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

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## Abstract

The presented study aims to evaluate the effects of the probiotic strain of L. acidophilus D2/CSL (CECT 4529) on nutritional condition and faecal quality in healthy cats. Ten healthy adult cats from the same cattery were included (age > 9 months; sex ratio M÷F = 3÷7). The animals were randomly assigned to a control group (CTR; N= 5; M÷F=1÷4, room 1 16 m2) and to a treated group (LACTO; N=5; M÷F=2÷3; room 2 16 m2) receiving the same commercial dry diet. LACTO group diet was supplemented with the probiotic; (5*109 CFU*kg-1 feed at least.). A five weeks experimental period was applied, nutritional status was monitored by Body weight (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. Obtained data were analyzed using MIXED, GLM and NPAR1WAY procedures (SAS® 9.4; P ≤ 0.05). BW and BCS data show no differences in the two groups. A clear effect of the probiotic supplementation on FM was recorded (LACTO 44% vs CRT group 46%; P= 0.04). FS in LACTO group (3.35) was close to ideal values (2-3) in comparison to CTR (3.75) group. Positive effects of L. acidophilus D2/CSL have been recorded in the increase of faecal lactobacilli counts and reduction of faecal Coli counts. In conclusion our preliminary results describe how L. acidophilus D2/CSL (CECT 4529) probiotic strain inclusion in cats' diets could effectively improve faecal quality parameters and consequently gut health in adult healthy cats.

**Key words**

*Lactobacillus acidophilus,* probiotic, cat, microbiota, faecal consistency, Coliforms

## Introduction

All animals are characterized by a complex variety of microorganism in their gastrointestinal (GI) tract. The equilibrium of this complex system and its interaction with the host have relevant consequences on general animal health and welfare (1) The microbiota, in fact, plays several functions leading to the improvement of host's general health and performance. Positive effects were recorded in counteracting activity against pathogens (e.g. *Salmonella* spp., *Campylobacter jejuni*, *Yersinia* spp.) (2), in food digestion and energy metabolism optimization and in enterocytes' nutritional status (3). A specie-specific microbiota composition has been described, furthermore a constant in microbiota composition was recorded in the same species even with very different geographical position (4). The microbes populating the GI tracts of cats and dogs are mostly belonging to the phyla Firmicutes, Bacteroidetes, Proteobacteria, and Fusobacteria, and Actinobacteria (1,5).

The well-known *Lactobacillus spp.* (*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 metabolites production and enteropathogens proliferation inhibition (7)

Several studies in dogs and cats pointed out the association between GI microbiota alteration of composition (called dysbiosis) and intestinal inflammatory and stress-associated diseases (2,8–13). Microbial imbalances have been manipulated throughout several approaches focusing on diets, prebiotics, probiotics, synbiotics, antibiotics and faecal microbiota transplantation (FMT) (9). An increasing inclusion of probiotics in both humans and animal's diets for their beneficial effects on the gut health has been reported. *Lactobacillus and Bifidobacterium* spp are the most commonly studied and used bacteria (11,14). In literature, for example, the administration of *Lactobacillus acidophilus* has been shown to improve the gastrointestinal microbial balance and induce immunostimulatory effects in dogs and to stimulate appetite and growth in puppies (11,15). Researches about cat microbiota are quite rare and the only specific clinical trial reports positive response on the general health of the animals under study (11). Specie specific trials are needed considering the high specificity of microbiota composition in the different animal species. The general positive trend in the market diffusion of probiotic products requires an scientific support in the evaluation of products efficacy and improvement, furthermore, the development of novel strains to be included in the animals' diets can supply adequate and effective action in the optimization of the positive effects of lactobacilli in animals' performance and general health status (5,16). The presented study was aimed to evaluate the effects of *Lactobacillus acidophilus* D2/CSL (CECT 4529) on nutritional conditions and faecal quality in healthy cats.

## Materials & Methods

*Animals and study design*

A total of 10 healthy adult cats were selected in the same cattery (age > 9 months; sex ratio M÷F = 3÷7). The animals were randomly assigned to a control group (CTR; N= 5; M÷F=1÷4, mean age: 43.2 months; room 1 16 $m^2$) and to a treated group (LACTO; N=5; M÷F=2÷3; mean age: 44.6 months; room 2 16 $m^2$) receiving the same commercial dry diet. LACTO group diet was supplemented with *L. acidophilus* CECT

4529. Cleaning and disinfecting procedures were carried out according to the routine practice. When the dietary acclimation period (2wks) started an antiparasitic treatment was carried out Animal's health and welfare conditions were daily evaluated by a veterinarian all over the experimental period.

*Feed supplement and Diet*

A standard premium commercial diet for adult cats (Table 1) was fed to both the experimental groups CTR and LACTO. An addition of *Lactobacillus acidophilus* CECT 4529, a freeze dried microbial preparation of *Lactobacillus acidophilus* D2/CSL, produced by Centro Sperimentale del Latte S.r.l. (Zelo Buon Persico, Lodi, Italy) has been included in LACTO group diet. The additive has been authorised by the Commission Implementing Regulation (EU) No 2015/38 (EU id. No 4b1715) in the functional group "*gut flora stabilisers*", and defined as *"micro-organisms or other chemically defined substances, which, when fed to animals, have a positive effect on the gut flora*".

During the whole experimental period, cats were fed a commercial dry pet food. Twice daily they received based upon their maintenance energy requirements [adult cats: 100kcal*$BW^{0.67}$ kg] (17)cats had free access to potable water.

Cats belonging to the LACTO group received the commercial food with the addition of 10g/100 kg of *L. acidophilus* CECT 4529, corresponding to (at least) $5*10^9$ $CFU*kg^{-1}$ food. The CTR group received the same commercial diet, with the supplementation of maltodextrin only (placebo). All over the experimental period 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 was corresponding to expectations.

*Data collection*

Cat performance was evaluated through nutritional parameters according AAHA Nutritional Assessment Guidelines for Dogs and Cats (18) Body weight (BW) and Body Condition Score (BCS) were recorded at week 0 (T0), 2 (T1), 4 (T2) and 5 (T3). The BW of each animal was measured by the same operator at the same time (morning, before feed administration). 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 is reflecting the ideal body condition (18).

To evaluated effect of the probiotic inclusion on faecal quality, Faecal Score (FS) and Faecal moisture (FM) were performed. Furthermore, identification and count of some gastrointestinal bacterial species were investigated.

On field, faecal firmness was firstly evaluated as FS using a 7-point score according to Bybee and colleagues (19) at T 0-3. In the laboratory, collected faecal samples were analysed to determine the Faecal Moisture (FM).

Faecal sampling was carried out at T0, T1, T2, and T3, collected samples were stocked at +4°C until their arrival at the laboratory, then stored at -20°C. 5-10g of stool were weighed and dried in an oven at a temperature of 105–110 °C for 20–24 h, cooled down in a desiccator for another 20–24 h, samples' faecal humidity was calculated as lost weight after exsiccation.

Microbiological analysis was performed at T1 and T3. 1 g of fresh stool was diluted in sterile saline solution with a ratio of 1:10. Diluted faeces were vortexed for 2 min to obtain a homogenous suspension. Then, they were streaked on different culture media for total bacterial count and for bacterial identification. Specifically, for *Escherichia coli* and total coliforms (Coli), EMB (Eosin Methylene Blue Agar, Oxoid, Italy) was used; after an incubation time of 24 h at 37 °C, *E. coli* colonies have grown with a green metallic reflex, while coliforms have grown with blue or red or uncoloured colonies. For *Lactobacilli* (LB), MRSA (Man Rogosa and Sharpe Agar, Oxoid, Italy) agar was used and plates were incubated under anaerobic condition at 37 °C for 48 hours.

*Statistical Analysis*

Obtained data were analyzed using MIXED, GLM and NPAR1WAY procedures (SAS® 9.4) with $P \leq 0.05$ considered statistically significant (20).

**Results**

All the cats were healthy throughout the trial and no side effects in the LACTO group were recorded. No residual pet food was found after consumption all over the experimental period. BCS did not vary during the trial in both groups, animals maintained their ideal body conditions. Body weights data (BW) show no differences between the two groups, the mean value for both groups all over the period was 6.9 kg.

As reported in table 2 FM was significantly lower throughout the trial in the LACTO group (44%) compared to the CRT group (46%) (P= 0.04). A lower humidity content has been found in the last week of the experimental period (T3) in the faecal samples of the LACTO group compared to the value recorded in the CTR group (43% vs 47%; P= 0.08). The same results describing the positive effects of *Lactobacillus acidophilus* D2/CSL supplementation are confirmed by FS evaluation (Tab 3). Cats in the LACTO group showed drier faeces compared to CTR cats with FS closer to the ideal one of 2-3 reported in literature (3.35 vs 3.75; (19)) in the overall treatment period. The results of the microbiological investigations are reported in table 4. The effects of the administration of *Lactobacillus acidophilus* D2/CSL have been recorded in the reduction of Coli counts in the LACTO group compared to the CTR group.

**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 humans and dogs affected by GI disorders, few studies on cats have been performed. The characteristics of probiotic supplementation require specie specific trials in a strictly carnivore pet as the cat with his own digestive physiology.

In our study we tested *L. acidophilus* D2/CSL (CECT 4529) as a feed additive in healthy cats, the strain has already a good evidence regarding its efficacy, especially on broilers and laying hens, showing improvement of their gut health and performance (23–25) Cats' body weight was consistent throughout the study period in both groups, the same results have been described by Marshall-Jones and co-authors (2006) who included *L. acidophilus* DSM13241 in healthy cats' diets too (11). The same constancy was recorded for BCS underlining the maintenance of ideal nutritional status in a carnivore like cat. The 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 (26), furthermore every pet owner could be able to evaluate the nutritional status of his pet. Many positive effects of *L. acidophilus.* inclusion have been described in different animal species where several Lactobacilli strains have, for example, demonstrated significant effects on of growth and appetite in puppies (27) in companion animals and growth performance in productive animals (24,28–31).

In our study we also evaluated the FM and FS as relevant gut functionality indicators, these could be altered from normal values depending mainly on diet type and occurring GI diseases or dysbiosis. Moisture content can determine whether faeces appear soft or firm. However, excluding infectious diarrhea, the possible causes of soft faeces in cats and dogs as such are still debated. Rolfe and collegue (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 absorption are not important determinant for faecal moisture. Indeed, higher fermentation activities of undigested soluble fibers or poorly digested proteins in the colon produces 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 the FM was observed through the whole study period. As for the FS, the LACTO group showed a mean score closer to the ideal compared to the CTR group. The change of these two parameters is a proof that *L. acidophilus CSL/D2* seem to influence and have 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, with the administration of L. acidophilus DSM13241, the FS remained unchanged (11). The same lack of effects on faecal quality parameters was described in a study performed on healthy dog where L. acidophilus NCDC 15 had no influence on the FS (11)

Culture-based identification methods were used in assessment of the gastrointestinal bacteria and microflora in our animals. Coliform populations were found to decrease in the treated group meaning that there was a slight protective effect of the probiotic on invasive bacteria spp. An increase in the lactobacilli count occurred in the LACTO group meaning that positive changes in the microbiota occurred, 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 (2006) (11). Bacterial enteropathogens (*Clostridium difficile, Cl. perfringens, Salmonella ser., Campylobacter jejuni, and pathogenic Escherichia coli*) have been frequently isolated from the faeces of clinically healthy dogs and cats. Dysbiosis, as the result of an unbalance among lactic acid bacteria (lactobacilli, in particular) and pathogenic bacteria, is commonly observed in animals. The altered intestinal microbiota can release toxic bacterial metabolites in a manner quantitatively dependent on the type of fermentations that occur 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 Marks and co-authors ten years later (36).

In this study, cats fed *L. acidophilus* D2/CSL (CECT 4529) showed a lower faecal moisture and better faecal score, healthy general conditions and gut functionality could be indirectly supposed.

It is argued that the probiotic balances the intestinal microbiota, reducing the number of putrefactive and pro-inflammatory bacteria and increases lactic acid bacteria population. The restoration of the intestinal eubiosis has immunomodulatory and anti-inflammatory effects due to the positive interaction of probiotic bacteria with epithelial cells and DCs and with monocytes/macrophages and lymphocytes.

## Conclusion

In conclusion, the dietary inclusion of the probiotic strain *L. acidophilus* D2/CSL (CECT 4529) seem to have improved the faecal quality parameters like FM and Fs in adult healthy cats. Furthermore, an apparent positive effect on lactobacilli counts was pointed out. As indirect observation, the supplemented specific strain of intestinal origin seemed to express a good ability to multiply in the feline intestine and to colonize it. All the animals kept their ideal BCS and BW during the 5 weeks' trial. Further studies with ann increment of the healthy cat sample size and a further comparison with cat with GI pathologies could be carried out to investigate the effect of the tested strain on a pure carnivore dysbiotic gut.

## 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, 12$^{th}$ October 2015).

## List of Tables

**Table 1.** Diet Chemical composition fed in

| | 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 | 3150 kcal/kg | |

**Table 2**: Effect of *Lactobacillus acidophilus* D2/CSL addition to diet on Faecal Moisture (FM) in cats: least square means (± SE) relative to control (CTR) and treated (LACTO) groups.

| ***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 | *0.3754* |
| T1 | 0.43 ± 0.013 | 0.42 ± 0.013 | *0.4782* |
| T2 | 0.46 ± 0.013 | 0.44 ± 0.013 | *0.2799* |
| T3 | 0.47 ± 0.015 | 0.43 ± 0.013 | *0.0859* |

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

| | ***FS*** | |
|---|---|---|
| | ***CRT*** | ***LACTO*** |
| Overall period | | |
| Mean ± SD | 3.75 ± 0.55 | 3.35 ± 0.59 |
| Median | $4^{a}$ | $3^{b}$ |
| 25% percentile;75% percentile | (3, 4) | (3, 4) |
| T0 | | |
| Mean ± SD | 3.80 ± 0.45 | 4.00 ± 0 |
| Median | 4 | 4 |

<table>
<tr><th></th><th></th><th></th><th></th></tr>
<tr><td></td><td>25% percentile;75% percentile</td><td>(4; 4)</td><td>(4; 4)</td></tr>
<tr><td>T1</td><td></td><td></td><td></td></tr>
<tr><td></td><td>Mean ± SD</td><td>3.80 ± 0.45</td><td>3.20 ± 0.45</td></tr>
<tr><td></td><td>Median</td><td>4</td><td>3</td></tr>
<tr><td></td><td>25% percentile;75% percentile</td><td>(4; 4)</td><td>(3; 4)</td></tr>
<tr><td>T2</td><td></td><td></td><td></td></tr>
<tr><td></td><td>Mean ± SD</td><td>4.00 ± 0.71</td><td>3.2 ± 0.45</td></tr>
<tr><td></td><td>Median</td><td>4[a]</td><td>3[b]</td></tr>
<tr><td></td><td>25% percentile;75% percentile</td><td>(4, 4)</td><td>(3; 3)</td></tr>
<tr><td>T3</td><td></td><td></td><td></td></tr>
<tr><td></td><td>Mean ± SD</td><td>3.40 ± 0.55</td><td>3.00 ± 0</td></tr>
<tr><td></td><td>Std Dev</td><td>XXX</td><td>XXX</td></tr>
<tr><td></td><td>25% percentile;75% percentile</td><td>(3; 4)</td><td>(3; 3)</td></tr>
</table>

[a, b] within each period medians with a different superscripts differ (P<0.10)

**Table 4**: Effects of *Lactobacillus acidophilus* D2/CSL in addition to diet on *Escherichia coli* and total coliforms (Coli) and Lactobacilli counts at day 7 and day 28: Mean ± Standard Deviation and Median (interquartile intervals) were reported.

<table>
<tr><th></th><th></th><th colspan="2">Coli [$log_{10}(N)$]</th><th colspan="2">LB [$log_{10}(N)$]</th></tr>
<tr><th></th><th></th><th>CRT</th><th>LACTO</th><th>CRT</th><th>LACTO</th></tr>
<tr><td>T1</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td>Mean ± SD</td><td>5.40 ± 0.55</td><td>4.94 ± 0.82</td><td>4.72 ± 0.94</td><td>5.60 ± 0.55</td></tr>
<tr><td></td><td>Median</td><td>5</td><td>5</td><td>5</td><td>6</td></tr>
<tr><td></td><td>25% percentile;75% percentile</td><td>(5; 6)</td><td>(5; 5)</td><td>(4; 5)</td><td>(5; 6)</td></tr>
<tr><td>T3</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td>Mean ± SD</td><td>3.00 ± 1.41</td><td>3.34 ± 0.48</td><td>3.17 ± 0.21</td><td>4.09 ± 1.44</td></tr>
<tr><td></td><td>Median</td><td>2.5</td><td>3</td><td>3.15</td><td>4</td></tr>
<tr><td></td><td>25% percentile;75% percentile</td><td>(2; 4)</td><td>(3; 3.7)</td><td>(3; 3.35)</td><td>(3; 5)</td></tr>
</table>

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