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

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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)

Commensal gastrointestinal tract microflora are vital to gastrointestinal health, aiding the host in digestion, nutrient metabolism, and vitamin production and in restricting colonization by pathogenic bacteria. Microbial populations in the gastrointestinal tract, however, are susceptible to change induced by factors such as poor nutrition, stress, gastrointestinal infections, antibiotic administration, predisposing illness, and immunosuppression. These fluctuations may impact the health of the host; therefore, enhancement of the colonic microflora by dietary interventions such as probiotics may be used to maintain gastrointestinal health.

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 potential 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 microflora and induce immunomodulatory effects in adult dogs, whereas dietary supplementation with another *L. acidophilus* strain was observed to stimulate appetite and growth in puppies up 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

ABBREVIATIONS

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

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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 > 10^9 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*. 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 in 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**—Conduct as described by Bailon et al.22

The probiotic strain was incorporated into the supplemented dry cat food at a concentration delivered at 4.5 × 10^9 CFU/kg of foodstuff at the start of the study, decreasing to 3.2 × 10^9 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.24 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, 58.73 ± 10.24 g). Probiotic administration equated to a daily intake of between 1.2 × 10^9 CFU and 2.8 × 10^9 CFU dependent on the individual requirements. Feeding occurred over a 2-hour period to minimize exposure of the probiotic to air and humidity. Food offered and refused was recorded for each cat daily, and body weight was measured weekly. Prior to the start of the study and during each study phase, cats received a physical examination and health assessment. All cats were healthy and had no record of chronic illness.

**Samples and measurements**—Fecal quality was assessed daily by use of a feces scoring system23 as described by Rolfe 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.25 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 10^7, and 50-µL aliquots were spread plated onto the appropriate media.25 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. Incubated agar plates were incubated at 38°C under anaerobic conditions (10% Hz, 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,26 through which taxonomic classification is determined by the characteristic ability of an isolate to ferment 49 carbohydrate substrates. Isolates that had fermentation patterns characteristic of the probiotic strain were characterized by DNA fingerprinting of the 16S rRNA gene.27 An automated ribotyping system27 was used in which genomic DNA was extracted and digested with restriction endonucleases; the resulting fragments were separated by gel electrophoresis. Hybridization with cyanine-3 labeled DNA probes designed against the 16S rRNA gene allowed observation of the DNA banding pattern, which was analyzed by use of proprietary software.27

Because growing evidence suggests that selective culture for assessment of microbial ecology is inadequate,27 measurement of bacterial populations by the molecular FISH method was used to further enumerate fecal bacteria. A series of 5 cyanine-3-labeled DNA probes28 targeted against the 16S rRNA gene allowed observation of the DNA banding pattern, which was analyzed by use of proprietary software.27

Briefly, 3 g of feces was diluted 10% (wt/vol) in PBS solution, and particulate material was removed by centrifugation at 1,000 × 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 × 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, 6-4-diamidino-2-phenylindole. Fluorescent-labeled and stained cells were counted by use of fluorescence microscopy.27

Fecal pH was measured in triplicate in fecal water extracted by centrifugation of 10 g of fresh feces at 40,000 × g for 2 hours. Fecal ammonia was measured in feces that was diluted 5% (wt/vol) in sterile distilled water containing 20%
at a wavelength of 540 nm. Exposure to 0.65% (wt/vol) sodium reagent set and colorimetric detection by spectrophotometry was performed by use of the Limulus amebocyte lysate test after pyrogen-free vessels were determined at external laboratories. Samples and was therefore used for data analysis purposes. Incubated for 20 minutes at 22°C. Cellular material was diluted 1:4 in sterile distilled water, and particulate material 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 and colorimetric detection by spectrophotometry at a wavelength of 670 nm. Sulfides were measured in parts per million against an Na₂S 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, which included a differential WBC count. Serum biochemical analysis included serum 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, and concentrations of the serum acute-phase reactants haptoglobin and C-reactive protein were measured via commercially available immunoassays. Total serum nitric oxide concentrations were determined by enzymatic conversion of nitrate to nitrite followed by colorimetric detection of diazotization products of the Griess reaction by spectrophotometry at a wavelength of 550 nm.

Assessment of RBC susceptibility to osmotic pressure 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 by use of the Limulus amebocyte lysate test after removal of endotoxin inhibitors by heating at 75°C for 30 minutes. Leukocyte phagocytic activity was investigated in vitro by use of a commercially available assay and flow cytometry. Heparinized whole blood was challenged with opsonized fluorescein isothiocyanate–labeled Escherichia coli and incubated at 37°C to support phagocytosis. Following quenching of extracellular bacteria, RBCs were lysed and fixed and WBC DNA was stained by use of propidium iodide solution. Phagocytic activity of granulocyte and monocyte populations was measured by flow cytometry by use of the blue-green excitation light (488 nm). In addition to analysis of phagocytic activity of the individual phagocyte populations, the median fluorescence intensity was measured for comparison of mean number of bacteria phagocytosed per WBC.

**Statistical analysis**—Data from the 3 phases were analyzed by use of a 2-factor general linear model to take into account the observation of the same cat in each of the following phases: baseline, probiotic treatment, and after probiotic treatment. Where measurements were taken over 2 study phases, data were analyzed by use of a paired Student t test. In the case of bacterial populations and species counts, data were logarithmically transformed prior to analysis, and fecal scores and body weights were averaged over each phase for each cat prior to analysis. Treatment effects found to be significant at the 5% significance level were followed up by use of the Student-Newman-Keuls multiple range test. Data were stored electronically and analyzed by use of the multifactor ANOVA procedure with proprietary analysis software. Results are expressed as mean ± SD values.

**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, 2.03 ± 0.14 g; probiotic, 2.02 ± 0.16 g; after probiotic, 2.06 ± 0.12 g; 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 (P = 0.017) decrease across the course of the study (baseline, 6.73 ± 0.34; probiotic, 6.57 ± 0.45; after probiotic, 6.48 ± 0.41).

**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 = 0.902), 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).

**Table 1**—Mean ± SD number of selected bacterial populations recovered from feces by bacterial culture during baseline, probiotic supplementation, and after probiotic treatment phases.

<table>
<thead>
<tr>
<th>Bacterial population</th>
<th>Baseline (Log₁₀/g)</th>
<th>Probiotic (Log₁₀/g)</th>
<th>After probiotic (Log₁₀/g)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacilli</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>Total anaerobes</td>
<td>10.04 ± 0.86</td>
<td>9.85 ± 0.61</td>
<td>9.73 ± 0.58</td>
<td>0.531</td>
</tr>
<tr>
<td>Clostridia</td>
<td>9.14 ± 0.97</td>
<td>8.34 ± 0.63</td>
<td>9.36 ± 0.36</td>
<td>0.043</td>
</tr>
<tr>
<td>Coliforms</td>
<td>5.12 ± 1.00</td>
<td>4.83 ± 1.54</td>
<td>4.23 ± 1.38</td>
<td>0.018</td>
</tr>
<tr>
<td>Enterococci</td>
<td>2.70 ± 1.44</td>
<td>2.06 ± 1.61</td>
<td>0.55 ± 1.17</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values represent data from 15 cats.

**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 FISH. Numbers of Bifidobacterium spp decreased as a percentage of the total population in the supplementation phase and increased to baseline numbers in the post-probiotic phase. As observed by bacterial culture, FISH analysis revealed a reduction in the opportunistic pathogen E. faecalis, in bacterial numbers and percentage population, throughout the course of the study. Technical difficulties were encountered in assessment of fecal clostridia by FISH during the baseline period. However, consistent with observations by selective culture, after probiotic administration, numbers of clostridia were increased significantly, compared with those during supplementation.

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 cellular immune factors IgG, IgM, and acute-phase reactants serum amyloid A, and haptoglobin remained constant throughout the study, with serum cytokine 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 ±

![Figure 1](image-url) Molecular fingerprint of the 16S rRNA of the probiotic strain (A) and 4 fecal isolates (B through E) cultured on MRS agar during probiotic supplementation. kb = Kilobase.

Table 2—Mean ± SD number of fecal bacterial populations as determined by use of FISH expressed in actual numbers and as a percentage of total population during baseline, probiotic supplementation, and after probiotic treatment phases.

<table>
<thead>
<tr>
<th>Bacterial spp</th>
<th>Mean ± SD (log10/g)</th>
<th>Total population (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Probiotic</td>
</tr>
<tr>
<td>Lactobacillus spp</td>
<td>8.21 ± 0.32a</td>
<td>8.57 ± 0.14a</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>0.47 ± 1.68b</td>
<td>0.72 ± 0.86b</td>
</tr>
<tr>
<td>Bifidobacterium spp</td>
<td>7.34 ± 0.09a</td>
<td>7.24 ± 0.14a</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>6.80 ± 0.95a</td>
<td>4.10 ± 3.69b</td>
</tr>
<tr>
<td>Clostridium spp</td>
<td>ND</td>
<td>8.13 ± 0.48a</td>
</tr>
</tbody>
</table>

ND = Not determined.
See Table 1 for remainder of key.
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 lumenal 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.

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 (× 10^9/L)</td>
<td>11.50 ± 2.27</td>
<td>11.21 ± 2.99</td>
<td>11.05 ± 2.63</td>
<td>0.828</td>
</tr>
<tr>
<td>Neutrophil (× 10^9/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 (× 10^9/L)</td>
<td>4.89 ± 1.81²</td>
<td>4.38 ± 1.94⁴</td>
<td>4.05 ± 1.69⁴</td>
<td>0.043</td>
</tr>
<tr>
<td>Monocyte (× 10^9/L)</td>
<td>0.17 ± 0.17</td>
<td>0.19 ± 0.17</td>
<td>0.14 ± 0.16</td>
<td>0.923</td>
</tr>
<tr>
<td>Eosinophil (× 10^9/L)</td>
<td>0.33 ± 0.27⁴</td>
<td>0.70 ± 0.37⁴</td>
<td>0.95 ± 0.71⁴</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>0.98 ± 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>22.3 ± 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 ± 2.80³</td>
<td>91.70 ± 5.20³</td>
<td>79.13 ± 6.91³</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 ± 1.74³</td>
<td>95.77 ± 5.84³</td>
<td>89.99 ± 5.18³</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fluorescence intensity</td>
<td>448.11 ± 126.08³</td>
<td>581.79 ± 128.38³</td>
<td>780.33 ± 131.61³</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.

10.60%; after probiotic, 11.83 ± 9.66%). Plasma endotoxin concentration was reduced during probiotic supplementation with concentrations below the detection limit in 6 cats; on return to the control diet, endotoxin concentrations increased to those characteristic of the baseline (Figure 2).

10.00%
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 targeted 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. difficiles*, 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 beneficial population was low, and the population was comparatively lower in number than the *Lactobacillus* population, suggesting a bias towards the latter in the healthy feline gastrointestinal tract.

The FISH-mediated detection of the probiotic species involved the use of a probe specific for a subgroup of lactobacilli comprising *L. acidophilus*, *L. crispatus*, *L. gillmarum*, and *L. helveticus*. Low numbers of these species observed in the baseline period, compared with the probiotic phase, indicate that an increase in the probiotic strain occurred. However, stimulation of *L. crispatus*, *L. gillmarum*, and *L. helveticus* populations during probiotic feeding cannot be discounted. The increased numbers of lactobacilli observed during probiotic feeding exceed those detected by the general *Lactobacillus* probe. It is therefore possible that feeding of the probiotic strain stimulated numbers of the inherent *Lactobacillus* populations.

The reduction in fecal pH likely reflects the observed changes in the microflora, such as increased numbers of lactic acid bacteria. The mechanism of persistence in fecal pH reduction is unclear; however, decreased colonic pH is considered a key factor in the effect of probiotic bacteria. This observation may therefore suggest an environment favoring the growth of beneficial species and inhibiting detrimental gastrointestinal tract species.

Probiotic bacteria, particularly *Lactobacillus* spp, have previously been shown to modulate nonspecific immune reactions in humans and other animals. The immunomodulatory effect of *L. acidophilus* DSM13241 in cats was apparent in several parameters measured in the current study. All systemic changes 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 study and by increased activity of peripheral blood phagocytes. Small changes in the phagocytically active proportion 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. Different *Lactobacillus* doses are known to be required for immune stimulation, compared with fecal colonization, with the former occurring at comparatively lower doses in human studies.

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 as a result of decreases in membrane damage caused by oxidants and blood toxins including endotoxin. The increased 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 continued increase in osmotic resistance following the return to base diet. Unidentified longer-term mechanisms are implicated in causing or assisting the observed effect.

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

\textit{a. Lactobacillus acidophilus} DSM13241, Christian Hansen, Horsholm, Copenhagen, Denmark.

\textit{b. Feces scoring system}, WALTHAM, Waltham-on-the-Wolds, Leicestershire, UK.

\textit{c. Oxoid Ltd}, Basingstoke, Hampshire, UK.

\textit{d. API 50 CH}, bioMérieux SA, Marcy l’Étoile, France.

\textit{e. Campden and Cherlowley Food Research Assoc}, Chipping Campden, UK.

\textit{f. Riboprint system}, Qualicon, Wilmington, Del.

\textit{g. MWG Biotech Ltd}, Milton Keynes, UK.

\textit{h. Zeiss, Oberkochen}, Germany.

\textit{i. 95-12 Ammonia electrode}, Orion, Thermoelectron Corp, Waltham, Mass.

\textit{j. Bach Co}, Loveland, Col.

\textit{k. IDEXX Laboratories Ltd}, Wetherby, UK.

\textit{l. VET-RID}, Bethyl Laboratories Inc, Montgomery, Tex.

\textit{m. Tridelta Development Ltd}, Greystones Co, Wicklow, Ireland.

\textit{n. Griess Reagent System}, Promega UK Ltd, Southampton, UK.

\textit{o. Biowhitaker Europe}, Verviers, Belgium.

\textit{p. PHAGOTEST}, Becton-Dickinson, Basel, Switzerland.

\textit{q. FACS caliber system}, Becton-Dickinson, Oxford, UK.

\textit{r. CellQuest}, Clearwater, Fla.

\textit{s. Excel 97}, Microsoft Corp, Redmond, Wash.


\section*{Appendix}

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

\begin{table}[h]
\centering
\begin{tabular}{|l|l|}
\hline
Target organism & Probe sequence (5'-3') \\
\hline
\textit{Lactobacillus} spp & GGT ATT AGC ACC(T)TG TCT CA CGG ATG CGA TCA TGC TCT TAC CCA CCEC TTA TGGA TGG ATT TTC CAT ACC TCA TCC TCA TCC CCA CCEC TTA TGG TGTT TGA TCC TCT TTAC TCC \\
\textit{Lactobacillus acidophilus} & ATG ATG CGA TCT GCT TCT TAA TCC TTT CCA CCEC TTA TGG TGTT TGA TCC TCT TTAC TCC \\
\textit{Bifidobacterium} spp & ATG ATG CGA TCT GCT TCT TAA TCC TTT CCA CCEC TTA TGG TGTT TGA TCC TCT TTAC TCC \\
\textit{Clostridium} spp & ATG ATG CGA TCT GCT TCT TAA TCC TTT CCA CCEC TTA TGG TGTT TGA TCC TCT TTAC TCC \\
\textit{Enterococcus faecalis} & ATG ATG CGA TCT GCT TCT TAA TCC TTT CCA CCEC TTA TGG TGTT TGA TCC TCT TTAC TCC \\
\hline
\end{tabular}
\end{table}

\section*{References}


28. Langendijk PS, Schut F, Jansen GJ, et al. Quantitative fluorescence in situ hybridization of \textit{Bifidobacterium} spp. with genus-


