Veterinary Record Open

OPEN ACCESS

Effects of *Lactobacillus acidophilus* D2/CSL (CECT 4529) supplementation on healthy cat performance

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To cite: Fusi E, Rizzi R, Polli M, *et al*. Effects of *Lactobacillus acidophilus* D2/CSL (CECT 4529) supplementation on healthy cat performance. *Veterinary Record Open* 2019;**6**:e000368. doi:10.1136/vetreco-2019-000368

Received 30 July 2019
Revised 28 November 2019
Accepted 29 November 2019

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ABSTRACT

Background The present study aimed to evaluate the effects of the probiotic strain *Lactobacillus acidophilus* D2/CSL (CECT 4529) on nutritional condition and faecal quality in cats.

Methods Ten healthy adult cats from the same cattery were included (aged >9 months; male:female sex ratio=3:7). The animals were randomly assigned to a control group (CTR; n=5; male:female=1:4; room 1: 16 m^2) and to a treated group (LACTO; n=5; male:female=2:3; room 2: 16 m^2) receiving the same commercial dry diet. The LACTO group diet was supplemented with the probiotic (5 x 10^9 cfu/kg feed at least). A five-week experimental period was applied, and nutritional status was monitored by bodyweight (BW) and body condition score (BCS). Faecal quality was evaluated using faecal score (FS) and faecal moisture (FM) parameters. Plate counts of some faecal bacteria species were carried out. The data obtained were analysed using MIXED, GLM and NPAR1WAY procedures (SAS V.9.4; P≤0.05).

Results The two groups did not show differences in BW and BCS data. A clear effect of the probiotic supplementation on FM was recorded (LACTO 44 per cent v CTR group 46 per cent; P=0.04). FS in the LACTO group (3.35) was close to ideal values (2–3) in comparison with the CTR group (3.75). Positive effects of *L acidophilus* D2/CSL have been recorded in terms of increase in faecal lactobacilli counts and reduction in faecal coli counts.

Conclusions This study's preliminary results describe how inclusion of *L acidophilus* D2/CSL (CECT 4529) probiotic strain in cats' diets could effectively improve faecal quality parameters and consequently gut health in adult healthy cats.

INTRODUCTION

All animals are characterised by a complex variety of microorganisms in their gastrointestinal (GI) tract. The equilibrium of this complex system and its interaction with the host have relevant consequences on the animal's general health and welfare.[1] The microbiota, in fact, plays several functions that lead to an improvement in the host's general health and performance. Positive effects were recorded in counteracting activity against pathogens (eg, *Salmonella* species, *Campylobacter jejuni*, *Yersinia* species),[2] in food digestion and energy metabolism optimisation, and in enterocytes' nutritional status.[3] A species-specific microbiota composition has been described. Furthermore, a similar microbiota composition has been recorded in the same species even with very different geographical locations.[4] Most of the microbes populating the GI tracts of cats and dogs belong to the phyla Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria and Actinobacteria.[1 5]

The well-known *Lactobacillus* species (*L acidophilus*, *L salivarius*, *L johnsonii*, *L reuteri* and *L sakei*), belonging to the Firmicutes phyla, have been described in canine, feline as well as in human intestine. Jacobsen and colleagues[6] reported the importance of lactobacilli in the correct maintenance of the intestinal microbial ecosystem. Within the many activities of lactobacilli, a pivotal role has been described in oxidative status regulation, antimicrobial metabolite production and enteropathogen proliferation inhibition.[7]

Several studies in dogs and cats have shown an association between alteration of GI microbiota composition (called dysbiosis) and intestinal inflammatory and stress-associated diseases.[2 8–13] Microbial imbalances have been manipulated using several approaches focusing on diets, prebiotics, probiotics, synbiotics, antibiotics and faecal microbiota transplantation.[9] Due to their beneficial effects on gut health, an increasing inclusion of probiotics in both human and animal diets has been reported. *Lactobacillus* and *Bifidobacterium* species are the most commonly studied and used bacteria.[11 14] In the literature, for example, the administration of *L acidophilus* has been shown to improve the GI microbial balance and to induce immunostimulatory effects in dogs and to stimulate appetite and growth in puppies.[11 15] Research on cat microbiota is quite rare, and the only specific clinical trial reported a positive response with

regard to the general health of the animals under study.[11] Species-specific trials are needed considering the high specificity of microbiota composition in different animal species. The general positive trend in the market infusion of probiotic products requires scientific support in evaluating product efficacy and improvement. Furthermore, there is still a need to develop novel strains that can be included in animals' diet and that can provide adequate and effective action to optimise the positive effects of lactobacilli on animals' performance and general health status.[5 16]

The present study aimed to evaluate the effects of *L acidophilus* D2/CSL (CECT 4529) on nutritional conditions and faecal quality in healthy cats.

MATERIALS AND METHODS

Animals and study design

A total of 10 healthy adult Maine Coon cats were selected from the same World Cat Federation (WCF)-registered cattery (aged >9 months; male:female sex ratio=3:7). The animals were randomly assigned to a control group (CTR; n=5; male:female=1:4; mean age: 43.2 months; room 1: 16 m^2) and to a treated group (LACTO; n=5; male:female=2:3; mean age: 44.6 months; room 2: 16 m^2) receiving the same commercial dry diet. The LACTO group diet was supplemented with *L acidophilus* CECT 4529. Cleaning and disinfecting procedures of the two rooms were carried out according to the routine practice of the cattery. When the dietary acclimation period of two weeks was completed, an antiparasitic (ecto and endo) treatment was administered and the cats were evaluated daily by a veterinarian for any health and welfare concerns throughout the experimental period (two-week acclimation and five-week study).

Feed supplement and diet

A standard premium commercial diet for adult cats (table 1) was fed to both the experimental groups, CTR and LACTO, throughout the study. *L acidophilus* CECT 4529 as a freeze-dried microbial preparation of *L acidophilus* D2/CSL, produced by Centro Sperimentale del Latte Srl (Zelo Buon Persico, Lodi, Italy), was added to the LACTO group diet. The additive has been authorised by the Commission Implementing Regulation (EU) No 2015/38 (EU ID No 4b1715) to belong to the 'gut flora stabilisers' functional group, and defined as 'microorganisms or other chemically defined substances, which, when fed to animals, have a positive effect on the gut flora'. Cats were fed a commercial dry pet food twice daily based on their maintenance energy requirements (adult cats: 100 kcal x $BW^{0.67}$ kg)[17] and they had free access to potable water.

Table 1 Diet chemical composition fed in cats

	As fed	Dry matter
Moisture (%)	9	
Crude protein (%)	31.6	34.73
Fat (%)	7.9	8.68
Fibre (crude) (%)	7.6	8.35
Calcium (%)	0.94	1.03
Phosphorus (%)	0.65	0.71
ME (kcal/kg)	3150	

ME, Metabolizable Energy.

Cats belonging to the LACTO group received commercial food with the addition of 10 g/100 kg of *L acidophilus* CECT 4529, corresponding to (at least) 5 x 10^9 colony-forming units (cfu)/kg food. The CTR group received the same commercial diet, with the supplementation of maltodextrin only (placebo). The experiment was double-blinded. Every week a sample of the LACTO diet was analysed in order to monitor the concentration of *L acidophilus* CECT 4529. The results showed that the concentration of the microorganism corresponded to expectations.

Data collection

Bodyweight (BW) and body condition score (BCS) were recorded at weeks 0 (T0), 2 (T1), 4 (T2) and 5 (T3), according to the American Animal Hospital Association (AAHA) Nutritional Assessment Guidelines for Dogs and Cats.[18] The BW of each animal was measured by the same person at the same time (morning, before feed administration), with the same instrument. At the same time, BCS assessment was carried out by visual examination and palpation of the animal on a scale between 1 and 9, where a score of 4 or 5 reflects an ideal body condition.[18] To evaluate the effect of the probiotic on faecal quality, an assessment of faecal score (FS) and faecal moisture (FM) was performed. Furthermore, some GI bacterial species were identified and their species count was investigated. On the cattery, faecal firmness was first evaluated as FS using a 7-point score according to Bybee and colleagues,[19] as described in table 2, at all four sampling times (T0–T3).

In the laboratory, collected faecal samples were analysed to determine FM. Faecal sampling was carried out at T0, T1, T2 and T3, and the collected samples were stored at +4°C until they are brought to the laboratory, where they are stored at −20°C. An aliquot of 5–10 g of stool was weighed and dried in an oven at a temperature of 105°C–110°C for 20–24 hours, cooled down in a desiccator for another 20–24 hours, after which the FM content was calculated as lost weight after desiccation.

Microbiological analysis was performed at T1 and T3. One gram of fresh stool was diluted in sterile saline solution with a ratio of 1:10. Diluted faeces were vortexed for two minutes to obtain a homogeneous suspension, which was then streaked on different culture media for total bacterial count and for bacterial identification. Specifically, for *Escherichia coli* and total coliforms (Coli), eosin methylene blue agar (Oxoid, Italy) was used. After an incubation time of 24 hours at 37°C, *E coli* colonies show growth with a green metallic reflex, while coliforms show

Table 2 Faecal scoring chart by Nestle Purina faecal score system (modified)

Score	Characteristics
1	► Very hard and dry. ► Often expelled as individual pellets. ► Requires much effort to expel from the body. ► Leaves no residue on ground when picked up.
2	► Firm, but not hard, pliable. ► Segmented in appearance. ► Little or no residue on ground when picked up.
3	► Log-shaped, moist surface. ► Little or no visible segmentation. ► Leaves residue on ground, but holds form when picked up.
4	► Very moist and soggy. ► Log-shaped. ► Leaves residue on ground and loses form when picked up.
5	► Very moist but has a distinct shape. ► Present in piles rather than logs. ► Leaves residue on ground and loses form when picked up.
6	► Has texture, but no defined shape. ► Present as piles or spots. ► Leaves residue on ground when picked up.
7	► Watery. ► No texture. ► Present in flat puddles.

growth with blue, red or uncoloured colonies. For lactobacilli, Man, Rogosa and Sharpe agar (Oxoid) was used and plates were incubated under anaerobic condition at 37°C for 48 hours.

Statistical analysis

The data obtained were analysed using MIXED, GLM and NPAR1WAY procedures (SAS V.9.4), with P<0.05 considered statistically significant.[20]

In particular, BW and FM of cats recorded over time were analysed by analysis of variance according to the repeated measurement model using the MIXED procedure. The statistical model was built as the following: yijkln = μ + Si + Gj + Tk + GTjk + klj + 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.

Microbiological parameters (Coli and lactobacilli) and FS were compared using Kruskal-Wallis test in relation to the group (CTR and LACTO) and time of data collection using the NPAR1WAY procedure. If the result of Kruskal-Wallis test was significant, a multiple comparison analysis based on pairwise two-sample Wilcoxon comparisons was performed.

Table 3 Effect of *Lactobacillus acidophilus* D2/CSL addition to diet on faecal moisture in cats: results of mixed models showing least square means (±se) in CTR and LACTO cats for the four individual sampling times and overall throughout the study, taking into account repeated measures

	Faecal moisture		
Time	CTR	LACTO	P value
Overall period	0.46±0.007	0.44±0.007	0.048
T0	0.47±0.017	0.45±0.017	0.4
T1	0.43±0.013	0.42±0.013	0.5
T2	0.46±0.013	0.44±0.013	0.3
T3	0.47±0.015	0.43±0.013	0.09

CTR, control group; LACTO, treated group; T0, week 0; T1, week 2; T2, week 4; T3, week 5.

RESULTS

All cats were healthy during the trial and no side effects in the LACTO group were recorded. No residual pet food was found after consumption throughout the experimental period. BCS did not change during the trial in either group, and the animals maintained an ideal body condition. BW data show no differences between the two groups, with a mean BW of 6.9 kg throughout the study. FM was significantly lower throughout the trial in the LACTO group (44 per cent) compared with the CTR group (46 per cent) (P=0.04; table 3). A lower humidity content was found in the last week of the experimental period (T3) in the faecal samples of the LACTO group compared with the value recorded in the CTR group (43 per cent v 47 per cent; P=0.08). A positive effect of *L acidophilus* D2/CSL supplementation on faecal quality was confirmed by FS evaluation; cats in the LACTO group showed drier faeces compared with cats in the CTR group

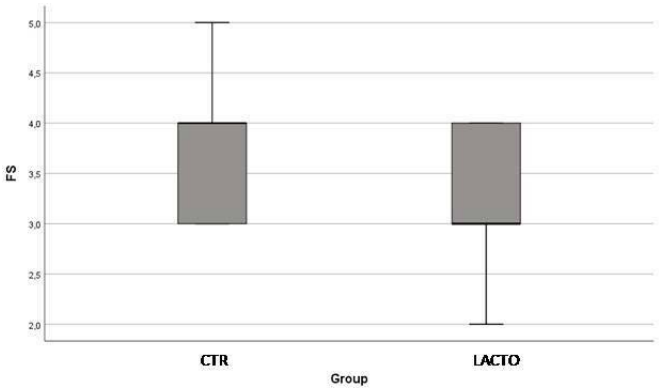

Figure 1 Box plot showing the effect of *Lactobacillus acidophilus* D2/CSL addition to diet on the faecal score (FS) of Maine Coon cats in the overall period (P<0.10; Kruskal-Wallis test). CTR, control group; LACTO, treated group.

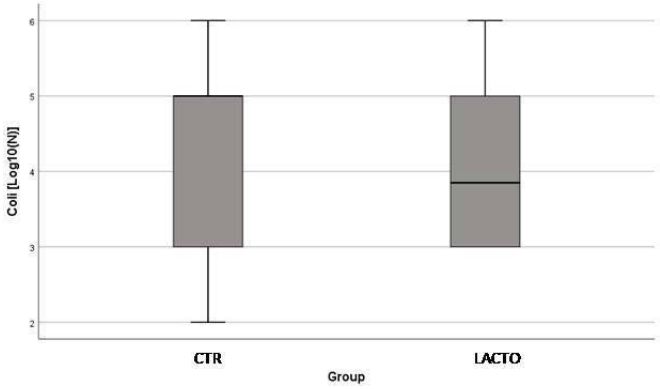

Figure 2 Box plot showing the effect of *Lactobacillus acidophilus* D2/CSL addition to diet on total coliform (Coli) in the overall period (P>0.10; Kruskal-Wallis test). CTR, control group; LACTO, treated group.

at T2 (figure 1). The results of the microbiological investigations are reported in figures 2 and 3.

DISCUSSION

Probiotics are commonly used in production animals to improve productive performance, but there is also an increasing interest in their supplementation in human and companion animals' diets.[6 9 12 14 21 22] Although several scientific studies reported beneficial effects of probiotics on gut health in human beings and dogs affected by GI disorders, few studies on cats have been performed. The characteristics of probiotic supplementation require species-specific trials, particularly for strictly carnivore pets such as cats.

In the present study the authors tested *L acidophilus* D2/CSL (CECT 4529) as a feed additive in healthy cats. This strain already has good evidence base with regard to its efficacy, especially on broilers and laying hens, showing improvement in gut health and performance.[23–25] BW was consistent throughout the study period in both groups of cats, and this is similar to what has been described by Marshall-Jones and coauthors,[11] who used *L acidophilus* DSM13241. A similar consistency was recorded for BCS,

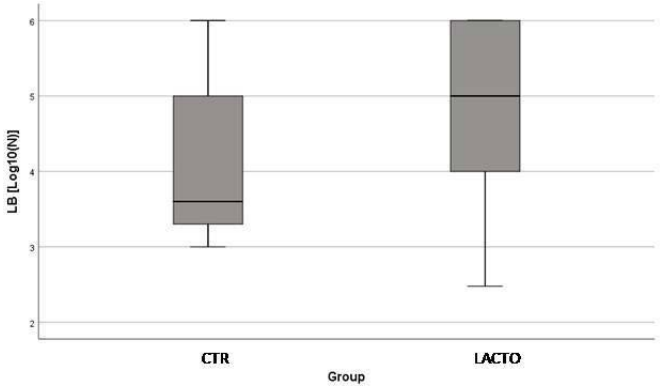

Figure 3 Box plot showing the effect of *Lactobacillus acidophilus* D2/CSL addition to diet on lactobacilli (LB) in the overall period (P>0.10; Kruskal-Wallis test). CTR, control group; LACTO, treated group.

underlining the maintenance of ideal nutritional status in carnivores such as cats. BCS is the most widely used method for assessing cats' nutritional status. It is an easily perceptible parameter commonly used to determine overweight and obesity status.[26] Furthermore, every pet owner should be able to evaluate the nutritional status of their pet using BCS. Many positive effects of inclusion of *L acidophilus* in the diet have been described in different animal species, where several lactobacilli strains have, for example, demonstrated significant effects on growth and appetite in puppies[27] and in companion animals and growth performance in production animals.[24 28–31]

In this study the authors also evaluated FM and FS as relevant gut functionality indicators as these could be altered from normal values depending mainly on the type of diet and occurrence of GI diseases or dysbiosis. Moisture content can determine whether faeces appear soft or firm. However, excluding infectious diarrhoea, the possible causes of soft faeces in cats and dogs are still debated. Rolfe and colleagues[22] stated that a shorter transit time reduces the capacity to absorb water and electrolytes in the colon, leading to the production of softer stools. However, others state that water and electrolyte absorptions are not important determinants of FM. Indeed, higher fermentation of undigested soluble fibres or poorly digested proteins in the colon produce excessive fermentation and can result in softer stools.[32] Thus, softness and increased moisture content of faeces are important criteria by which the US National Research Council has established safe upper limits for the inclusion of carbohydrates in pet foods.[32] A significant reduction in FM was observed throughout the study period. As for medians in FS, the LACTO group showed a mean score closer to the ideal compared with the CTR group. The change of these two parameters is a proof that *L acidophilus* CSL/D2 seems to influence and has a good effect on the moisture content of stools in healthy cats, making the stools more consistent. On the contrary, in another study on healthy cats, the FS remained unchanged with the administration of *L acidophilus* DSM13241.[11] The same lack of effects on faecal quality parameters was described in a study performed on healthy dogs where *L acidophilus* NCDC 15 had no influence on the FS.[11]

Culture-based identification methods were used to assess GI bacteria and microflora in the animals of the present study. The effects of the administration of *L acidophilus* D2/CSL in reducing the total coliform counts and increasing the lactobacilli counts in the LACTO group compared with the CTR group have been reported. Coliform populations were found to decrease in the treated group, meaning that probiotics have a slight protective effect on invasive bacteria species. An increase in the lactobacilli count occurred in the LACTO group, meaning that positive changes in the microbiota occurred, and this can help animals to restore their correct microbiome balance in case of dysbiosis. Similar results were observed in the study performed on cats by Marshall-Jones and colleagues.[11] Bacterial enteropathogens (*Clostridium difficile, C perfringens, Salmonella ser, C jejuni*

and pathogenic *E coli*) have been frequently isolated from the faeces of clinically healthy dogs and cats. Dysbiosis, as a result of an imbalance among lactic acid bacteria (lactobacilli, in particular) and pathogenic bacteria, is commonly observed in animals. An altered intestinal microbiota can release toxic bacterial metabolites in a manner quantitatively dependent on the type of fermentation that occurs in the bowel.[33] Putrefactive fermentation profiles can have detrimental effects on the intestinal mucosa and faecal consistency,[34] leading to excretion of softer or watery stools as reported for dogs and cats by Weese and colleagues in 2004[35] and by Marks and coauthors 10 years later.[36] It could be argued that the probiotic balances the intestinal microbiota, reducing the number of putrefactive and proinflammatory bacteria and increasing the population of lactic acid bacteria. Restoration of intestinal eubiosis has immunomodulatory and anti-inflammatory effects due to the positive interaction of probiotic bacteria with epithelial cells and dendritic cells and with monocytes/macrophages and lymphocytes.

CONCLUSION

In conclusion, dietary inclusion of the probiotic strain *L acidophilus* D2/CSL (CECT 4529) appears to improve faecal quality parameters such as FM and FS in adult healthy cats. Furthermore, an apparent positive effect on increasing lactobacilli counts and decreasing total coliform counts has been shown. This study's findings suggest that the supplemented specific strain of intestinal origin seemed to express a good ability to multiply in the feline intestine and to colonise it. All cats maintained an ideal BCS and BW during the five-week trial. Further studies with a larger sample of healthy cats and a comparison with cats experiencing GI pathology could be carried out to investigate the effect of the tested strain on carnivore dysbiotic gut.

Contributors All authors contributed to the paper in the same proportion. EF and SPM: study planning, data collection and manuscript writing. MP: data collection. SC: conducted the survey. AG and NB: supplement preparation and analysis.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Ethics 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, October 12, 2015).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement No data are available.

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