Effects of *Lactobacillus acidophilus* DSM13241 as a probiotic in healthy adult cats

Zoe V. Marshall-Jones, PhD; Marie-Louise A. Baillon, PhD; Julie M. Croft, BSc; Richard F. Butterwick, PhD

**Objective**—To evaluate the effect of dietary supplementation with the probiotic strain *Lactobacillus acidophilus* DSM13241 in healthy adult cats.

**Animals**—15 adult cats.

**Procedures**—Cats were fed a nutritionally complete dry food for 5 weeks. Fecal character was assessed daily, and a single fecal sample and 3-mL blood sample were collected for bacterial enumeration and hematologic analysis, respectively. Cats were then fed the same diet supplemented with *L acidophilus DSM13241* (2 x 10^8 CFU/d) for 4.5 weeks. Repeat fecal and hematologic measurements were taken prior to the return to control diet for a 4-week period.

**Results**—The probiotic species was recovered from feces, demonstrating survival through the feline gastrointestinal tract. Probiotic supplementation was associated with increased numbers of beneficial *Lactobacillus* and *L acidophilus* groups in feces and decreased numbers of *Clostridium* spp and *Enterococcus faecalis*, indicating an altered bacterial balance in the gastrointestinal tract microflora. Fecal pH was also decreased suggesting a colonic environment selective for the beneficial lactic acid bacterial population. Systemic and immunomodulatory effects were associated with administration of *L acidophilus DSM13241* including altered cell numbers within WBC subsets and enhanced phagocytic capacity in the peripheral granulocyte population. In addition, plasma endotoxin concentrations were decreased during probiotic feeding, and RBCs had a decreased susceptibility to osmotic pressure.

**Conclusions and Clinical Relevance**—Probiotic strain *L acidophilus DSM13241* fed at 2 x 10^8 CFU/d can alter the balance of gastrointestinal microflora in healthy cats. Furthermore, administration of this probiotic results in beneficial systemic and immunomodulatory effects in cats. *(Am J Vet Res 2006;67:1005–1012)*

**Abbreviations**

- **FISH** Fluorescence in situ hybridization
- **MRS** De Man, Rogosa, and Sharpe
- **rRNA** Ribosomal RNA

Probiotics are defined as live microbial feed supplements, which beneficially affect the host animal by improving its gastrointestinal microbial balance. The bacterial species most often used as health-promoting probiotics are *Lactobacillus and Bifidobacterium* spp, although probiotic yeasts and *Enterococcus* spp are also used to promote weight gain in production animals.

Probiotics are increasingly used therapeutically for the treatment of gastrointestinal complaints, including antimicrobial and stress-associated diarrhea, as well as bacterial and viral diarrheal diseases. Dietary supplementation with probiotics has also been reported to alleviate inflammatory bowel disease and reduce the occurrence of colonic carcinomas.

These microorganisms may exert positive effects on the host by various means. Firstly, they increase numbers of beneficial bacteria such as lactobacilli and bifidobacteria, compared with putrefying or potentially detrimental bacteria. This improved microbial balance may occur through inhibition of detrimental species by competition for nutrients and gastrointestinal binding sites. However, the production of metabolites such as lactic acid, which reduce the pH of the colon favoring growth of other lactic acid bacteria, and secretion of antimicrobial peptides that directly target bacterial pathogens may also be involved. Secondly, probiotics may regulate host immune function. Although the exact mechanisms underlying immune effects are currently unclear, lactobacilli and bifidobacteria are known to modulate the expression of immunoregulatory cytokines by dendritic cells and macrophages.

Probiotics are commonly used in production animals, and more recently, interest in their usage for promotion of companion animal health has increased. Although several studies have investigated probiotic usage in dogs, few have shown beneficial effects. Administration of *Lactobacillus acidophilus DSM13241* has been shown to improve the gastrointestinal microbial balance and induce immunostimulatory effects in adult dogs, whereas dietary supplementation with another *L acidophilus* strain was observed to stimulate appetite and growth in puppies to 19 weeks of age. The probiotic *Enterococcus faecium* (SF68/NCIMB 10415) was reported to stimulate immune function in young dogs, with a second study on the same *E faecium* probiotic reporting modification of the canine fecal microflora. In that study, however, the beneficial...
effect of the probiotic remained questionable with fecal Salmonella and Campylobacter spp higher in most dogs following probiotic supplementation.

Studies on probiotic usage in cats have not been reported to date to our knowledge, and because of differences in host physiologic characteristics and diet, probiotic efficacy in cats cannot be extrapolated from studies in dogs. In addition, differences exist in the resident colonic microflora, which, in cats, is thought to comprise high numbers of anaerobic bacteria similar to those representing bacterial overgrowth in the small intestine of humans and dogs.21

The purpose of the study reported here was to determine whether dietary supplementation with the probiotic L. acidophilus DSM13241 would induce health-related effects in adult cats. The probiotic was incorporated into dry cat food at a concentration delivering > 10^8 CFU/d.

Materials and Methods

Study design—Fifteen domestic shorthair cats were involved in a 3-phase longitudinal study to assess the effects of supplementation with the probiotic strain L. acidophilus DSM13241.22 A within-subject study design was used to remove an important source of between-subject variation, with each cat representing its own control. Cats were fed a nutritionally complete commercial dry diet for a period of 5 weeks (baseline phase) before switching to the same diet supplemented with the probiotic strain for 4.5 weeks (probiotic phase). Following probiotic supplementation, cats returned to the base diet without probiotics for a period of 4 weeks (postprobiotic phase). Fecal samples were collected at the final 2 weeks of each phase for the assessment of gastrointestinal health status. Small-volume blood samples were collected 5 days prior to the end of each study phase for assessment of systemic effects and immune status. Comparisons between the data collected during each phase allowed assessment of the effects of supplementation with L. acidophilus DSM13241 in cats.

Kibble preparations, packaging, and validation of probiotic inclusion was conducted as described by Baillon et al.22 The probiotic strain was incorporated into the supplemented diet to a concentration of 1.1 X 10^8 CFU/kg of foodstuff at the start of the study, decreasing to 3.2 X 10^6 CFU/kg by the end of the study.

Animals and husbandry considerations—Cats involved in the study were between 4 and 5.5 years old (mean ± SD, 4.5 ± 0.4 years), weighing between 3.6 and 7.3 kg (3.6 ± 1.1 kg). For ease of feeding and feces measurements, cats were housed individually in 2-room, environmentally enriched lodges as described by Loveridge.23 Full animal welfare considerations were in place. The study was approved by the Waltham Centre for Pet Nutrition Ethical Review Committee, in accordance with the UK Home Office Animals (Scientific Procedures) Act of 1986. Cats were socialized daily, and fresh water was available at all times. Cats were fed once daily at energy levels required to maintain body weight (food offered, 50.73 ± 10.24 g). Probiotic administration equated to a daily intake of between 1.2 X 10^8 CFU and 2.8 X 10^9 CFU/kg of foodstuff at the start of the study, decreasing to 3.2 X 10^6 CFU/kg by the end of the study.

Samples and measurements—Fecal quality was assessed daily by use of a feces scoring system as described by Rolle et al.24 Trained assessors graded feces on a scale of 1 to 5, in which grade 1 represented dry, crumbly feces and grade 5 represented diarrhea. Each major sector (1 through 5) was subdivided into 4 subsectors to allow accurate scoring of fecal form. Mean fecal score was calculated for each cat during each phase of the study. During the final 2 weeks of each phase, fecal samples were collected within 30 minutes of production for assessment of bacterial populations, pH, and ammonia and hydrogen sulfide contents.

Fecal bacterial populations were enumerated by use of selective bacterial culture and FISH. For selective culture, fecal material was prepared by 10% (wt/vol) dilution in 50% (vol/vol) maximum recovery diluent. Anaerobic culture of total anaerobes, lactobacilli, and clostridia was supported by the addition of 0.025% (wt/vol) cysteine hydrochloride and prior reduction of reagents in anaerobic conditions. Duplicate serial dilutions of the fecal solution were prepared to a dilution of 10^5, and 50-µL aliquots were spread plated onto the appropriate media. Total anaerobes were selected on fastidious anaerobe agar; lactobacilli were selected on MRS agar acidified to pH 5, and clostridia were selected by use of a Clostridium perfringens agar base containing tryptose-sulfite-cycloserine selective supplement. Inoculated agar plates were incubated at 38°C under anaerobic conditions (10% H2, 10% CO2, and 80% N2) for 18 to 48 hours depending on bacterial growth characteristics. Aerobic culture of enterococci and coliforms was supported by selection on K-F Streptococcus agar and MacConkey No. 3 agar, respectively; these cultures were incubated under aerobic conditions at 38°C for 18 to 48 hours.

Putative lactobacilli selected on acidified MRS medium were retained for characterization by biochemical profiling, through which taxonomic classification is determined by the characteristic ability of an isolate to ferment 50 carbohydrate substrates. Isolates that had fermentation patterns characteristic of the probiotic strain were characterized by DNA fingerprinting of the 16S rRNA gene.25 An automated ribotyping system was used in which genomic DNA was extracted and digested with restriction endonucleases; the resulting fragments were separated by gel electrophoresis. Hybridization with fluorescein-labeled probes designed against the 16S rRNA gene allowed observation of the DNA banding pattern, which was analyzed by use of proprietary software.

Because growing evidence suggests that selective culture for assessment of microbial ecology is inadequate, measurement of bacterial populations by the molecular FISH method was used to further enumerate fecal bacteria. A series of 5 cyanine-3-labeled DNA probes26 targeted against the 16S rRNA were used in the analysis of Lactobacillus spp, L. acidophilus, bifidobacteria, clostridia, and Enterococcus faecalis (Appendix).27-30

Briefly, 3 g of feces was diluted 10% (wt/vol) in PBS solution, and particulate material was removed by centrifugation at 1,000 X g for 2 minutes. The supernatant was diluted 1:4 in 4% paraformaldehyde. Bacterial cells were harvested from the supernatant by centrifugation at 12,500 X g for 5 minutes. The harvested cells were washed in PBS solution and suspended in a 900-µL volume of 50% (vol/vol) ethanol-PBS solution. Cellular material was then hybridized overnight at 45°C with 1 µg of a cyanine-3–labeled DNA probe and with the total bacterial stain, 4'-diamidino-2-phenylindole. Fluorescent-labeled and stained cells were counted by use of fluorescence microscopy.

Fecal pH was measured in triplicate in fecal water extracted by centrifugation of 10 g of fresh feces at 40,000 X g for 2 hours. Fecal ammonia was measured in feces that was diluted 5% (wt/vol) in sterile distilled water containing 20%
(vol/vol) ammonia pH adjusting solution by use of an ammonia electrode. Total sulfide (H<SUB>2</SUB>S and H<SUB>2</SUB>S<sup>2-</sup>) production in feces was measured by use of the methylene blue method. Briefly, 0.3 g of feces was diluted 1:10 in pre-reduced sodium phosphate buffer (pH 7.4) and agitated to mix. The resulting slurry was used as an inoculum diluted 10% (vol/vol) in anaerobe basal broth, and the culture was incubated at 38°C under anaerobic conditions for 24 hours. Cultures were diluted 1:4 in sterile distilled water, and particulate material was removed by centrifugation at 13,800 × g for 15 minutes. Total sulfides were detected by use of the hydrogen sulfide reagent set<sup>1</sup> and colorimetric detection by spectrophotometry at a wavelength of 670 nm. Sulfides were measured in parts per million against an Na<sub>2</sub>S standard curve.

A 3-mL blood sample was collected by venipuncture of the cephalic vein during the final week of each study phase. Sample analyses included measurement of acellular immune factors and hemogram,<sup>4</sup> which included a differential WBC count. Serum biochemical analysis included concentrations of total protein, albumin, urea, creatinine, cholesterol, calcium, and inorganic phosphorus; serum activities of alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase were also analyzed. Serum immunoglobulin concentrations (IgG, IgA, and IgM) were determined via commercially available radial immunodiffusion assays,<sup>1</sup> and concentrations of the serum acute-phase reactants haptoglobin and C-reactive protein were measured via commercially available immunoassays.<sup>1</sup> Total serum nitric oxide concentrations were determined by enzymatic conversion of nitrate to nitrite followed by colorimetric detection of diazonium products of the Griess reaction<sup>1</sup> by spectrophotometry at a wavelength of 550 nm.

Assessment of RBC susceptibility to osmotic pressure<sup>31</sup> was performed on whole-blood samples collected in EDTA. Blood samples (20 µL) were diluted to 1% (vol/vol) in sodium chloride solution of various concentrations (0.25%, 0.6%, 0.65%, 0.7%, and 0.9% [wt/vol]), and samples were incubated for 20 minutes at 22°C. Cellular material was removed by centrifugation at 500 × g, and the optical density of the supernatant was determined by spectrophotometry at a wavelength of 540 nm. Exposure to 0.65% (wt/vol) sodium chloride resulted in hemolysis of >0% and <100% for all samples and was therefore used for data analysis purposes.

Endotoxin (bacterial lipopolysaccharide) concentrations in serum from 1-mL whole-blood samples collected in pyrogen-free vessels were determined at external laboratories in serum from 1-mL whole-blood samples collected in EDTA. Serum immunoassay<sup>1</sup> and concentrations of the serum acute-phase reactants haptoglobin and C-reactive protein were measured via commercially available radial immunodiffusion assays,<sup>1</sup> and concentrations of the serum acute-phase reactants haptoglobin and C-reactive protein were measured via commercially available immunoassays.<sup>1</sup> Total serum nitric oxide concentrations were determined by enzymatic conversion of nitrate to nitrite followed by colorimetric detection of diazonium products of the Griess reaction<sup>1</sup> by spectrophotometry at a wavelength of 550 nm.

### Results

**Health status**—Probiotic supplementation was not associated with changes in the health status of cats, as demonstrated by findings on physical examination, CBC determination, and serum biochemical analysis. Food intake was consistent throughout the study (baseline, 47.2 ± 11 g; probiotic, 47.6 ± 10.8 g; after probiotic, 47.8 ± 9.2 g; P = 0.902), and body weight was not significantly (P = 0.178) altered by probiotic feeding (baseline, 5.27 ± 1.06 kg; probiotic, 5.22 ± 1.09 kg; after probiotic, 5.21 ± 1.12 kg).

**Fecal measurements**—Fecal quality was excellent, and fecal scores remained unchanged throughout the study (baseline, 2.03 ± 0.14; probiotic, 2.02 ± 0.16; after probiotic, 2.06 ± 0.12; P = 0.388). Hydrogen sulfide (baseline, 34.21 ± 10.04 µg/g; probiotic, 31.18 ± 10.86 µg/g; after probiotic, 36.57 ± 10.52 µg/g; P = 0.394) and ammonia (baseline, 11.48 ± 4.89 mM; probiotic, 10.40 ± 6.03 mM; after probiotic, 9.13 ± 4.32 mM; P = 0.239) concentrations were also constant among fecal samples throughout the study. Fecal pH had a slight but significant (P = 0.017) decrease across the course of the study (baseline, 6.73 ± 0.14; probiotic, 6.48 ± 0.41; after probiotic, 6.32 ± 0.45).

Assessment of fecal bacterial populations by selective culture (Table 1) revealed that clostridia numbers were significantly reduced during the probiotic-feeding phase and increased following the cessation of probiotic supplementation, whereas coiform and Enterococcus populations decreased during the course of the study.

<table>
<thead>
<tr>
<th>Bacterial Numbers (Log&lt;sub&gt;10&lt;/sub&gt;/g)</th>
<th>Baseline</th>
<th>Probiotic</th>
<th>After Probiotic</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactobacilli</strong></td>
<td>6.83 ± 0.68</td>
<td>6.78 ± 0.61</td>
<td>6.03 ± 1.10</td>
<td>0.171</td>
</tr>
<tr>
<td><strong>Total anaerobes</strong></td>
<td>10.04 ± 0.86</td>
<td>9.86 ± 0.61</td>
<td>9.73 ± 0.56</td>
<td>0.531</td>
</tr>
<tr>
<td><strong>Clostridia</strong></td>
<td>9.14 ± 0.97</td>
<td>8.34 ± 0.65</td>
<td>8.63 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.045</td>
</tr>
<tr>
<td><strong>Coliforms</strong></td>
<td>5.52 ± 1.00</td>
<td>4.83 ± 1.54</td>
<td>4.23 ± 1.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>Enterococci</strong></td>
<td>2.70 ± 1.44</td>
<td>2.06 ± 1.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.55 ± 1.17</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values represent data from 15 cats.

<sup>a</sup>Significant (P < 0.05) difference among phases.
No significant changes in numbers of culturable lactobacilli and total anaerobes were observed by selective culture.

Biochemical profiling was used to assess the carbohydrate fermentation pattern of randomly selected isolates cultured on MRS agar from each cat. During the baseline, phase isolates were predominantly identified as Lactobacillus plantarum, Lactobacillus brevis, and Pediococcus pentosaceus, while isolates with profiles characteristic of the probiotic strain were not identified. During the probiotic feeding phase, however, 11 of the 28 isolates tested had fermentation profiles characteristic of the probiotic strain. Molecular fingerprinting of the 16S rRNA gene revealed that these isolates were identical to the probiotic strain (Figure 1). On returning to the base diet, biochemical profiling suggested a return to Lactobacillus populations characteristic of the baseline period.

Molecular methods for bacterial enumeration had several differences in bacterial populations, compared with bacterial culture. Numbers of lactobacilli as observed by FISH increased significantly during probiotic-feeding in actual numbers and as a percentage of the total bacterial population (Table 2). Similarly, numbers of the L. acidophilus group (also including Lactobacillus crispatus, Lactobacillus gallinarum, and Lactobacillus helveticus) increased significantly during probiotic feeding as measured by administration of the probiotic supplement.

Blood parameters—Results of blood biochemical analysis and CBC determination were within reference ranges and revealed no significant changes associated with probiotic supplementation. Significant changes were observed in the lymphocyte population with a reduction in cell numbers across the course of the study and a similar increase in numbers of eosinophils (Table 3). The proportion of the monocyte and granulocyte populations having phagocytic activity in vitro remained unchanged, compared with baseline numbers during probiotic feeding (Table 4). During the postprobiotic phase, however, the percentage of cells from both populations that were active in phagocytosis was slightly, yet significantly, reduced, compared with baseline. The mean fluorescent intensity, which correlates to the mean number of bacteria phagocytosed per WBC, was unchanged in the monocyte population throughout the study. However, mean numbers of bacteria ingested by the granulocyte population increased significantly in response to probiotic administration and again following the cessation of probiotic feeding. The acellular immune factors IgG, IgM, and acute-phase reactants serum amyloid A, and haptoglobin remained constant throughout the study, with serum nitric oxide concentrations also unchanged by administration of the probiotic supplement.

The susceptibility of RBCs to hemolysis on exposure to 0.65% (wt/vol) NaCl solution was reduced significantly (P = 0.028) throughout the course of the study (baseline, 17.14 ± 11.32%; probiotic, 14.18 ±
Table 3—Mean ± SD WBC population numbers and acellular immune factor concentrations in blood samples obtained during baseline, probiotic supplementation, and after probiotic treatment phases.

<table>
<thead>
<tr>
<th>Immune factor</th>
<th>Baseline</th>
<th>Probiotic</th>
<th>After probiotic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCs (X 10⁹/L)</td>
<td>11.50 ± 2.27</td>
<td>11.21 ± 2.99</td>
<td>11.05 ± 3.63</td>
<td>0.828</td>
</tr>
<tr>
<td>Neutrophil (X 10⁹/L)</td>
<td>5.94 ± 1.67</td>
<td>5.96 ± 1.67</td>
<td>5.91 ± 2.19</td>
<td>0.926</td>
</tr>
<tr>
<td>Lymphocyte (X 10⁹/L)</td>
<td>4.89 ± 1.81</td>
<td>4.38 ± 1.94</td>
<td>4.05 ± 1.69</td>
<td>0.043</td>
</tr>
<tr>
<td>Monocyte (X 10⁹/L)</td>
<td>0.17 ± 0.17</td>
<td>0.19 ± 0.17</td>
<td>0.14 ± 0.18</td>
<td>0.923</td>
</tr>
<tr>
<td>Eosinophil (X 10⁹/L)</td>
<td>0.33 ± 0.27</td>
<td>0.70 ± 0.63</td>
<td>0.95 ± 0.71</td>
<td>0.002</td>
</tr>
<tr>
<td>IgG (mg/mL)</td>
<td>9.37 ± 2.65</td>
<td>8.61 ± 1.89</td>
<td>9.47 ± 2.84</td>
<td>0.154</td>
</tr>
<tr>
<td>IgM (mg/mL)</td>
<td>1.05 ± 0.40</td>
<td>0.96 ± 0.35</td>
<td>0.99 ± 0.35</td>
<td>0.200</td>
</tr>
<tr>
<td>Serum amyloid A (µg/mL)</td>
<td>1.48 ± 0.06</td>
<td>1.56 ± 0.05</td>
<td>1.54 ± 0.06</td>
<td>0.508</td>
</tr>
<tr>
<td>Haptoglobin (mg/mL)</td>
<td>1.32 ± 0.53</td>
<td>1.32 ± 0.62</td>
<td>1.21 ± 0.68</td>
<td>0.288</td>
</tr>
<tr>
<td>Nitric oxide (µM)</td>
<td>17.4 ± 14.8</td>
<td>21.5 ± 17.3</td>
<td>22.2 ± 17.0</td>
<td>0.254</td>
</tr>
</tbody>
</table>

See Table 1 for key.

Table 4—Mean ± SD values of phagocytic activity of monocyte and granulocyte populations during baseline, probiotic supplementation, and after probiotic treatment phases.

<table>
<thead>
<tr>
<th>Phagocytic activity</th>
<th>Baseline</th>
<th>Probiotic</th>
<th>After probiotic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of population (%)</td>
<td>91.94 ± 2.80</td>
<td>91.70 ± 5.20</td>
<td>79.13 ± 6.91</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fluorescence intensity</td>
<td>557.42 ± 129.56</td>
<td>546.05 ± 150.86</td>
<td>606.56 ± 82.19</td>
<td>0.273</td>
</tr>
<tr>
<td>Granulocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of population (%)</td>
<td>98.30 ± 1.74</td>
<td>95.77 ± 5.84</td>
<td>89.99 ± 5.18</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fluorescence intensity</td>
<td>448.11 ± 126.08</td>
<td>581.79 ± 128.38</td>
<td>780.33 ± 131.61</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

See Table 1 for key.

Figure 2—Mean plasma endotoxin concentrations in blood samples from 15 cats during the baseline (closed bar), probiotic feeding (hatched bar), and post-probiotic (open bar) phases. *Significant (P < 0.001) difference between phases.

Discussion

Dietary supplementation with *L. acidophilus* DSM13241 resulted in various health-related effects that may be considered beneficial to the host. A variety of changes were observed in the commensal gastrointestinal tract flora and health or immune status of cats in response to probiotic administration. The probiotic was recovered from feces of cats demonstrating survival through the feline gastrointestinal tract. Persistence of the probiotic, however, appeared to cease following the withdrawal of supplementation.

Changes associated with the probiotic feeding phase alone and persisting following withdrawal of the probiotic were observed in association with the probiotic *L. acidophilus* DSM13241. Persistence for the period following withdrawal of the probiotic was largely observed for immunomodulatory factors altered during the study. This suggests a mechanism not requiring direct action of the probiotic or facilitated by colonization at numbers below the detectable limit. The exception to this was blood endotoxin concentrations, which were reduced only in the probiotic-feeding phase, consistent with a direct mode of action such as enhanced gastrointestinal barrier function or reduced luminal bacterial endotoxin. Conversely, changes in gastrointestinal bacterial populations were largely associated with the supplementation phase, with fewer measurements or those of lower magnitude providing evidence of a continued effect on the gastrointestinal tract.
Confounding effects as a result of adaptation to environmental factors or diet, although not impossible, are considered unlikely. All cats were maintained in the same environmentally enriched housing as that used during the study and had previously been fed nutritionally complete commercial dry pet food of similar composition to the base diet. The additional adaptation to base diet for 3 to 5 weeks prior to collection of feces and 5 weeks prior to obtaining hematologic measurements makes this increasingly unlikely.

A combination of culture-based and molecular identification methods were used in assessment of the gastrointestinal microflora to counteract inaccuracies inherent in the use of selective culture alone to investigate the microflora of companion animals. Some evidence of this inaccuracy was observed in our study where numbers of lactobacilli remained unchanged as measured by bacterial culture while FISH revealed increased numbers of lactobacilli and L. acidophilus during probiotic supplementation. Selective bacterial culture is reliant on the provision of optimal growth conditions (which include temperature and nutrient and atmospheric factors) for target bacterial populations within the complex gastrointestinal microflora. Furthermore, culture media are largely developed for the selection of human isolates adding to these inadequacies. Because FISH uses specific DNA probes that hybridize the 16S rRNA, the technique can be used to detect viable, yet nonculturable, bacteria on the basis of 16S rRNA nucleotide sequence.

Enumeration of enterococci, E. faecalis, and Clostridium spp by FISH and selective culture revealed the techniques to be largely similar in describing the effect of probiotic feeding on these populations. Bacterial groups enumerated were selected as markers of microflora health with Clostridium and Enterococcus spp representing detrimental bacterial markers and lactobacilli and Bifidobacterium spp representing beneficial species. In contrast to the health-promoting short-chain fatty acids produced by lactic acid bacteria, the proteolytic nature of Clostridium spp results in the production of putrefying metabolites. Clostridium perfringens, C. difficile, and E. faecalis are also considered opportunistic pathogens, the former 2 being associated with gastrointestinal infection and diarrheal disease and the latter with wound and systemic infections in companion animals. The observed reductions in the clostridia and E. faecalis populations, combined with increased numbers of lactobacilli and L. acidophilus observed by FISH, are highly suggestive of a healthier balance of the gastrointestinal microflora occurring during probiotic supplementation. The reduction in Bifidobacteria as a percentage of the total population is not considered to counteract the suggestion of a healthier balance. The magnitude of change in this healthy population, however, were relatively small in magnitude, and measured values were within reference range.

Immunomodulation was suggested by altered lymphocyte and eosinophil populations with reduced and increased numbers, respectively, throughout the course of the study and by increased activity of peripheral blood phagocytes. Small changes in the phagocytically active portion of granulocytes and monocytes occurred across the course of the study. The granulocyte population, however, had substantially increased numbers of fluorescent-labeled E. coli phagocytosed per cell when challenged in vitro. Furthermore, this increase was observed in a background of stable granulocyte (neutrophil) numbers. These data suggest that L. acidophilus administration in cats may enhance the phagocytic capacity of circulating granulocytes and that the effect is maintained at least 4 weeks after the cessation of probiotic feeding. Because granulocytes account for approximately 50% of the WBC population in healthy adult cats, the phagocytic capacity of peripheral blood is almost entirely the result of the stimulated population. The nonspecific immune stimulation observed in the postprobiotic period, therefore, involves newly developed or maturing cells not directly influenced by probiotic feeding. It is not known whether the prolonged nature of this effect is the result of the nature of the immunomodulatory mechanism or because of continued presence of the probiotic in the gastrointestinal tract at levels below the detection limit.

Effects on general health were observed in cats in response to probiotic feeding, with blood endotoxin concentrations reduced during probiotic feeding and improved resistance to osmotic lysis in RBCs over the
course of the study. The lowered endotoxin concentrations may be consistent with an enhanced gastrointestinal barrier or with decreased amounts of luminal endotoxin and, hence, lowered translocation through the gastrointestinal wall. Reduced concentrations of circulating toxins have previously been reported to improve the osmotic resistance of RBCs possibly as a result of decreases in membrane damage caused by oxidants and blood toxins including endotoxin. The integrated robustness of RBCs in the probiotic and postprobiotic phases may therefore be linked to altered plasma endotoxin concentrations. A direct correlation, however, would not explain the observed effect.

In summary, the administration of *L. acidophilus* DSM13241 at a daily dose of 2 × 10^8 CFU in healthy adult cats resulted in a beneficial balance of the gastrointestinal microflora with concurrent beneficial health and immunomodulatory effects. Probiotic supplementation with *L. acidophilus* DSM13241 may therefore promote feline health and improve resistance to disease.

a. *Lactobacillus acidophilus* DSM13241, Christian Hansen, Horsholm, Copenhagen, Denmark.
b. Feces scoring system, WALTHAM, Waltham-on-the-Wolds, Leicestershire, UK.
c. Oxoid Ltd, Basingstoke, Hampshire, UK.
d. API 50 CH, bioMérieux SA, Marcy l’Etoile, France.
e. Campden and Chorleywood Food Research Assoc, Chipping Campden, UK.
f. Riboprint system, Qualicon, Wilmington, Del.
g. MWG Biotech Ltd, Milton Keynes, UK.
h. Zeiss, Oberkochen, Germany.
i. 95-12 Ammonia electrode, Orion, Thermoelectron Corp, Waltham, Mass.
j. Hach Co, Loveland, Colo.
k. IDEXX Laboratories Ltd, Wethery, UK.
l. VET-RID, Bethyl Laboratories Inc, Montgomery, Tex.
m. Tridelta Development Ltd, Greystones Co, Wicklow, Ireland.
n. Griess Reagent System, Promega UK Ltd, Southampton, UK.
o. BioWhittaker Europe, Verviers, Belgium.
p. PHAGOTEST, Becton-Dickinson, Basel, Switzerland.
q. FACScaniber, Becton-Dickinson, Oxford, UK.
r. CellQuest, Clearwater, Fla.
s. Excel 97, Microsoft Corp, Redmond, Wash.
t. Statgraphics Plus, version 4.1, Manugistics Inc, Rockville, Md.

Appendix

Target bacterial group and deoxyribonucleotide sequence of DNA probes used in bacterial enumeration by FISH.

<table>
<thead>
<tr>
<th>Target organism</th>
<th>Probe sequence (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus</em> spp</td>
<td>GGT ATT AGC ACG(T/C)T GC TGG TTA CA²</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em></td>
<td>ATG ATG CGA TCT GCT TTT TAC</td>
</tr>
<tr>
<td><em>Bifidobacterium</em> spp</td>
<td>CAT GCC GCA TTA CCA CCA CCA²</td>
</tr>
<tr>
<td><em>Clostridium</em> spp</td>
<td>TTA TGG GGT ATT AAT CTA CCT TT²</td>
</tr>
<tr>
<td><em>Enterococcus</em> faecalis</td>
<td>ATG ATG CGA TCT GCT TTA CCA TAC</td>
</tr>
</tbody>
</table>

References

28. Langendijk PS, Schut F, Jansen GJ, et al. Quantitative fluorescence in silt hybridization of *Bifidobacterium* spp. with genus-


