Clinical Benefits of Probiotic Canine-Derived 
*Bifidobacterium animalis* Strain AHC7 in Dogs 
with Acute Idiopathic Diarrhea*

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This study evaluated the effect of supplementation with canine-derived probiotic 
*Bifidobacterium animalis* strain AHC7 (Iams Prostora, Procter & Gamble Pet 
Care) on the resolution rate of acute idiopathic diarrhea in dogs randomly as -
signed to receive a placebo (n = 18) or the probiotic (n = 13). Nutritional man -
agement with the probiotic fed at 2 × 10¹⁰ CFU/day significantly reduced the time 
to resolution (3.9 ± 2.3 versus 6.6 ± 2.7 days; *P* < .01) and reduced the per -
centage of dogs that were administered metronidazole (38.5% versus 50.0%) 
compared with placebo. Probiotic *B. animalis* AHC7 may provide veterinarians 
another tool for management of acute diarrhea in dogs.

INTRODUCTION

Gastrointestinal (GI) disorders are some of the most common health concerns in dogs and 
comprise a group of ailments with varying and 

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often unrelated underlying causes. Regardless 
of the cause, most GI disorders manifest as 
acute or chronic diarrhea or, in some cases, 
vomiting or anorexia. Acute diarrhea is a par -
ticularly frustrating problem for dog owners 
and veterinarians and occurs commonly in 
puppies and working dogs. Most cases are mild 
and self-limiting, but some may require varying 
degrees of intervention, especially in dogs 
that become dehydrated. The vast majority of 
cases are idiopathic in nature, although dietary
indiscretion, dietary intolerance, parasitic or protozoal infections, microbial imbalance, and stress are frequently associated with acute diarrhea.\(^1\)

Despite the fact that very few cases of acute diarrhea pose serious health risks, owners are continually seeking ways to resolve this condition as quickly and safely as possible. Antimicrobial agents have commonly been used to combat the increase in pathogenic bacteria that often accompanies acute diarrhea, regardless of the direct cause. Recently, however, dog owners and veterinarians have expressed concern about antimicrobial agents and their potential adverse effects and are becoming increasingly interested in “natural” or “alternative” interventions, such as prebiotics and probiotics.\(^2\)

Probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts. Probiotics have long been consumed by humans in the form of fermented foods such as yogurt, kefir, buttermilk, sauerkraut, and cheese, and several species of probiotic organisms are now available in pet foods and supplements. The theory behind these products is that probiotics can help reestablish the bacterial balance in the digestive system and lead to better digestive health after disruption of normal function by such factors as stress, infection, or antibiotic therapy. However, little or no objective research has been conducted in companion animals. Indeed, scant information is available on the populations of bacteria within the various regions of the gut in dogs or the responses of canine microbiota to dietary factors. Molecular profiling of canine intestinal microbiota indicate that the number and diversity of bacteria are highly variable and unique to each individual and do not depend on breed or size.\(^3\) It is generally accepted that commensal organisms may exert species-specific effects and, therefore, a successful canine probiotic organism would ideally be derived from the canine GI tract.\(^4\) In addition, evidence indicates that probiotic effects are strain specific.\(^5\) Most commercial probiotic strains for dogs, however, do not have a canine origin, and most products list only the bacterial genus and species, not the strain. This has led to concern about the quality, labeling, and verification of claims attributed to some of these products.\(^6\) Accurate information about the benefits of each strain will assist veterinary practitioners in determining which probiotic product to use for a specific medical condition.

The two genera of gram-positive lactic acid bacteria used most commonly as probiotics are *Lactobacillus* and *Bifidobacterium*. A recent study conducted to isolate and screen lactobacilli and bifidobacteria adherent to healthy canine GI tissue for strains with commensal activity found that *Bifidobacterium animalis* strain AHC7 had significant potential as a probiotic for improving canine GI health.\(^7\) To be considered a probiotic, an organism must be capable of surviving passage through the gut and able to proliferate and colonize the digestive tract.\(^8,9\) A probiotic organism also must be safe and effective and demonstrate an ability to maintain effectiveness and potency for the duration of the product’s shelf-life. *B. animalis*
AHC7 survived a low pH (2.5) environment and 1% bile exposure and exerted antimicrobial activity against indicator pathogenic organisms.7 This strain survived freeze-drying and withstood storage at room temperature. In addition, B. animalis AHC7 transited the murine GI tract in high numbers, preferentially adhered to intestinal epithelial cells, and significantly inhibited Salmonella typhimurium translocation to the liver and spleen.7 Further testing in dogs that consumed B. animalis AHC7 for 5 weeks revealed a significant reduction in the total number of anaerobic bacteria counted in fecal samples.7 In addition, total clostridia counts were significantly reduced,7 particularly Clostridium difficile, which has been described as the causative agent of antibiotic-associated diarrhea and pseudomembranous colitis in susceptible humans.10,11 These results suggested that B. animalis AHC7 could potentially enhance canine GI health; therefore, the purpose of this study was to determine the effect of daily supplementation of probiotic B. animalis AHC7 on the resolution rate of acute idiopathic diarrhea in dogs.

### MATERIALS AND METHODS

Forty-five young adult dogs (mean age, 1.73 ± 0.72 years) were recruited over a 3-month period from a large guide dog organization colony residing on a single training campus after referral to their veterinary clinic for acute GI issues. Recruited dogs were randomly assigned by blinded personnel to receive either the probiotic B. animalis AHC7 (Iams Prostora, Procter & Gamble Pet Care) or a placebo (same vehicle as the test agent without the probiotic). All dogs then were assessed medically, including fecal microscopy, and those that presented with acute diarrhea, defined by a stool score of 4 (i.e., watery, liquid stool with little or no particulate matter), were eligible to participate provided that they had no other medical conditions and were not being treated for other medical conditions. Dogs with stool scores ≤3 (1 = ideal, firm stool; 2 = soft, amorphous stool; 3 = viscous liquid with some particulate matter) were excluded. Thirty-six dogs met the inclusion criteria and were considered to have acute diarrhea at study entry. Of these, 5 dogs were excluded from the statistical analysis: stool score data were missing for 4 dogs, and 1 dog was withdrawn after discovering that it had been previously included in the study and reenrolled. A total of 31 dogs representing four breeds completed the study and had analyzable data: 10 German shepherds, 2 golden retrievers, 11 Labrador retrievers, and 8 Labrador–golden retriever crosses.

Of the 31 dogs that completed the study, 18 received placebo and 13 received probiotic B. animalis AHC7. Probiotic B. animalis AHC7 (1 × 10^{10} CFU) and placebo (0 CFU) were administered as cocoa butter treats twice daily (i.e., 2 × 10^{10} CFU/day for dogs assigned to receive probiotic B. animalis AHC7) for a maximum of 2 weeks or until diarrhea resolved. Dogs received their fixed daily rations of EuKanuba or Iams maintenance diets (both by Procter & Gamble Pet Care) for the duration of the study. Dogs were monitored daily by trained kennel staff for changes in behavior or clinical condition. Stool scores were recorded by trained personnel who were blinded to treatment group assignment. Resolution of diarrhea was defined as stool scores that improved from 4 to ≤2 and remained ≤2 for at least 5 consecutive days.

After the study began, administration of the antinfecive drug metronidazole was permitted at the veterinarian’s discretion based on the following considerations: number of abnormal stools, degree of stool looseness, health of the dog, and whether other dogs housed nearby were experiencing outbreaks of diarrhea. The decision to treat with metronidazole was made by veteri-
nary personnel who were unaware of the dog’s treatment group assignment. Metronidazole was a treatment choice in the standard care for uncomplicated diarrhea at this guide dog facility.

All dogs were screened for giardial and intestinal parasite infection by standard fecal flotation and microscopy techniques. All dogs were also routinely treated with ivermectin/pyrantel for internal parasite control. Previous investigation has shown that *B. animalis* AHC7 is resistant to metronidazole.12 This study was approved by the Procter & Gamble Pet Care Animal Care and Use Committee.

Data were analyzed by group (probiotic *B. animalis* AHC7 versus placebo) for all dogs combined and separately by breed (if the number of subjects was sufficient) with the Proc GLM procedure of SAS (Release 8.2, SAS Institute, Cary, NC) using a model accounting for the dependent variables of age and days to resolution of diarrhea and the fixed effect of treatment. All data are reported as least squares mean ± standard error. A *P* value ≤ .05 was declared significant.

### RESULTS

A total of 31 young adult dogs (mean age, 1.58 ± 0.18 years) with acute uncomplicated idiopathic diarrhea completed the study. Baseline ages and body weights were similar between the groups for all dogs combined and separately for each breed (Table 1). No other medical conditions emerged during the 2-week supplementation period. None of the dogs became dehydrated during the study, and all ate and drank normally. No abnormal behavior was observed in any of the dogs.

#### Resolution of Diarrhea

Supplementation with probiotic *B. animalis* AHC7 significantly reduced the mean number of days to resolution of diarrhea compared with placebo (3.9 ± 2.3 versus 6.6 ± 2.7 days, respectively; *P* < .01; Table 2). Daily mean

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**TABLE 1. Baseline Characteristics of Dogs Randomly Assigned to Receive Either Probiotic *B. animalis* AHC7 or Placebo**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Probiotic <em>B. animalis</em> AHC7</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.55 ± 0.05 (<em>n</em> = 13)</td>
<td>1.61 ± 0.04 (<em>n</em> = 18)</td>
</tr>
<tr>
<td>German shepherds</td>
<td>1.52 ± 0.12 (<em>n</em> = 4)</td>
<td>1.61 ± 0.10 (<em>n</em> = 6)</td>
</tr>
<tr>
<td>Labrador retrievers</td>
<td>1.68 ± 0.07 (<em>n</em> = 4)</td>
<td>1.64 ± 0.05 (<em>n</em> = 7)</td>
</tr>
<tr>
<td>Labrador–golden retrievers</td>
<td>1.48 ± 0.07 (<em>n</em> = 4)</td>
<td>1.61 ± 0.07 (<em>n</em> = 4)</td>
</tr>
<tr>
<td>Golden retrievers</td>
<td>1.49 ± 0.00 (<em>n</em> = 1)</td>
<td>1.30 ± 0.00 (<em>n</em> = 1)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>26.95 ± 2.80 (<em>n</em> = 13)</td>
<td>28.13 ± 2.38 (<em>n</em> = 18)</td>
</tr>
<tr>
<td>German shepherds</td>
<td>28.12 ± 2.46 (<em>n</em> = 4)</td>
<td>28.92 ± 2.01 (<em>n</em> = 6)</td>
</tr>
<tr>
<td>Labrador retrievers</td>
<td>27.42 ± 1.85 (<em>n</em> = 4)</td>
<td>29.09 ± 1.40 (<em>n</em> = 7)</td>
</tr>
<tr>
<td>Labrador–golden retrievers</td>
<td>27.17 ± 2.69 (<em>n</em> = 4)</td>
<td>26.81 ± 2.69 (<em>n</em> = 4)</td>
</tr>
<tr>
<td>Golden retrievers</td>
<td>19.50 ± 0.00 (<em>n</em> = 1)</td>
<td>21.95 ± 0.00 (<em>n</em> = 1)</td>
</tr>
</tbody>
</table>

*Values are least squares mean (±SE) unless otherwise noted.
stool scores by group are depicted in Figure 1. Subset analysis also demonstrated that supplementation with probiotic *B. animalis* AHC7 significantly reduced the mean number of days to resolution of diarrhea compared with placebo for German shepherds (3.3 ± 1.9 versus 7.7 ± 2.8 days, respectively; *P* = .03) and Labrador retrievers (3.3 ± 1.3 versus 6.3 ± 2.4 days, respectively; *P* = .05).

### TABLE 2. Days to Resolution of Diarrhea in Dogs Randomly Assigned to Either Probiotic *B. animalis* AHC7 or Placebo*

<table>
<thead>
<tr>
<th>Dog Breeds</th>
<th>Days to Resolution of Diarrhea†</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>All dogs</td>
<td>3.9 ± 2.3 (n = 13)</td>
<td>6.6 ± 2.7 (n = 18)</td>
</tr>
<tr>
<td>German shepherds</td>
<td>3.3 ± 1.9 (n = 4)</td>
<td>7.7 ± 2.8 (n = 6)</td>
</tr>
<tr>
<td>Labrador retrievers</td>
<td>3.3 ± 1.3 (n = 4)</td>
<td>6.3 ± 2.4 (n = 7)</td>
</tr>
<tr>
<td>Labrador–golden retrievers</td>
<td>5.3 ± 1.5 (n = 4)</td>
<td>6.0 ± 1.5 (n = 4)</td>
</tr>
<tr>
<td>Golden retrievers</td>
<td>1.0 ± 0.0 (n = 1)</td>
<td>6.0 ± 0.0 (n = 1)</td>
</tr>
</tbody>
</table>

*Values are least squares mean (±SE) unless otherwise noted.
†Resolution of diarrhea was defined as stool scores that improved from 4 to ≤2 and remained ≤2 for at least 5 consecutive days. Stool score categories: 1 = ideal, firm stool; 2 = soft, amorphous stool; 3 = viscous liquid with some particulate matter; 4 = watery, liquid stool with little or no particulate matter.
‡*P* values are based on the Proc GLM procedure of SAS (Release 8.2, SAS Institute, Cary, NC) using a model accounting for the dependent variables of age and days to resolution of diarrhea and the fixed effect of treatment. A *P* value ≤.05 was declared significant.

### Antiinfective Usage

The antiinfective drug metronidazole was administered to fewer dogs in the probiotic *B. animalis* AHC7 group (5 of 13 [38.5%]; Figure 2A) compared with the placebo group (9 of 18 [50.0%]; Figure 2B). In the majority of cases, metronidazole administration (750 mg bid, typically for 7 days) was associated with multiple case outbreaks of diarrhea in dogs housed in proximity to each other, with only three cases of documented giardiasis. The dogs with giardiasis continued the study with regular daily monitoring by the trained kennel staff.

### DISCUSSION

Idiopathic acute diarrhea with no additional known health conditions is common in dogs and often is considered to be related to diet or...
stress, although bacterial, viral, or protozoal infections may also be involved. Stress, such as physical or exercise stress, kennel stress, stress related to transport, reproductive stress, and separation anxiety, among others, is often associated with acute diarrhea in dogs. Many stress conditions have been shown to affect water absorption, osmotic balance, intestinal permeability, and bacterial translocation in humans and other species. In dogs, increased intestinal permeability has been associated with traumatic injury and sustained exercise. Although the mechanisms associated with stress and GI dysfunction have not been clearly identified, findings in restraint-stressed rats suggest that increased intestinal permeability may involve cholinergic nerve pathways. Santos et al.\textsuperscript{16} reported reduced colonic barrier function in stressed rats, with results that could be mimicked by administration of corticotropin-releasing hormone and inhibited by corticotropin-releasing hormone antagonists. Meddings and Swain\textsuperscript{17} reported that adrenalectomized rats, or rats treated with high doses of glucocorticoid receptor antagonists, maintained normal barrier function following stress compared with a control group. Regardless of the exact mechanism, it is becoming more evident that stress can reduce gut function and likely plays a role in some cases of acute diarrhea.

Although acute diarrhea can occur in all dogs, certain groups or breeds appear to be more susceptible than others. Acute diarrhea is quite common in many performance dogs (show, working, or sporting dogs).\textsuperscript{18} These dogs are routinely introduced to new environments that potentially expose them to new pathogenic organisms, including some that have been associated with diarrhea in dogs, such as \textit{Clostridium} spp\textsuperscript{19} and specific strains of \textit{Escherichia coli}.\textsuperscript{20} In the present study, it was presumed that stress related to guide dog training led to the acute diarrhea observed.

Exposure to stress is known to alter the composition of commensal gut microbiota by reducing the number of beneficial bacteria and
increasing the growth, epithelial adherence, and mucosal uptake of pathogenic bacteria.\textsuperscript{21} This imbalance can result in a wide range of digestive disturbances, including diarrhea, constipation, vomiting, and gas or bloating, and can compromise immune function. Lower levels of bifidobacteria, for example, have been specifically linked to such digestive problems in humans as irritable bowel syndrome and inflammatory bowel disease.\textsuperscript{22} A balanced microbial environment prevents invasion by pathogens, provides nutrients essential for proper nutrition by their metabolism (which aids in the synthesis of B and K vitamins), and strengthens the immune system by interacting with key immunomodulatory cells.

Probiotics are being used more frequently to help restore and maintain the normal balance between pathogenic and nonpathogenic intestinal microbiota.\textsuperscript{17} The mechanisms by which probiotics may protect the host from potentially harmful organisms are not completely understood but may involve immune-enhancing and antiinflammatory activities, modifications to intestinal pH, suppression of pathogenic bacteria through production of inhibitory substances, competition with pathogens for essential nutrients and mucosal attachment sites, production of lactase, induction of epithelial heat-shock proteins, restoration of tight junction protein structure, up-regulation of mucin genes, and regulation of the nuclear factor $\kappa$B signaling pathway.\textsuperscript{23,24} These effects are strain specific with respect to the types of GI benefits provided. A study in which a combination of \textit{Lactobacillus acidophilus} NCC2628, \textit{L. acidophilus} NCC2766, and \textit{Lactobacillus johnsonii} NCC2767 was administered to dogs with confirmed inflammatory bowel disease found that these strains were able to modulate proportions of regulatory and proinflammatory cytokines.\textsuperscript{25} When investigators administered these strains to dogs with food-responsive diarrhea, those that received the probiotics appeared to experience greater clinical improvements while on elimination diets. Rinkinen and colleagues\textsuperscript{26} administered canine isolates of \textit{Lactobacillus fermentum}, \textit{Lactobacillus salivarius}, \textit{Lactobacillus rhamnosus}, \textit{Lactobacillus mucosae}, and \textit{Weissella confusa} to dogs and found transient changes in indigenous lactic acid–producing bacteria. However, the strains \textit{L. rhamnosus} GG, \textit{Lactobacillus pentosus} UK1A, \textit{L. pentosus} SK2A, \textit{Bifidobacterium lactis} Bb12, \textit{Enterococcus faecium} M74, and \textit{E. faecium} SF273 reduced adhesion by \textit{Clostridium perfringens}, although none inhibited adhesion by \textit{Salmonella typhimurium} or \textit{Staphylococcus intermedius}. Two strains of \textit{E. faecium} examined by this group in an earlier study were found to increase binding by \textit{Campylobacter jejuni}.\textsuperscript{27} Baillon and Butterwick\textsuperscript{28} reported that \textit{L. acidophilus} DSM13241 reduced shedding of \textit{Campylobacter} in infected cats. Vahjen and Männer\textsuperscript{29} investigated the effects of \textit{E. faecium} NCIB 10415 on numbers of fecal \textit{Salmonella}, \textit{Campylobacter}, and \textit{Clostridium} spp in dogs and found significant decreases in clostridia but increases in \textit{Salmonella} and \textit{Campylobacter} spp.
The present study revealed that nutritional management with the probiotic *B. animalis* strain AHC7 fed at $2 \times 10^{10}$ CFU/day significantly reduced the time to resolution of acute diarrhea compared with placebo (3.9 ± 2.3 versus 6.6 ± 2.7 days, respectively; $P < .01$). Even breeds with commonly recognized sensitivity in the GI area, such as German shepherds, derived faster diarrhea resolution benefits with probiotic *B. animalis* AHC7 compared with placebo (3.3 ± 1.9 versus 7.7 ± 2.8 days, respectively; $P = .03$). Probiotic *B. animalis* AHC7 provides a proactive approach to GI health concerns that may also lessen reliance on antimicrobial drugs and reduce potential undesirable side effects associated with the use of such agents. The percentage of dogs in the study that were administered metronidazole was lower in the group supplemented with probiotic *B. animalis* AHC7 compared with placebo. Based on the present study, probiotic *B. animalis* AHC7 may provide veterinarians another tool for management of acute diarrhea in dogs.

The disproportionate prescribing of metronidazole in the placebo group versus the probiotic group is a limitation of this clinical study. Because the cause of the acute diarrhea was not conclusively determined in each case, it is possible that different etiologies, some of which may have been responsive to metronidazole, were involved and may have complicated these results. However, when dogs given metronidazole are excluded from the analysis, only 3 of 9 in the placebo group had diarrhea resolution by day 4 versus 7 of 9 in the probiotic group, supporting the conclusion that probiotic *B. animalis* AHC7 was associated with more rapid resolution of diarrhea.

Acute diarrhea will likely remain a common issue in dogs given the array of contributing factors, particularly among stressed individuals. However, improvements in kennel design and socialization practices, along with a better understanding of the physiologic responses induced by stress, will likely aid in the reduction of occurrences or severity of diarrhea outbreaks. Dog owners now have access to numerous commercial dietary formulations that are highly digestible and contain specialized fiber sources to help promote gut health. In addition, commercially available probiotics are becoming more common.

**Probiotic *B. animalis* AHC7 provides a proactive approach to GI health concerns that may also lessen reliance on antimicrobial drugs and reduce potential undesirable side effects associated with the use of such agents.**

The use of beneficial bacteria, perhaps alone or in combination with prebiotics, may offer the greatest chance of reducing the number or severity of outbreaks of acute diarrhea. However, not all strains of probiotics or types of prebiotics provide equal benefits. A given strain may promote improved immunologic function, whereas another may reduce pathogenic populations or activity of inflammatory pathways. Veterinarians should seek assurance of the effectiveness of specific strains, that products contain adequate amounts necessary to deliver the benefit, and that the claimed benefits are supported by documented objective research and not just implied. Advancements in these areas will allow transition from a reactive approach to diarrhea management to one of prevention.
CONCLUSION
Dogs whose diets were supplemented with probiotic *B. animalis* AHC7 had significantly (P < .05) more rapid resolution of acute diarrhea than dogs that received placebo. The percentage of dogs that were administered metronidazole was lower in the group supplemented with probiotic *B. animalis* AHC7 compared with placebo. The benefits of probiotic *B. animalis* AHC7 supplementation observed in German shepherds and Labrador retrievers suggest potential health benefits in dogs prone to GI disorders.

REFERENCES


