.99	12.31 ± 0.82	.8056
NECK (mm)						
Overall period	15.09 ± 0.58	13.15 ± 0.48	0.0270*	5.33 ± 0.44	4.62 ± 0.37	.2509
T0	15.09 ± 0.82	13.65 ± 0.67	0.1890	5.67 ± 0.62	4.85 ± 0.51	.3283
T3	15.09 ± 0.82	12.65 ± 0.67	0.0355*	5.00 ± 0.62	4.40 ± 0.51	.4719
THORAX (mm)						
Overall period	14.68 ± 0.56	12.96 ± 0.46	0.0374*	6.17 ± 0.42	4.96 ± 0.35	.0592*
T0	15.02 ± 0.78	13.63 ± 0.64	0.1875	6.50 ± 0.60	5.29 ± 0.49	.1429
T3	14.35 ± 0.78	12.29 ± 0.64	0.0601*	5.83 ± 0.60	4.63 ± 0.49	.1429
FM (%)						
Overall period	0.68 ± 0.012	0.64 ± 0.010	0.0365*	0.66 ± 0.004	0.63 ± 0.004	.0084*
T0	0.68 ± 0.019	0.65 ± 0.015	0.2157	0.65 ± 0.012	0.65 ± 0.010	.9108
T1	0.64 ± 0.019	0.59 ± 0.015	0.0345*	0.60 ± 0.012	0.60 ± 0.010	.7490
T2	0.72 ± 0.019	0.68 ± 0.015	0.0495*	0.71 ± 0.012	0.62 ± 0.010	<.0001*
T3	0.67 ± 0.019	0.66 ± 0.015	0.5328	0.67 ± 0.012	0.66 ± 0.010	.6372

Source. Least square means ± SE from the performed mixed model in control (CTR) and treated (LACTO) groups. Results are presented for the entire trial period and for each experimental time. T0 (day 0), T1 (day 14), T2 (day 28) and T3 (day 35).
**p*-values <.10 were considered significant.

Table 2. Effect of the addition of *Lactobacillus acidophilus* D2/CSL to diet on body condition score (BCS). Descriptive statistics and results from the Kruskall-Wallis test.

Experimental period	Group	Mean	SD	Median (25th; 75th)
Labrador Retriever (n = 15)				
Overall	CTR	4.63	0.58	5.5^a (4.0;5.0)
	LACTO	4.94	0.53	5.0^b (5.0;5.0)
T0	CTR	4.83	0.41	5.0 (5.0;5.0)
	LACTO	4.89	0.33	5.0 (5.0;5.0)
T1	CTR	4.33	0.82	4.5^a (4.0;5.0)
	LACTO	4.89	0.33	5.0^b (5.0;5.0)
T2	CTR	4.67	0.52	5.0 (4.0;5.0)
	LACTO	5.00	0.71	5.0 (5.0;5.0)
T3	CTR	4.67	0.52	5.0 (4.0;5.0)
	LACTO	5.00	0.71	5.0 (5.0;5.0)
English Cocker Spaniel (n = 15)				
Overall	CTR	5.67	0.76	5.5^a (5.0;6.0)
	LACTO	5.28	0.85	5.0^b (5.0;6.0)
T0	CTR	5.50	0.55	5.5 (5.0;6.0)
	LACTO	5.44	0.53	5.0 (5.0;6.0)
T1	CTR	5.50	0.55	5.5 (5.0;6.0)
	LACTO	5.44	0.53	5.0 (5.0;6.0)
T2	CTR	6.50	0.84	7.0 (6.0;7.0)
	LACTO	5.55	1.33	5.0 (5.0;7.0)
T3	CTR	5.17	0.41	5.0^a (5.0;5.0)
	LACTO	4.67	0.50	5.0^b (4.0;5.0)

Source. CTR: control group; LACTO: treated group; T0 (day 0), T1 (day 14), T2 (day 28) and T3 (day 35).
[a,b]Within each period, medians different if superscript differ ($p < .10$)

in ECS and 20% in LR, given the fact that both breeds included in the study are prone to overweight as reported by literature (Edney and Smith 1986; German 2006). Daily feed consumption per dog was 200 g and 350 g for the ECS and the LR, respectively.

The LACTO group received the commercial diet with the probiotic *Lactobacillus acidophilus* D2/CSL (CECT 4529). This feed additive is a freeze-dried microbial preparation. It has been added to the functional group 'gut flora stabilisers' and has been authorised by the Commission Implementing Regulation (EU) 2015/38 (EU id No4bl715). It is produced by the Centro Sperimentale del Latte S.r.l. (Zelo Buon Persico, Lodi, Italy). The process to obtain the right probiotic amount consisted in using 50 g of the feed additive (standard concentration 5.0×10^{10} CFU g^{-1}), pre-mixed in laboratory with 9950 g of maltodextrins. Then, a total of 20 g of this pre-mixture was daily added to each 980 g of commercial food in the bowl.

The CTR group received the commercial diet, with the addition of 10 g of maltodextrin in 100 Kg of dog food (placebo). Five samples of the food belonging to the LACTO group were sent to the Quality Control Laboratory of the Centro Sperimentale del Latte S.r.l., in order to verify the number of lactobacilli in the preparation and the absence of undesired bacteria.

Data collection

The evaluation of the nutritional status was performed by the same veterinarian following standard guidelines (Baldwin et al. 2010). Body weight (BW), Body Condition Score (BCS) and Skinfold thickness were recorded at T0 (day 0), T1 (day 14), T2 (day 28) and T3 (day 35).

The BW of each dog in Kg was measured using the same scale (large pet scale, four-sensor, maximum of 100 kg, d = 100 g; Momert, Dunaújváros, Hungary) and at the same time of the day avoiding excessive animal manipulation.

The BCS is an efficacious assessment of the body fat using a visual examination and palpation of the animal. Scores between 1 and 9 were assigned by the same trained veterinarian who carried out the BW measurement, where a score of 4 or 5 represents the ideal ones (WSAVA. 2013). The measurements were taken at the same time as the BW.

In addition, at the beginning and the end of the trial, skinfold thickness was measured with a calliper at the 4th cervical vertebra (NECK) and at the 7th/8th rib (THORAX) as reported by Wilkinson and McEwan (1991).

Two faecal parameters, Faecal Score (FS) and Faecal Moisture (FM) were evaluated at T0, T1, T2, T3. In particular, FS was determined using a 7 point scale on fresh faeces, just before the collection (Davies et al. 1986; Greco 2011; Cappai et al. 2013). One fresh faecal sample per individual dog per week was collected in a plastic bag and stored at 4 °C until the transport to the laboratory. Here, the same technician who could not identify the samples determined the faecal moisture (in per cent). In particular, 5–10g fresh stool sample was weighed with a precision balance (Sartorius CP224S, maximum of 200 g, d = 0.1 mg; Sartorius, Bohemia, New York, USA), then dried in an oven at a temperature of 105–110 °C for 20–24 h and then weighed. The resulting dry material was weighed two times, and their mean was considered as the final measurement.

Statistical analysis

Data on nutritional status and faecal parameters were analysed to see differences between CTR and LACTO group over time. BW, NECK, THORAX, and FM of dogs recorded at different times during the trial were analysed using the analysis of the variance ANOVA according to repeated measures (MIXED procedure in SAS 9.4, 2013).

The statistical model was built as the following:

$$\text{yijkln} = \mu + \text{Si} + \text{Gj} + \text{Tk} + \text{GTjk} + \text{klj} + \text{eijkln},$$

where: yijkl = dependent variable, FM; μ = overall mean; Si = fixed effect of the ith sex (i = 1, 2); Gj = fixed effect of the jth group (j = 1, 2); Tk = fixed effect of the kth time (k = 1, 4); GTjk = fixed effect of the interaction between the jth treatment and kth time; and eijkl = error.

Time was used as repeated measurement and replicate within group was used as repeated subject. The autoregressive covariance structure was used. Least square means were separated using Student's t-test.

BCS and FS for the CTR and LACTO group were compared using Kruscall Wallis test in relation to the overall study period by means of PROC NPAR1WAY. In the case of a significant result, a multiple comparison analysis based on pairwise two-sample Wilcoxon test was performed. Test statistics from two-tailed tests that yielded p-values $<.10$ were considered significant. Analyses were performed separately by breed given the different animal size. Similar analyses were performed in a previous study on healthy cats (Fusi et al. 2019).

Results

All the dogs were healthy throughout the study, and no side effects (i.e. vomiting, diarrhoea) in both groups were recorded. No waste of food was found in any box for the whole period. No change in feed administration/consumption was recorded.

A summary table for the effects of the addition of the probiotic to the diet on BW, Skinfold thickness (NECK and THORAX), and FM by breed are presented in Table 1.

No difference in BW between CTR and LACTO group for the whole study period and at different time points was recorded in both breeds (Table 1).

A significant decrease in values for both NECK (13.15 mm vs 15.09 mm, $p = .027$) and THORAX (12.96 mm vs 14.68 mm, $p = .037$) was recorded in the LR group throughout the trial. At the end of the trial (T3) the CTR group showed a thicker skin compared to dogs belonging to the LACTO group (NECK: 15.09 mm vs 12.65 mm, =0.033; THORAX: 14.35 mm vs 12.29 mm, $p = .06$). In the ECS group, the skin thickness at the thorax level (THORAX) was significantly thinner ($p = .059$) in the LACTO group considering the overall period, being 4.96 mm ± 0.35 for the LACTO group and 6.17 mm ± 0.42 for the CTR group. No significant differences were found for NECK, instead (Table 1).

The BCS was affected by the addition of *Lactobacillus acidophilus* to the diet. Indeed, dogs in the LACTO group maintained an ideal BCS score closer to 5 during the whole experimental period compared to the CTR group (Table 2).

Regarding the faecal parameters, a significant improvement of the FM was recorded in the LACTO compared to the CTR group in the overall period for both dog breeds (LR: 0.64 vs 0.68, $p = .036$; ECS: 0.63

Table 3. Effect of the addition of *Lactobacillus acidophilus* D2/CSL to diet on faecal score (FS). Descriptive statistics and results from the Kruskall-Wallis test.

Experimental period	Group	Mean	SD	Median (25th; 75th)
Labrador Retriever (n = 15)				
Overall	CTR	3.58	0.72	3.0[a] (3.0;4.0)
	LACTO	3.19	0.62	3.0[b] (3.0;3.5)
T0	CTR	4.33	0.52	4.0 (4.0;5.0)
	LACTO	3.89	0.60	4.0 (4.0;4.0)
T1	CTR	3.67	0.82	3.5[a] (3.0;4.0)
	LACTO	2.67	0.50	3.0[b] (2.0;3.0)
T2	CTR	3.17	0.41	3.0 (3.0;3.0)
	LACTO	3.11	0.33	3.0 (3.0;3.0)
T3	CTR	3.17	0.41	3.0 (3.0;3.0)
	LACTO	3.11	0.33	3.0 (3.0;3.0)
English Cocker Spaniel (n = 15)				
Overall	CTR	3.13	0.68	3.0[a] (3.0;3.0)
	LACTO	2.75	0.65	3.0[b] (2.0;3.0)
T0	CTR	3.00	0.00	3.0 (3.0;3.0)
	LACTO	3.00	0.00	3.0 (3.0;3.0)
T1	CTR	2.67	0.82	2.5 (2.0;3.0)
	LACTO	2.22	0.44	2.0 (2.0;2.0)
T2	CTR	3.83	0.75	4.0[a] (3.0;4.0)
	LACTO	2.56	0.88	2.0[b] (2.0;3.0)
T3	CTR	3.00	0.00	3.0 (3.0;3.0)
	LACTO	3.22	0.44	3.0 (3.0;3.0)

Source. CTR: control group; LACTO: treated group; T0 (day 0), T1 (day 14), T2 (day 28) and T3 (day 35).
[a,b] Within each period, medians different if superscript differ ($p < .10$)

vs 0.66, $p = .008$). In particular, FM least square means differed at T1 (LR: 0.59 vs 0.64, $p = .034$) and at T2 (LR: 0.68 vs 0.72, $p = .049$; ECS: 0.62 vs 0.71, $p < .0001$) in LACTO and CTR group, respectively (Table 1).

The FS significantly differed between CTR and LACTO group during the overall period for both dog breeds (ECS: 3.13 vs 2.75; LR:3.58 vs 3.19; $p < .10$). In particular, a decrease in score was found in the LACTO group at T2 for the ECS group (3.83 vs 2.56) and at T1 for the LR group (3.67 vs 2.67, $p < .10$) (Table 3).

Discussion

In recent years, an increased use of probiotics in animal diets as supplements able to help to maintain and promote the optimal gastro-intestinal health and well-being in both healthy pets or pets with disorders have been reported (Grześkowiak et al. 2015; Brusaferro et al. 2018; Fusi et al. 2019).

In the present study *Lactobacillus acidophilus* D2/CSL as a feed additive in healthy dogs to see its effect on the nutritional status and faecal consistency have been tested. This probiotic has already shown its safety and efficacy in cats, dogs and poultry. Specifically, it has improved the health and faecal parameters of cats and dogs (Fusi et al. 2019; Marelli et al. 2020), gut health and performance of broilers and laying hens (Gallazzi et al. 2008; De Cesare et al. 2017).

In literature, the positive effect of the addition of probiotics to diet have already been recorded on non-healthy dogs, in the prevention and treatment of acute gastroenteritis (Herstad et al. 2010) in the treatment of inflammatory bowel diseases (IBD) (Rossi et al. 2014) and diarrhoea (Rose et al. 2017), and in the prevention and alleviation of allergy symptoms (Kim et al. 2015). On the other hand, lots of studies performed on experimental animals clearly demonstrate that the administration of probiotics could have positive effects in the prevention and treatment of overweight given their favourable metabolic effect and being the intestinal microbiota involved in the regulation of fat storage (Aronsson et al. 2010; Ji et al. 2012; Miyoshi et al. 2014; John et al. 2018). Indeed, it has been shown that probiotics could be used in direct regulation of the fasting-induced adipose factor (FIAF) and/or the expression of one or more genes encoding the FIAF in a subject (Arulampalam et al. 2010; John et al. 2018). However, it is necessary to consider the specificity in the activity of different probiotics on different animal species when their effects are described (Brusaferro et al. 2018). Diabetic rats fed with yoghurt supplemented with *Lactobacillus acidophilus* and *Lactobacillus casei* showed a marked reduction of hyperglycaemia and hyperinsulinaemia (Yadav et al. 2007) and the use of *L. gasseri* (LG2055) prevented body weight gain, fat accumulation and pro-inflammatory gene expression in the adipose tissue in the tested obese mice (Miyoshi et al. 2014). Data on experimental animals can give us only useful information on the potential future use of products in pet animals, but no clear and direct data extrapolation could be made from these studies. Indeed, a not clear effect on the prevention or control of overweight has been demonstrated in dogs and cats so far. There is a clear need for more *in vivo* studies on pet animals to provide enough evidence for prescribing probiotics for this reason (Handl et al. 2013; Grześkowiak et al. 2015; Wang et al. 2015; Singleton et al. 2019). Despite this, our data are very promising given the fact that we observed a reduction of the skin thickness (NECK and THORAX) in dogs belonging to the groups treated with *Lactobacillus acidophilus* D2/CSL, taking into account differences in breeds (Weese et al. 2001). These measurements are normally used by veterinarians to assess the nutritional status of animals, and it could also be performed when monitoring animal overweight (Wilkinson and McEwan 1991). In addition, the BCS of dogs belonging to both breeds remained ideal ($\simeq$5) for the whole duration of the study in the treatment group, so reflecting a good maintenance of the nutritional conditions in these dogs that are prone to overweight. No effect on the body weight was recorded, instead. Based on these results and given the fact that this study was performed on healthy dogs with no recorded overweight, we would expect a more significant effect on these parameters administering the supplement to dogs already overweight.

With regards to the effect of the probiotic on faecal parameters, the FM and FS of the dogs receiving the *Lactobacillus* supplementation demonstrated a significant improvement, meaning that, even in healthy dogs with no gastrointestinal disorder, the addition of this supplement to the diet helps to maintain the optimal balance of their intestinal microbiota. The present data are in agreement with a study where faecal consistency was improved in dogs with the addition of *Lactobacillus acidophilus* strain DSM 13241 (Pascher et al. 2008) and in another trial on dogs belonging to the Boxer breed only treated with *Lactobacillus acidophilus* D2/CSL (Marelli et al. 2020), while no effect on FS was recorded on dogs with a diet supplemented with *Lactobacillus acidophilus* NCDC 15 (Kumar et al. 2017).

Conclusions

Our study showed a good maintenance of the nutritional status and a significant improvement of faecal parameters of dogs, meaning that the supplement helps to maintain the optimal balance of their intestinal microbiota. Further studies are needed to increase the sample size and to test the long term *Lactobacillus acidophilus* D2/CSL effects on weight control and on faecal parameters of healthy dogs, and on gut health status of dogs with intestinal disorders or dysbiosis. Based on present data and other recent research results, the use of probiotics, prebiotics, or synbiotic developed for modulating the gut microbiomes, could be considered as a novel approach and a valid alternative or additional therapy for canine overweight and other metabolic disorders in the near future.

Ethical approval

The experimental procedures used in this trial were reviewed and approved by the institutional Committee for Animal Care of the University of Milan (approval 48/15, 12th October 2015).

Disclosure statement

Candioli Pharma S.r.l. and Centro Sperimentale del Latte Srl may be affected by the research reported.

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