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ORIGINAL RESEARCH

Effects of probiotic *Lactobacillus acidophilus* D2/CSL (CECT 4529) on the nutritional and health status of boxer dogs

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Abstract

Background The aim of the present study was to investigate the effects of *Lactobacillus acidophilus* D2/CSL (CECT 4529) probiotic strain on nutritional status and faecal and microbiological parameters in a group of purebred boxers.

Methods Forty healthy adult boxer dogs were randomly assigned to a treated (LACTO) group receiving a commercial diet supplemented with *L acidophilus* D2/CSL (CECT 4529) to a final concentration of 5.0 x 10^9 colony-forming unit/kg of food, and a control (CTR) group receiving the same diet but without the probiotic (placebo). Nutritional status (body weight, skinfold thickness, body condition score) and faecal quality parameters were analysed.

Results No differences in body weight and skin thickness were found during the whole experimental period. Dogs in the LACTO group showed a significantly higher body condition score than those in the CTR group (4.86±0.55 v 4.65±0.65), and no significant differences were recorded in body weight and skinfold thickness. The LACTO group showed a significantly lower faecal moisture (in per cent) compared with the CTR group (0.67±0.007 v 0.69±0.007). Faecal hardness (in kg) was higher in the LACTO group than in the CTR group (0.86±0.047 v 0.70±0.051), and faecal score also improved in the LACTO group (3.78±0.95 v 4.25±0.91). A significant difference in total *Escherichia coli* counts as well as in lactobacilli counts between the CTR and LACTO groups was only detected at 28 days.

Conclusion Supplementation of *L acidophilus* D2/CSL (CECT 4529) significantly improved the nutritional status and faecal parameters of dogs.

Introduction

The gastrointestinal (GI) microbiota of animals is a complex ecosystem composed of a consortium of bacteria, archaea, eukarya (especially fungi) and viruses. It has a strong influence on maintenance of normal gut function and the general health of hosts.[1 2] One of the main roles of a mature and balanced GI microbiota is colonisation resistance, also defined as competitive exclusion or barrier effect.[3] Specifically, the GI microbiota works together with the host's other non-specific defences and with the gut-associated immune system in order to resist the invasion of dangerous organisms.[4] However, the balance among the microbial GI communities within a host, or eubiosis, changes over time due to physiological and/or environmental causes, including ageing, changes in feed formula, dietary restrictions, stress and immunodepression, infections, and antibiotic treatments.[5–7] Some alterations in the GI microbiota, or dysbiosis, can affect animal wellbeing by promoting obesity or increasing faecal water content. The reduction of beneficial bacteria and the increase of proinflammatory/pathogenic bacteria in the gut are consistently associated with the development of adipose tissue, systemic inflammation and metabolic comorbidities in both people and mice.[8–11]

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In dogs, it has been shown that administration of some diets differentiated in supplementation with chicory (inulin), non-digestible oligosaccharide and glucose, or in the protein content can modify the faecal counts of *Clostridium perfringens* and reduce *Bifidobacterium* species. Such dysbiosis can induce a reduction in stool consistency.[12 13] In addition, the effects of severe dysbiosis on gut physiology could be severely health-threatening, potentially causing acute and chronic GI inflammation, atopic diseases and intestinal cancer.[5 6 14] As an example, human and canine inflammatory bowel disease has been associated with an increase in Proteobacteria (eg, *Escherichia coli*) and a decrease in Firmicutes in the intestine.[15 16]

At present, there is an increasing interest in finding a way to naturally modify the GI microbiota, with positive effects on health and welfare as a result.[17] Feeding animals with probiotic lactobacilli (LB) could be one of the possible ways. *Lactobacillus* species, such as *L acidophilus*, can be identified in the GI tract of healthy dogs.[18] Similar to people and other mammals, including rodents and cats, it could be supposed that, even considering interindividual and intraindividual variations, LB becomes established in the GI tract of dogs soon after birth and reaches compositional stability with growth,[19] and its principal activity is inhibition of undesirable microorganism proliferation.[20 21]

However, the relative abundance of intestinal LB varies over time. Dysbiosis, as a result of an unbalance between lactic acid bacteria and pathogenic bacteria (eg, *C perfringens* and *E coli*), is commonly observed in companion animals. It leads to excretion of softer or watery stools, as reported in dogs and cats by Weese *et al*[22] and Marks *et al*.[23] Live LB, as recently written by the Food and Agriculture Organization,[24] '*when administered in an adequate amount, confer a beneficial health effect to the host*' and can be defined as probiotics. Modes of action of probiotic LB strains include competitive exclusion towards undesirable bacteria, alteration of microbial and host metabolism, and immunity stimulation.[25] In human medicine, probiotic LB has been largely used to manage a number of disorders related to GI dysbiosis, such as antibiotic-associated diarrhoea, intestinal infections and inflammations.[26 27] Probiotic LB is able to suppress mucosal inflammation and restore cytokine balance towards an anti-inflammatory state.[28] It is interesting to note that, along with its subtherapeutic benefits, the antiobesity effects of probiotic LB have also been reported in the scientific literature about mice.[11 29]

In general, the microbial strain, dose (colony-forming unit (cfu)/day) and duration of treatment are among the critical factors influencing the efficacy of probiotics.[30] Indeed, probiotic effects are strain-specific and the outcomes vary depending on the targeted animal species.[24] To the authors' knowledge, no experimental trials on dogs have been performed yet. In this study, the authors investigated the change in selected parameters they considered as potential indicators of animal welfare especially in terms of 'gut health', following the administration of *L acidophilus* D2/CSL probiotic to dogs. Specifically, the authors evaluated the nutritional status, faecal consistence and moisture content of stool samples, as well as some faecal microbiological parameters (faecal total coliform (coli) and LB count) related to intestinal dysbiosis.

Aim

The aim of the present study was to investigate the effects of *L acidophilus* D2/CSL (CECT 4529) probiotic strain on nutritional status and faecal and microbiological parameters in a group of purebred boxer dogs.

Materials and methods

Experimental design

The experimental design comprised two consecutive stages: a seven-day adaptation period, followed by a 35-day data collection experimental period.

Animals

Forty healthy adult dogs (breed: boxer; male to female sex ratio (M:F) of 1:5; age >1 year; two dogs per box, M+F, F+F; box measurement: indoor+outdoor=6 m^2+6 m^2) were randomly assigned to a control group (CTR: n=20, weight 23.1±0.7) fed a balanced commercial diet for 35 days and a treated group (LACTO: n=20, weight 23.4±0.6) receiving the same commercial diet supplemented with *L acidophilus* D2/CSL (CECT 4529). A daily health check-up was conducted by the kennel vet. Before starting the study (two weeks), an antiparasitic treatment was carried out using commercial molecule drugs with no antibacterial effect (Frontline Combo, Boehringer Ingelheim, spot on, one administration per dog; Drontal Plus Flavour, Bayer Animal Health, tablet, one administration per dog). A one-week acclimation period was applied before the data collection period.

Diets and supplementation

Dogs received a commercial extruded dry pet food with the amount calculated according to the energy maintenance requirements of adult dogs (130 kcal x $BW^{0.75}$ kg; European Pet Food Federation (FEDIAF) 2017 and National Research Council (NRC) guidelines, 350–370 g/day/dog). Feed ingredients and chemical composition are reported in table 1. The LACTO group received food supplemented with *L acidophilus* CECT 4529 to a final concentration of 5.0 x 10^9 cfu/kg of food. The dogs of the CTR group received the same diet with the same supplementation of maltodextrin but without *L acidophilus* (10 g; placebo). Probiotic dose was verified by analysing every week five samples of LACTO food (European Standard No EN 15787:2009: E-Animal feeding stuffs-Isolation and enumeration of *Lactobacillus* species). Feed intake was recorded.

Table 1 Ingredients and chemical composition of the diet fed during the trial

Ingredients: chicken (chicken 26%, total poultry 39%): maize, poultry meat meal, maize gluten meal, animal fat, digest, vegetable oil, minerals, beet pulp, flaxseed, rice, vitamins and trace elements. With natural preservatives and antioxidants.	
Analytical constituents	
	Dry matter
Protein	23.80%
Fat	16.40%
Carbohydrate (NFE)	52.80%
Fibre (crude)	1.80%
Calcium	0.81%
Phosphorus	0.70%
Sodium	0.31%
Potassium	0.75%
Magnesium	0.09%
Omega-3 fatty acids	0.51%
Omega-6 fatty acids	3.70%
Vitamin A	7180 iu/kg
Vitamin D	797 iu/kg
Vitamin E	656 mg/kg
Vitamin C	98 mg/kg
Beta-carotene	1.6 mg/kg
ME	3750 kcal/kg

ME, metabolisable energy; NFE, nitrogen-free extract.

Standard animal husbandry procedures were carried out by the same operator in both the experimental groups according to daily routine protocols for the entire duration of the experimental period.

Data collection and analysis

Nutritional status was monitored according to the Nutritional Assessment Guidelines for Dogs and Cats.[31] Body weight (BW in kg) (measured using a large pet scale, four-sensor, maximum of 100 kg, d=100 g; Momert, Dunaújváros, Hungary) and body condition score (BCS) (n=1–9; measured by the same trained operator) were monitored on days 0, 7, 14, 21, 28 and 35.

Skinfold thickness was measured using a calliper at the level of the fourth cervical vertebra (neck) and of the seventh/eighth rib on the right side (thorax) on days 7 and 35.[32]

Faecal score (FS), faecal moisture (FM) and faecal hardness (FH) and the count and identification of coli and LB were considered as indicators of the dog's gut health status.

Faecal analyses were performed on 0, 7, 14, 21, 28 and 35 days of the probiotic administration. Single samples of fresh faeces per dog were collected after deposition (08.30–09.00, 30–60 minutes after feed administration). All samples were kept in a numbered (box/dog-coded number) plastic bag, then stored at 4°C until their transport to the laboratory. Faeces analysis was carried out following a blinded sample identification protocol.

Faecal firmness was evaluated as FS using a 7-point score,[33–35] and as FM (in per cent). To measure FM, 5–10 g stool sample was weighed and dried in an oven at a temperature of 105°C–110°C for 20–24 hours and then weighed using Sartorius CP224S (maximum of 200 g, d=0.1 mg; Sartorius, Bohemia, New York, USA). Furthermore, at 0, 7, 21 and 35 days, FH (in kg) was measured on fresh faeces (50 g) with a fruit penetrometer 53220 FTA (GUSS Manufacturing, South Africa), replacing the supplied punch (cone) with a 4 x 4 cm plate. This modification was necessary to facilitate assessment of faecal consistency because the faeces are softer than the fruits pulp; three repetitions per sample were performed.

Microbiological analysis

Faeces were collected at 7 and 28 days following the described procedure and were analysed from each dog. An aliquot of fresh faeces (1 g) was diluted in sterile saline solution with a ratio of 1:10. Diluted faeces were vortexed for two minutes to obtain a homogeneous suspension and were streaked on different culture media for total bacterial count and for bacterial identification. For *E coli* and total coli, eosin methylene blue agar (Oxoid, Italy) was used. After an incubation time (24 hours) at 37°C, *E coli* colonies have grown with a green metallic reflex, while coli have grown with blue or red or uncoloured colonies. De Man, Rogosa and Sharpe agar (Oxoid) was used for the growth and enumeration of *Lactobacillus* species, incubating plates under anaerobic conditions at 37°C for 48 hours.

The data obtained were analysed using MIXED, GLM and NPAR1WAY procedures (SAS V.9.4), with P≤0.05 considered statistically significant.

Results and discussion

All dogs were healthy throughout the study. During the study no changes in feed consumption were recorded (350–370 g/day/dog; waste=0 g throughout the experimental period).

No differences in BW (CTR=23.5±0.7 kg v LACTO=23.9±0.6 kg) and skin thickness (neck, CTR=4.97±0.34 mm v LACTO=5.37±0.33 mm; thorax, CTR=4.80±0.38 mm v LACTO=4.40±0.37 mm) were found in dogs receiving treatment and control diets during the whole experimental period. The average skin thickness in dogs varies from 0.5 to 5 mm depending on the breed,[22] and these results are consistent throughout the experimental period in the two experimental groups characterised by standardisation of breed, sex ratio, feed, environment and management. Skinfold measurements are well-known procedures in nutritional status evaluation and obesity quantification and monitoring.[36]

Dogs in the LACTO group showed a significantly higher BCS than those in the CTR group throughout the experimental period (4.65±0.65 v 4.86±0.55) and at 7 and 14 days (4.75±0.45 v 5±0.00) (table 2). These results suggest that the supplementation of *L*

Table 2 Effect of *Lactobacillus acidophilus* D2/CSL addition to diet on body condition score: descriptive statistics and results from Kruskal-Wallis test

Period	Group	Mean	sd	Median	Percentile (25th, 75th)
Overall period	CTR	4.65	0.65	5[a]	4, 5
	LACTO	4.86	0.55	5[b]	5, 5
0 day	CTR	4.88	0.34	5	5, 5
	LACTO	4.96	0.20	5	5, 5
7 days	CTR	4.75	0.45	5[a]	4.5, 5
	LACTO	5	0	5[b]	5, 5
14 days	CTR	4.75	0.45	5[a]	4.5, 5
	LACTO	5	0	5[b]	5, 5
21 days	CTR	4.63	0.62	5	4, 5
	LACTO	4.71	0.69	5	4.5, 5
28 days	CTR	4.44	0.89	5	4, 5
	LACTO	4.75	0.79	5	4, 5
35 days	CTR	4.44	0.89	5	4, 5
	LACTO	4.75	0.79	5	4, 5

Within each period, medians with different superscript letters differ (P<0.05).
CTR, control group; LACTO, treated group.

Table 3 Effect of *Lactobacillus acidophilus* D2/CSL addition to diet on dog performance: least square means (±se) relative to CTR and LACTO groups for FM and FH

	Groups		P value
	CTR	LACTO	
FM (%)			
Overall period	0.69±0.007	0.67±0.007	0.0198
0 day	0.66±0.013	0.68±0.013	0.5169
7 days	0.71±0.016	0.72±0.012	0.3354
14 days	0.66±0.012	0.63±0.012	0.0756
21 days	0.70±0.013	0.65±0.012	0.0010
28 days	0.73±0.013	0.68±0.012	0.0040
35 days	0.69±0.013	0.68±0.012	0.7295
FH (kg)*			
Overall period	0.70±0.051	0.86±0.047	0.0035
0 day	0.62±0.066	0.69±0.057	0.2958
7 days	0.49±0.066	0.57±0.057	0.2741
21 days	0.88±0.066	1.11±0.057	0.0024
35 days	0.82±0.066	1.09±0.057	0.0002

*Pressure related to a 4 x 4 cm plate.
CTR, control group; FH, faecal hardness; FM, faecal moisture; LACTO, treated group.

acidophilus CECT 4529 could improve the nutritional status of dogs. BCS is a direct method for evaluating nutritional status, with scores ranging from 1 to 9 and with the ideal body condition being a score of 4 or 5 depending on the breed.[37] In the ideal range, the body fat ratio can be assumed to range between 15 and 25 per cent, and in these scores (4–5) the ideal BW can be assumed. Jeusette *et al*[38] estimated a 19±8 per cent fat mass in dogs with a BCS score of 5.

Some authors even observed an antiobesity effect of probiotic LB. For example, a strain of *L gasseri* (LG2055) significantly prevented BW gain, fat accumulation and proinflammatory gene expression in the adipose tissue of obese mice.[39] In dogs, Park *et al*[40] reported how the gut microbiome, through vagal afferent neurons, is able to regulate neuronal signalling to the brain. They described how obesity could be linked to microbiota composition and serotonin concentrations in the CNS.

Wang *et al*[11] demonstrated the utility of *L paracasei* CNCM I-4270, *L rhamnosus* I-3690 and *Bifidobacterium animalis lactis* I-2494 strains to individually attenuate high-fat diet-induced obesity, inflammation and metabolic syndrome in mice. Although a definitive explanation of the antiobesity effect of some probiotic strains does not exist, it is known that the intestinal microbiota is involved in the regulation of fat storage in dogs.[40 41] Microbiota of obese mice leads to an increased concentration of fermentation end products butyrate and acetate, so it is more efficient at extracting energy from a given diet than the microbiota of lean animals.[8]

The results of this study are consistent with the observations of different authors,[9 42 43] and suggest that administration of probiotic may favour a different equilibrium in the intestinal microbiota and is less effective in fermenting the indigestible residues of the diet, therefore providing better control of weight in adult boxer dogs.

Considering faecal parameters, a lower FM in the LACTO group was recorded compared with the CTR group (0.69±0.007 v 0.67±0.007, P≤0.05) throughout the experimental period. Similar results were pointed out in FH and FS. Throughout the experimental period, FH was also higher in the LACTO group (table 3) compared with the CTR group (0.86±0.047 kg v 0.70±0.051 kg, P≤0.05).

Significant differences in FS were found in the five-week period (table 4): lower scores were detected in the LACTO group compared with the CTR group (3.78±0.95 v 4.25±0.91). All the results concerning faecal parameters (FS, FM, FH) indicate an improvement in faecal consistency in the LACTO group.

A significant difference in total coli log counts was only detected at 28 days between the CTR (4.92) and LACTO (5.59) groups (table 5). LB was detected in stools at 28 days, and counts of these bacteria were

Table 4 Effect of *Lactobacillus acidophilus* D2/CSL addition to diet on faecal score: descriptive statistics and results from Kruskal-Wallis test

Period	Group	Mean	sd	Median	Percentile (25th, 75th)
Overall period	CTR	4.25	0.91	4[a]	4, 5
	LACTO	3.78	0.95	4[b]	3, 5
0 day	CTR	4.88	0.34	5	5, 5
	LACTO	4.83	0.64	5	4, 5
7 days	CTR	4.94	0.25	5	5, 5
	LACTO	4.83	0.48	5	5, 5
14 days	CTR	3.93	1.62	4	3, 5
	LACTO	3.11	0.88	3	2, 4
21 days	CTR	3.86	0.53	4[a]	4, 4
	LACTO	3.25	0.44	3[b]	3, 3.5
28 days	CTR	3.86	0.53	4[a]	4, 4
	LACTO	3.25	0.44	3[b]	3, 3.5
35 days	CTR	3.86	0.53	4[a]	4, 4
	LACTO	3.25	0.44	3[b]	3, 3.5

Within each period, medians with different superscript letters differ (P<0.05).
CTR, control group; LACTO, treated group.

Table 5 Effect of *Lactobacillus acidophilus* D2/CSL addition to diet on dog performance: least square means (±se) relative to faecal total coliform (coli) and lactobacilli (LB) counts

	Groups		
	CTR	LACTO	P value
Coli (log_{10}(N))			
Overall period	4.54±0.24	4.71±0.15	0.3053
7 days	4.16±0.17	3.84±0.17	0.1227
28 days	4.92±0.16	5.59±0.17	0.0023
LB (log_{10}(N))			
28 days	4.50±0.22	5.64±0.26	0.0005

CTR, control group; LACTO, treated group.

significantly (P≤0.01) higher in the LACTO group (5.64±0.26) than in the CTR group (4.50±0.22).

Dysbiosis, or the unbalance between lactic acid bacteria and putrefactive and/or pathogenic ones, is commonly observed in people and animals. Bacterial enteropathogens (*C difficile*, *C perfringens*, *Salmonella* ser, *Campylobacter jejuni* and pathogenic *E coli*) have been frequently isolated from the faeces of clinically healthy dogs and cats.[23] Release of toxic bacterial metabolites is quantitatively dependent on the type of fermentations that occur in the bowel,[5] and putrefactive fermentation profiles can have detrimental effects on the intestinal mucosa and faecal consistency. Ammonia and valeric acid concentrations were higher in soft stools, suggesting a higher level of protein fermentation in softer faeces.[44]

Reported results are in accordance with those written by different researchers who described the ability of *L acidophilus* to inhibit the growth of potentially pathogenic bacteria[45] and to improve immune function and intestinal health in dogs.[46] Some of the tested parameters have also been used by Pascher *et al* in 2008[47] to evaluate feed tolerance in dogs with non-specific dietary sensitivity. In agreement with the results of the present study, they found that faecal consistency and faecal dry matter were improved by inclusion of *L acidophilus* in dogs' diet.

The nutritional status and the gut status parameters that the authors have evaluated in healthy dogs were improved by addition of *L acidophilus* D2/CSL (CECT 4529) to diet. Moreover, considering the findings of Herstad *et al* (2010),[48] a further potential use in case of self-limiting diarrhoea could be suggested.

Conclusions

The inclusion of *L acidophilus* D2/CSL (CECT 4529) at the recommended dosage of (at least) 5.0 x 10^9 cfu/kg of dry food showed a significant positive effect on faecal consistency (FS, FH and FM) in adult dogs. In addition, the count of faecal LB was higher in dogs fed with diet supplemented with *L acidophilus* D2/CSL. A significant positive effect on the nutritional status of dogs was highlighted, given the ideal BCS of around 5 reported in the results. Further studies could be carried out focusing on the antiobesity effects of *L acidophilus* strains. Considering faecal quality, the importance of faecal dryness in dogs management in indoor (soiling pet animals) situation and in urban areas where faeces consistency could favour collection and elimination procedures is helpful.[49]

In conclusion, the supplementation of *L acidophilus* D2/CSL (CECT 4529) significantly improved the welfare of boxer dogs, improving their gut health and in turn the quality of their stools. Furthermore, the nutritional status of dogs was positively influenced.

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Competing interests None declared.

Ethics approval The experimental procedures used in this study were reviewed and approved by the Institutional Committee for Animal Care of the University of Milan, Italy (approval number 48/15, 12 October 2015).

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