Effects of Varying Doses of a Probiotic Supplement Fed to Healthy Dogs Undergoing Kenneling Stress

Russ Kelley MS[1]

Kerinne Levy[2]

Paul Mundell[2]

Michael G. Hayek PhD[1]

[1]P&G Pet Care, DV1-1Y4, 8700 Mason Montgomery Road, Mason, Ohio 45040, 513-622-1301, hayek.mg@pg.com

[2] Canine Companions for Independence, 2965 Dutton Avenue, Santa Rosa, CA 95407, 707-577-1785, klevy@cci.org

Corresponding author: Russ Kelley, 513-622-1621, kelley.rl@pg.com

KEY WORDS: Dogs, Probiotic, Kenneling, stress.

ABSTRACT

Dogs undergoing stress may experience gastrointestinal disturbances that can manifest as loose stool or diarrhea. The objective of this study was to examine the efficacy of feeding varying doses of a canine-derived probiotic supplement (*Bifidobacterium animalis* strain AHC7) for reducing stress-related gastrointestinal disturbances in dogs that were relocated from homes to a kennel environment. Healthy, young adult dogs (n = 134) were randomly assigned to one of four supplements containing either < 10^3 (control), 1.5 x 10^7, 1.5 x 10^8, or 1.5 x 10^9 CFU of AHC7. Dogs received an oral supplement one time per day for 5 weeks prior to relocation and for the first 3 weeks following relocation. Fecal scores, number of defecations per day, fecal microbial populations, and serum cortisol levels were measured before and after kennel relocation. Fecal populations of AHC7 were significantly elevated in all three treatment groups when compared with the control group, and concentrations suggested a dose-response effect. Fecal scores were significantly higher (closer to optimal) in dogs supplemented with AHC7 when compared with the control group during Week 3 and over the entire 3-week relocation period. Significantly fewer dogs that were supplemented with AHC7 passed unacceptable stools during the first week of relocation when compared with the control group. Supplementing healthy dogs with *Bifidobacterium animalis* AHC7 prior to and during kennel relocation at doses between 107 and 109 CFU/day supported optimal stool production and may help to prevent stress-related gastrointestinal upsets and diarrhea.

INTRODUCTION

Relocation and kenneling are stressful events for dogs, as evidenced both by behavioral signs and changes in cortisol levels.[1,2] In addition, stress associated with kennel environments may cause changes in gut function, manifesting as poor stool quality or diarrhea.[3] Typically, signs of gastro-

intestinal stress related to kenneling in dogs are mild and self-limiting. When needed, treatment includes dietary modification and/or rehydration.[4] Antimicrobial agents may also be prescribed to treat the overgrowth of potentially pathogenic bacteria that can accompany cases of acute enteritis. Although not a serious health risk, stress-related diarrhea is of concern to many pet owners as well as to professional trainers, breeders, and handlers who routinely need to transport or kennel their dogs.

In recent years, concern about antibiotic overuse and potential adverse health effects of antimicrobial drugs has led to increased interest in alternative or supportive treatments such as probiotics and prebiotics.[5] Probiotics are live microorganisms that, when ingested in sufficient amounts, exert beneficial health effects to the host animal by modulating the intestinal microbial environment in favor of non-pathogenic bacterial species.[6] To be effective, a probiotic must be capable of surviving passage through the gastrointestinal tract (which includes exposure to stomach acid and bile) and able to proliferate and colonize safely within the host's intestinal tract.[7,8] When used therapeutically, probiotic supplementation is intended to support or re-establish a healthy bacterial balance following disruption caused by environmental stress or infection. The microbes that are used are bacterial strains that are typically found in intestinal microbiota and known to be beneficial.

In dogs, a variety of bacteria are used as probiotics, most commonly species and strains of lactic acid bacteria of the genera *Lactobacillus*, *Bifidobacterium*, or *Enterococcus*.[9,10] Although limited studies have been conducted, there is evidence that probiotics can help to reduce the incidence and duration of non-specific diarrhea in dogs.[11,12] Specific benefits that probiotics can provide to the stressed gut involve support of gastrointestinal tract barrier function, prevention of bacterial translocation, and reduced severity and duration of enteritis.[13,14] The underlying molecular and cellular mechanisms through which probiotic organisms exert benefit are not completely understood, but it appears that most organisms have multiple effects and that these vary with both species and strain of organism. Some potential mechanisms of action include suppression of pathogenic bacteria through production of inhibitory substances, modulation of the host's mucosal immune responses, modification of luminal pH through lactic acid production, and competition with pathogenic bacteria for essential nutrients and mucosal attachment sites.[15,16]

A strain of canine-derived *Bifidobacterium animalis* AHC7 has been shown to survive the environment of the canine gastrointestinal tract as well as conditions associated with commercial processing.[17] As a probiotic supplement, it has been demonstrated to be safe for use with dogs, and was shown to effectively reduce the total number of fecal anaerobic bacteria and clostridia species such as *Clostridium difficile* in healthy dogs following 5 weeks of supplementation.[17,18] A subsequent clinical trial reported that *Bifidobacterium animalis* AHC7 significantly improved recovery time and reduced the need for antimicrobial drug therapy in dogs with acute idiopathic diarrhea.[19]

In addition to surviving exposure to bile acids and digestive enzymes, it is essential that a probiotic organism is administered at a dose that contains concentrations high enough to allow gut proliferation and colonization, without overwhelming or imbalancing the normal intestinal microbiota. Most of the probiotic supplements that have been studied delivered between 1×10^7 and 1×10^{11} CFU per dose, regardless of the bacterial species. There are few studies that examine a range of effective probiotic doses in dogs, and none known to the authors that have examined a range of doses for *Bifidobacterium* spp. The purpose of the current study was to examine the effects of varying doses of a canine-derived strain of *Bifidobacterium animalis* (strain AHC7) on gut function in a group of adult dogs undergoing environmental stress associated with reloca-

tion from homes to a kennel environment.

MATERIALS AND METHODS

Animals and Treatments

A group of 134 young adult dogs (mean age 17.96 $\pm$ 1.06 months) was recruited for the study. All of the dogs were Labrador Retrievers or Labrador-Golden Retriever crosses that had been bred at a large, private service dog organization and were placed in private puppy raising homes shortly after weaning (approximately 8 weeks of age). Puppy Raiser (PR) homes were located in 16 different states that served four large, regional training centers. Dogs remained in their assigned home until they were approximately 60 weeks of age, and were enrolled in the study approximately 5 weeks prior to relocation. Relocation involved transfer from the PR's home to a regional training campus for evaluation as a candidate service dog.

At study enrollment, each PR was provided with 27.2 kg of study diet (Eukanuba® Large Breed Adult Maintenance), feeding equipment, a study journal, and a 6-week supply of study treats (probiotic supplement). Dogs that had not previously been fed the study diet were transitioned to the new food approximately 2 weeks prior to study initiation. Dogs were fed only the prescribed diet, and the daily probiotic treat for the duration of the study.

Dogs were randomly assigned to one of four treatment groups, with consideration for balancing across regional kennel locations. Dogs in each treatment group received a daily supplement containing either 0 (Control), 1 x 10^7 (Lg7), 1 x 10^8 (Lg8), or 1 x 10^9 (Lg9) colony forming units (CFU) of *Bifidobacterium animalis* AHC7 (AHC7) with their morning meal. Dogs were supplemented daily for 5 weeks prior to relocation and for 20 days following relocation to the kennel environment (a total of 8 weeks). Per the service dog organization's normal procedures, kenneled dogs were pair-housed following relocation with kennel assignments restricted within treatment group to minimize potential for treatment contamination during the 20-day kennel period.

Study treats were composed of sucrose, vegetable oil, dried skim milk, dried reduced mineral whey, soy lecithin, artificial vanilla flavor, artificial color, cocoa butter, and appropriate amounts of the probiotic strain, *B. animalis* AHC7 (Knechtel Research Sciences, Inc., Skokie, Illinois, USA 60076). Control treats contained no added probiotic. Following production, treats were refrigerated until distribution for study use and samples were obtained for *B. animalis* AHC7 concentration analysis (P&G SOP RD LAB MB.038.0 – *Bifidobacterium* enumeration; Iams Central Laboratory, Mason, Ohio, 45040).

Sample Collection and Analysis

Fecal scores, number of defecations per day, the number of fecal scores that were unacceptable (scores of 2 or less), fecal microbial populations, and serum cortisol levels were measured before and after kennel relocation. Puppy raisers evaluated fecal quality for five consecutive days prior to their dog's relocation and on the day of relocation. A 5-point fecal score scale was used with the following assigned scores:

- 1 = liquid
- 2 = soft, without shape
- 3 = soft, with shape
- 4 = firm, ideal stool
- 5 = extremely dry.

A stool score of 4 was considered to be ideal. Following relocation to kennels, daily fecal scores were assessed by trained kennel staff for 20 days, beginning the first day of relocation. Because dogs were pair-housed, a single fecal score was recorded for each kennel. Daily kennel fecal scores were used to calculate mean daily, weekly and period fecal scores for statistical analysis.

Duplicate fecal samples were collected from each dog at the start of the study (prior to supplementation), approximately 5 weeks prior to relocation. During the kenneling period, fecal samples were collected on day 3, 4, 10, 11, 19, and 20. Following collection, fecal samples were sub-sampled and imme-

Table 1. *Fecal quality scores during three-week kennel relocation period in dogs fed varying levels of a probiotic supplement**

Week	Treatment**				P Value
	Lg9	Lg8	Lg7	Control	
Week 1	3.93 ± 0.07	3.93 ± 0.07	3.77 ± 0.07	3.84 ± 0.07	P<0.29
Week 2	3.95 ± 0.06	3.92 ± 0.06	3.90 ± 0.05	3.85 ± 0.05	P<0.69
Week 3	3.92 ± 0.07[b]	3.87 ± 0.07[b]	3.89 ± 0.07[b]	3.67 ± 0.07[a]	**P<0.05**
Overall***	3.94 ± 0.05[b]	3.91 ± 0.05[b]	3.87± 0.05[b]	3.75 ± 0.04[a]	**P<0.03**

** Reported as LSMeans ± SEM of weekly average within kennel of fecal score using the following 5-point scale: 1 = liquid; 2 = soft, without shape; 3 = soft, with shape; 4 = firm, ideal stool; and 5 = extremely dry.*
*** Different superscripts within rows indicate significant differences between treatments (P<0.05)*
**** Significant treatment effect for three-week period (P<0.05)*

diately placed on dry ice and stored at -70°C until analysis. Fecal microbial populations were measured in samples collected at the start of the study (baseline) and on days 3, 10, and 20 of the kenneling period (The GI Lab, Department of Small Animal Surgery, Texas A&M University, College Station, Texas 77843). Fecal microbial populations were determined for *Bifidobacterium animalis* AHC7, total *Bifidobacteria* spp, total *Lactobacillus* spp, *Bacteroides fragilis*, *Clostridium perfringens, Escherichia coli,* and the *Clostridium coccoides-Eubacterium* group using qPCR techniques that were previously described.[20,21]

Blood samples were collected by cephalic venipuncture from all dogs 5 weeks prior to relocation (baseline) and on days 3, 10, and 20 of the kenneling period. Following collection, samples were transferred to centrifugation tubes and allowed to clot for 15 minutes. Serum was separated via centrifugation (10 minutes @ 2,000g), transferred to sterile cryogenic tubes (2 ml), and stored at -70°C until analysis. Serum cortisol was measured in duplicate using a commercially available cortisol assay (ParameterTM Cortisol Assay, R&D Systems Inc., Minneapolis, MN 55413).

Statistics

Data were analyzed utilizing the GLM procedures of SAS (V9.1, SAS Institute, Gary, NC 27513). The initial statistical model included region, treatment group, and sex. Because no significant region (relocation center) or sex differences were found, the final statistical model included treatment only. When statistically significant treatment effects were found, post-hoc treatment differences were examined using Least Square means (p<0.10).

RESULTS

Dogs

One hundred twenty-one (121) of the 134 enrolled dogs (90.3%) completed the study. Seven dogs were excluded from analysis due incomplete data, four dogs were excluded as a result of PR noncompliance, and two dogs were released by the service dog organization prior to the end of the study because of behavior problems.

Fecal Scores

Mean fecal scores collected during the 5 days prior to relocation were all within a range that is considered to be optimal (3.7 to 3.9) and did not differ significantly among treatment groups (data not shown, P > 0.10). During the 3-week relocation period, mean fecal scores of the three probiotic treated groups were significantly higher when compared with the control group (P<0.03; Table 1). When compared within the week, all three supplemented groups had significantly higher mean fecal scores when compared with mean fecal scores of the control group for week 3 (P < 0.05), with no significant differences in fecal score among groups during week 1 or week 2 of the kennel relocation period. Probiotic supplementation also

Table 2. *Mean number of unacceptable stools produced during a three-week kennel relocation period in dogs fed varying levels of a probiotic supplement**

Week	Treatment**				P Value
	Lg9	Lg8	Lg7	Control	
Week 1	0.06 ± 0.3^{b}	0.31 ± 0.3^{b}	0.47 ± 0.3^{ab}	1.10 ± 0.3^{a}	**P<0.08**
Week 2	0.33 ± 0.3	0.50 ± 0.3	0.44 ± 0.3	1.00 ± 0.3	P<0.33
Week 3	0.88 ± 0.5	0.38 ± 0.5	0.61 ± 0.4	1.10 ± 0.4	P<0.67
Overall***	1.18 ± 0.7^{b}	1.19 ± 0.7^{b}	1.50 ± 0.6^{b}	3.1 ± 0.6^{a}	**P<0.10**

** Reported as LSMeans ± SEM of the number of unacceptable stools per dog with a score ≤ 2*
*** Different superscripts within rows indicate significant differences between treatments (P<0.10)*
**** Significant treatment effect for three-week period (P<0.10)*

significantly affected the number of unacceptable stools that were produced during the first week of kennel relocation (Table 2).

Dogs supplemented with either 10^8 (Lg8) or 10^9 (Lg9) CFU of AHC7 produced significantly fewer unacceptable stools during the first week of relocation (0.31 + 0.3 and 0.06 + 0.3, respectively) when compared with the mean number of unacceptable stools produced by dogs in the control group (1.10 + 0.3). No significant difference was found between number of unacceptable stools produced by dogs supplemented with 10^7 (Lg7) CFU and any of the other treatment groups. The mean number of unacceptable stools produced per dog over the entire 3-week period was significantly lower for the three groups treated with AHC7 when compared with the control group (P < 0.10). The number (percent) of dogs that passed one or more unacceptable stools was also significantly lower during the first week of relocation in the Lg8 and Lg9 treatment groups when compared with the control group (1.56 + 4.4 and 4.46 + 4.4 vs. 15.7 + 3.97, respectively; Table 3). Cumulatively, the total number of unacceptable stools that were passed during the relocation period by dogs in the supplemented groups was lower in all three treatment groups when compared with the control group (Figure 1).

Fecal Microbial Counts

No significant differences in fecal microbial populations were found among the four treatment groups at the start of the study period (baseline) prior to initiation of supplementation (Table 4). During the supplementation period, the fecal AHC7 number increased significantly in all three supplemented groups above baseline, and

Table 3. *Percent of dogs producing unacceptable stools during a three-week kennel relocation period in dogs fed varying levels of a probiotic supplement**

Week	Treatment**				P Value
	Lg9	Lg8	Lg7	Control	
Week 1	1.56 ± 4.4^{a}	4.46 ± 4.4^{a}	6.72 ± 4.3^{ab}	15.7 ± 3.97^{b}	**P<0.10**
Week 2	4.76 ± 4.3	7.14 ± 4.1	6.35 ± 3.9	14.30 ± 3.8	P<0.33
Week 3	14.58 ± 7.6	6.25 ± 7.6	10.19 ± 7.2	18.42 ± 7.0	P<0.67
Overall***	8.75 ± 3.5	5.95 ± 3.6	7.63 ± 3.4	16.42 ± 3.3	P<0.15

** Reported as LSMeans ± SEM of the percent of dogs within treatment group producing one or more unacceptable stools (score ≤ 2)*
*** Different superscripts within rows indicate significant differences between treatments (P≤0.10)*
**** Significant treatment effect for three-week period (P≤0.10)*

Table 4. *Fecal microbial populations prior to and during a three-week kennel relocation period in dogs fed varying levels of a probiotic supplement**

Organism/Day	**Treatment Group****				
***B. animalis* AHC7**	**Lg9**	**Lg8**	**Lg7**	**Control**	**P Value**
Baseline	0.105 ± 0.03	0.038 ± 0.04	0.060 ± 0.03	0.102 ± 0.03	P≤0.46
3 days	0.700 ± 0.04[d]	0.600 ± 0.04[c]	0.376 ± 0.04[b]	0.163 ± 0.03[a]	**P≤0.001**
10 days	0.693 ± 0.04[d]	0.559 ± 0.05[c]	0.347 ± 0.04[b]	0.158 ± 0.04[a]	**P≤0.001**
20 days	0.709 ± 0.05[d]	0.547 ± 0.05[c]	0.349 ± 0.05[b]	0.213 ± 0.04[a]	**P≤0.001**
***Bifidobacterium* spp.**	**Lg9**	**Lg8**	**Lg7**	**Control**	**P Value**
Baseline	0.271 ± 0.05	0.206 ± 0.06	0.321 ± 0.05	0.390 ± 0.05	P≤0.11
3 days	0.726 ± 0.04[c]	0.577 ± 0.04[b]	0.481 ± 0.04[b]	0.348 ± 0.04[a]	**P≤0.001**
10 days	0.731± 0.05[c]	0.574 ± 0.05[b]	0.487 ± 0.05[b]	0.345 ± 0.04[a]	**P≤0.001**
20 days	0.726 ± 0.05[c]	0.566 ± 0.05[b]	0.493 ± 0.05[b]	0.363 ± 0.05[a]	**P≤0.001**
***Lactobacillus* spp.**	**Lg9**	**Lg8**	**Lg7**	**Control**	**P Value**
Baseline	0.320 ± 0.06	0.338 ± 0.07	0.313 ± 0.06	0.378 ± 0.06	P≤0.87
3 days	0.501 ± 0.06	0.536 ± 0.07	0.395 ± 0.06	0.574 ± 0.06	P≤0.21
10 days	0.356 ± 0.07	0.446 ± 0.08	0.499 ± 0.07	0.539 ± 0.07	P≤0.29
20 days	0.331 ± 0.07[b]	0.470 ± 0.07[a]	0.642 ± 0.07[a]	0.501 ± 0.06[a]	**P≤0.02**
***Bacteroides* spp.**	**Lg9**	**Lg8**	**Lg7**	**Control**	**P Value**
Baseline	0.593 ± 0.03	0.615 ± 0.04	0.592 ± 0.03	0.601 ± 0.03	P≤0.97
3 days	0.636 ± 0.03	0.577 ± 0.03	0.614 ± 0.03	0.652 ± 0.03	P≤0.40
10 days	0.643 ± 0.03	0.650 ± 0.04	0.667 ± 0.03	0.629 ± 0.03	P≤0.86
20 days	0.624 ± 0.03	0.626 ± 0.03	0.621 ± 0.03	0.663 ± 0.02	P≤0.61
C. coccoides-Eubact.	**Lg9**	**Lg8**	**Lg7**	**Control**	**P Value**
Baseline	0.834 ± 0.04	0.753 ± 0.04	0.829 ± 0.04	0.845 ± 0.03	P≤0.33
3 days	0.852 ± 0.03	0.781 ± 0.03	0.776 ± 0.03	0.841 ± 0.03	P≤0.20
10 days	0.867 ± 0.04[b]	0.838 ± 0.04[b]	0.77 ± 0.04[ab]	0.723 ± 0.04[a]	**P≤0.04**
20 days	0.837 ± 0.03	0.838 ± 0.03	0.760 ± 0.03	0.802 ± 0.03	P≤0.23
***C. perfringens* spp.**	**Lg9**	**Lg8**	**Lg7**	**Control**	**P Value**
Baseline	0.399 ± 0.04	0.458 ± 0.05	0.484 ± 0.04	0.420 ± 0.04	P≤0.53
3 days	0.286 ± 0.04	0.352 ± 0.04	0.378 ± 0.04	0.392 ± 0.04	P≤0.25
10 days	0.278 ± 0.04	0.338 ± 0.05	0.321 ± 0.04	0.233 ± 0.04	P≤0.32
20 days	0.321 ± 0.04	0.259 ± 0.04	0.371 ± 0.04	0.346 ± 0.04	P≤0.27
E. coli	**Lg9**	**Lg8**	**Lg7**	**Control**	**P Value**
Baseline	0.380 ± 0.05	0.283 ± 0.06	0.392 + 0.05	0.306 ± 0.05	P≤0.42
3 days	0.340 ± 0.05	0.305 ± 0.05	0.341 + 0.05	0.401 ± 0.05	P≤0.58
10 days	0.275 ± 0.05	0.183 ± 0.05	0.295 + 0.05	0.354 ± 0.05	P≤0.13
20 days	0.210 ± 0.05	0.214 ± 0.05	0.197 + 0.05	0.267 ± 0.04	P≤0.71

** Reported as LSMeans ± SEM (fg DNA/5 ng bacterial DNA)*
*** Different superscripts within rows indicate significant differences between treatments (P<0.10)*

the values suggest a dose-response effect (Lg9>Lg8>Lg7>Control). The supplemented groups maintained fecal AHC7 populations throughout the study that were significantly higher than populations in the control group (Figure 2). Total Bifidobacteria populations paralleled the values reported for AHC7 (Table 4). *Lactobacillus* populations did not differ among the four groups at any sampling times, with the exception of on Day 20, when dogs in the Lg9 group had significantly lower numbers of *Lactobacillus* when compared with the other groups ($P<0.02$).

Within treatment groups, dogs in the Lg7 group had elevated levels of *Lactobacillus* on Day 20 when compared with baseline values ($P<0.05$). No significant differences were found among treatment groups or within groups when compared with baseline values during the relocation period in populations of *Bacteroides* spp. No differences among treatment groups were found in counts of the family group *C. coccoides* and *Eubacterium* at most time points. However, there was a significant decrease in count in the Control group on Day 10 when compared with the baseline count. This change in the Control group resulted in a significant difference between the Control and the Lg8 and Lg9 treatment groups on Day 10 ($P<0.04$). *C. perfringens* populations did not differ significantly among treatment groups at any time point. Significant decreases in *C. perfringens* were observed in the Control group and in the Lg7 treatment group on Day 10 when compared with baseline values ($P < 0.05$). Similarly, no differences in *E. coli* populations were observed among the four groups during the relocations period. However, *E. coli* decreased significantly on Day 20 in the Lg7 group when compared with baseline ($P<0.05$).

Serum Cortisol

No significant differences in serum cortisol levels were observed among the four groups prior to or during relocation. During the relocation period, all groups had significantly elevated serum cortisol concentrations when compared with baseline values on the third day of relocation (Table 5). Concentrations in all dogs returned to values that were not statistically different from baseline values on Day 10 and Day 20 of relocation.

DISCUSSION

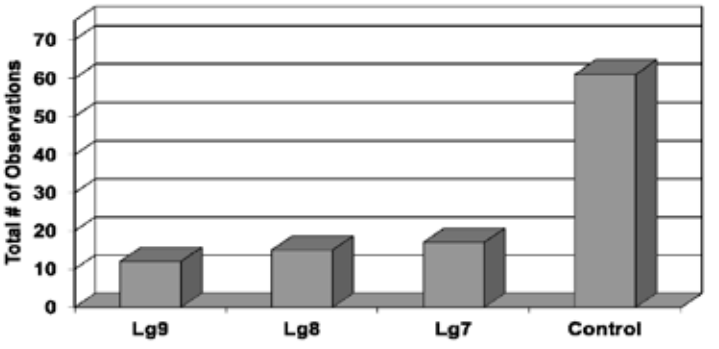
The two most common kenneling situations that dogs experience are pet dogs that are boarded at a commercial kennel and homeless dogs housed at animal shelters. It is generally accepted that these transitions are stressful to dogs, and that this stress may manifest as both physiologic and behavioral signs.[1,22] Measures of stress that have been used with dogs in shelters include serum or salivary cortisol levels, barking, and behavioral signs (pacing, panting).[23,24,25] In addition, gastrointestinal responses to stress can include loose stools and diarrhea.[3,26] Although the exact underlying mechanisms of these gastrointestinal changes in dogs have not been studied, research with other species has shown that psychological stress can compromise colonic barrier function through activation of mast cells, which can contrib-

Table 5. *Serum cortisol concentrations (ug/dl) during a three-week kennel relocation period in dogs fed varying levels of a probiotic supplement**

Cortisol (ug/dl)	Treatment**				P Value
	Lg9	Lg8	Lg7	Control	
Baseline	1.43 ± 0.11	1.56 ± 0.13	1.34 ± 0.11	1.36 ± 0.11	P<0.57
KT+3	2.12 ± 0.19	2.14 ± 0.21	2.13 ± 0.19	2.07 ± 0.18	P<0.99
KT+10	2.00 ± 0.16	2.07 ± 0.19	1.70 ± 0.15	2.00 ± 0.15	P<0.36
KT+20	1.63 ± 0.15	1.83 ± 0.17	1.64 ± 0.14	1.60 ± 0.14	P<0.74

** Reported as LSMeans ± SEM (ug/dl)*

Figure 1. *Total number of unacceptable stools passed during a three-week kennel relocation period in dogs fed varying levels of a probiotic supplement*



ute to large bowel diarrhea.[27,28,29] Exposure to stress may also alter the composition of commensal gut microbiota by reducing the number of beneficial bacteria and allowing the proliferation and epithelial adherence of potentially pathogenic bacterial species.[30]

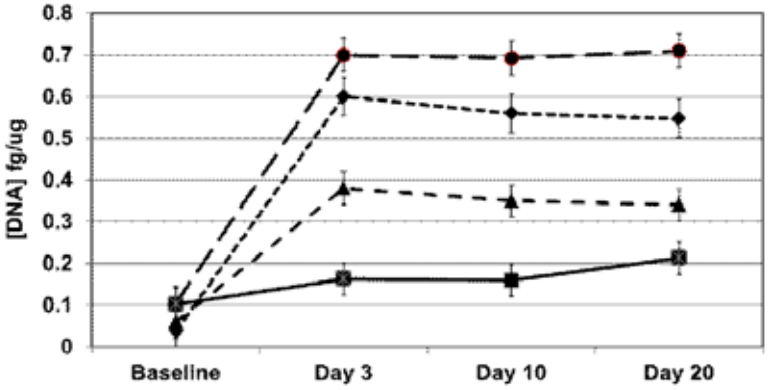
An earlier pilot study conducted by the authors found that service dogs undergoing relocation from homes to kennel environments experienced reduced stool scores and tended to show increased serum cortisol concentrations. These changes occurred even though the dogs were all fed a high quality diet, received regular veterinary care, and were in optimal health. As a result, the study reported here was conducted to examine effects of varying doses of *B. animalis* AHC7 supplementation on gut function and physiology, as measured by stool scores and fecal microflora, in a similar group of dogs undergoing kennel relocation. Results corroborated the pilot study data, showing that all of the dogs in the present study experienced some degree of stress, as evidenced by elevated serum cortisol concentrations on the third day of kennel relocation and varying effects on fecal quality.

There is evidence in dogs that probiotics can help to restore normal gut function during episodes of idiopathic acute enteritis.[14,19,26] Probiotic therapy may also have efficacy in the management of other forms of enteritis in dogs, including dietary sensitivity.[31,32,33] A range of different lactic acid bacteria species have been studied, most of which are within the genera *Lactobacillus*, *Enterococcus* or *Bifidobacterium*. Because the intestinal microbiota of every mammalian species is unique and distinct, it is presumed that selection of a probiotic organism that is derived from the species of its intended use should provide the greatest benefit.[34,35] For example, when a group of canine-derived lactic acid bacteria species were screened for use as probiotics in dogs, two *Lactobacillus* species became established in the intestine during the feeding period.[36] Following supplementation, these strains declined in numbers, to be rapidly replaced by a proliferation of indigenous *Lactobacillus acidophilus* in the canine gut. Although these results are preliminary, the authors suggested that the canine-derived lactic acid bacteria that were fed as a probiotic functioned to enhance the proliferation of the beneficial indigenous population of *Lactobacillus acidophilus*. This unique effect may be related to the use of species-specific organisms that are already adapted to existing as commensal organisms. This specificity may enhance a probiotic's ability to support the normal microbiota balance.

Recent evidence has shown that *B. animalis* AHC7, the canine-derived probiotic used in the current study, possesses the characteristics that are needed for an effective probiotic. The organism remains viable following freeze-drying and processing, survives exposure to stomach and bile acids, and is capable of adhering to intestinal epithelial cells.[17] The ACH7 strain has also been shown to be well tolerated and safe when fed to growing dogs at a concentration of 5 x 10^{10} CFU per day.[18] To provide beneficial

health effects, a probiotic must be capable of remaining viable long enough to colonize the gut microbiota of the dog, at least temporarily.[37] Results of the study reported here show that daily supplementation with *B. animalis* strain AHC7 for 5 weeks effectively establishes a viable population of AHC7 in the lower gut, and that the population is maintained with continued supplementation, as measured using fecal microbial counts.

Figure 2. *Change in fecal microbial populations of Bifidobacterium animalis AHC7 from baseline at 3,10 & 20 days of a three-week kennel relocation period in dogs fed varying levels of AHC7 probiotic supplement**



** Data are reported as LSMeans ± SE (Δ from baseline), with different superscripts denoting a significant difference (P<0.05) between treatment groups.*

Few studies have been conducted that examine effective dose ranges for probiotics, and those that are available have studied different organisms and host species. For example, a dosing recommendation for a human strain of *Lactobacillus rhamnosis* in dogs is 5 x 10^{10} CFU per day, while an approximately 10-fold increase of the same organism is recommended for horses.[38,39] Effective doses may also be affected by the original source of the probiotic microbe. When a probiotic preparation containing either 0, 1 x 10^{9}, 1 x 10^{10}, 5 x 10^{10}, or 5 x 10^{11} CFU/day of a human strain of *Lactobacillus rhamnosus* was fed to healthy dogs, the probiotic organism was detected in the feces of at least 50% of dogs treated with the three lowest concentrations, and in 100% of the dogs treated with the preparation containing the highest concentration (5 x 10^{11} CFU).[38]

Fecal concentrations of *L. rhamnosus* were also significantly higher in this group when compared with the control and with the other three supplemented groups. The authors reported that the dose required by dogs was substantially higher than the dose needed to achieve colonization in human subjects, suggesting that colonization with a human strain of *L. rhamnosus* in dogs was less efficient. In addition, the persistence of *L. rhamnosus* colonization in dogs is of shorter duration than that reported in humans.[40] These differences suggest that *L. rhamnosus* (and perhaps other probiotic organisms) of human origin are less well adapted to colonize the canine gastrointestinal tract than the human intestine, and thus require a higher dose for colonization in dogs when compared with doses that are effective in human subjects. This theory is supported by results from another study that fed healthy dogs 1 x 10^{9} CFU/ml (2 to 3 ml doses/day) of an *Enterococcus faecium* strain that had been isolated from dog food.[41] Dogs were supplemented for a period of only 7 days, but presence of the organism was detected in treated dogs' feces for up to 3 months following cessation of supplementation. Colonization appeared to occur rapidly and persisted for a longer period of time than that reported for probiotics of human origin.

Finally, the mode of delivery and duration of treatment may also influence the dosing range for probiotics. When a dry dog food containing 1 x 10^{6} CFU/gm of a human

strain of *Lactobacillus acidophilus* was fed to dogs with non-specific dietary sensitivity for 12 weeks, dogs showed significant improvements in fecal quality and defecation frequency.[32] However, while there were numerical increases in fecal lactobacilli, statistically significant changes in fecal microbial populations were not observed.

Results of the present study showed that feeding between 1 x 10^7 and 1 x 10^9 CFU/day of a canine origin *B. animalis* strain resulted in significant increases in fecal populations of Bifidobacteria that persisted throughout the dosing period. A dose-response trend was also observed. Dogs supplemented with higher concentrations of AHC7 generally had higher AHC7 fecal concentrations. Dogs fed a supplement containing 1 x 10^9 CFU (Lg9) had the highest fecal concentrations of AHC7 throughout the relocation period when compared with the control group and when compared with dogs supplemented with lower concentrations of AHC7. Similar to reports with other canine-derived probiotic organisms, a relatively low dose (1 x 10^7 CFU/day) of AHC7 was capable of effecting changes in the gastrointestinal microbiota and (as indicated through fecal counts) appeared to support colonization. Fecal counts following supplementation were not measured in this study. Collecting this type of data in future studies would help to determine if and for how long changes to the intestinal microbiota persist in dogs.

While evidence of colonization is important, the clinical response to a probiotic and how dosing level affects clinical signs is of equal or greater interest. Fecal scoring provides a non-invasive and frequently used method for measuring intestinal wellness and presence of diarrhea in dogs. Stool scoring systems employ a standard scale that typically includes between 3 and 7 points. Each point is accompanied by a description of the stool's shape and texture and illustrative photographs. Although this type of scoring system is subjective, there is evidence that fecal scores provide a superior measure of fecal quality in dogs than do fecal dry matter or fecal moisture content.[42,43] In the present study, supplementation with AHC7 resulted in the maintenance of optimal fecal scores during kennel relocation. Dogs supplemented with AHC7 maintained excellent stool scores, while un-supplemented dogs showed a decline in fecal quality during the third week of relocation, leading to significant differences between supplemented and non-supplemented groups. In addition, during the first week of relocation, a higher proportion of dogs that did not receive the probiotic supplement produced stools of poor quality when compared with dogs in the probiotic supplemented groups.

It is of importance that the dogs in this study were all young and healthy, fed a high quality dog food, and from genetic lines that have been specifically selected for the stable temperament that is needed for service dog work. Therefore, the effects of relocation on gastrointestinal health were expected to be moderate in the dogs included in this study. Yet results showed that probiotic supplementation helped to maintain normal gut function when faced with environmental stressors. These data suggest that supplementation with 1 x 10^7 CFU/day of AHC7 can provide benefit, while supplementation with 1 x 10^8 to 1 x 10^9 CFU per day AHC7 may lead to a more consistent response.

CONCLUSION

In this study, significant positive effects of probiotic supplementation were observed in healthy adult dogs that were exposed to the stress of relocation from a home environment to a kennel environment. A dose-response effect was also suggested, as measured by fecal concentrations of *Bifidobacterium animalis* AHC7 and gastrointestinal response. Supplemented dogs maintained optimal fecal quality throughout relocation, and a significantly lower proportion of supplemented dogs produced poorly formed or loose stools. Supplementation with a canine-derived probiotic comprised of *B. animalis* AHC7 can provide a welfare benefit to working dogs, dogs being board-

ed, or other dogs being subjected to environmental changes such as transport, kenneling, or other stressors.

FOOTNOTES

a Unpublished data; P&G Pet Care and Nutrition Center, Lewisburg, Ohio

REFERENCES

1. Rooney NJ, Gaines SA, Bradshaw JWS. Behavioural and glucocorticoid responses of dogs (Canis familiaris) to kenneling: Investigating mitigation of stress by prior habituation. *Physiol Behav* 2007; 92:847-854.
2. Beerda B, Schilder MB, van Hooff JA, et al. Chronic stress in dogs subjected to social and spatial restriction. I. Behavioral responses. *Physiol Behav* 1999;66:233-242.
3. Sokolow SH, Rand C, Marks SL, et al. Epidemiologic evaluation of diarrhea in dogs in an animal shelter. *Am J Vet Res* 2005;66:1018-1024.
4. Cave N, Marks S,L, ass PH, et al. Evaluation of a routine diagnostic fecal panel for dogs with diarrhea, *J Am Vet Med Assoc* 2002;221:52-59.
5. Trejo AV, Hayek MG, Kelley RL. Managing microbes for wellness. *Compend Contin Educ Vet* 2008;30(suppl):1-4.
6. Floch MH, Walker WA, Guandalini S. Recommendation for probiotic use; 2008. *J Clin Gastroenterol* 42:S14-S108, 2008.
7. Chou LS, Weimer B. Isolation and characterization of acid and bile-tolerant isolates from strains of *Lactobacillus acidophilus*. *J Dairy Sci* 1999;82:23-31.
8. Saarela M, Mogensen G, Fonden R, et al. Probiotic bacterial: Safety, functional and technological properties. *J Biotechnol* 2000;84:197-215.
9. Manninen TJK, Rinkinen ML, Shea S, et al. Alteration of the canine small-intestine lactic acid bacterium microbiota by feeding potential probiotics. *Appl Envir Microbiol* 2006;72:6539-6543.
10. Biagi G, Cipollini I, Pompei A, et al. Effect of *Lactobacillus animalis* strain on composition and metabolism of the intestinal microflora in adult dogs. *Vet Microbiol* 2007;124: 160-165.
11. Swanson KS, Grieshop CM, Flickinger EA, et al. Fructooligosaccharides and *Lactobacillus acidophilus* modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs, *J Nutr* 2002;132:3721-3731.
12. Baillon ML, Marshall-Jones ZV, Butterwick RF. Effects of probiotic *Lactobacillus acidophilus* strain DSM13241 in healthy adult dogs. *Am J Vet Res* 2004;65:338-343.
13. Zareie MK, Johnson-Henry J, Jury PC, et al. Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. *Gut* 2006;55:1553-1560.
14. Herstad HK, Nesheim BB, Abee-Lund TL, et al. Effects of a probiotic intervention in acute canine gastroenteritis – a controlled clinical trial. *J Small Anim Pract* 2010;51:34-38.
15. Reid G, Jass J, Sebulsky MT, McCormick JK. Potential uses of probiotics in clinical practice. *Clini Microbiol Rev* 2003;16:658-672.
16. Murphy C, Murphy S, O'Brien F, et al. Metabolic activity of probiotics – oxalate degradation. *Vet Microbiol* 2009;136:1001-107.
17. O'Mahony D, Murphy KB, Macsharry J, et al. Portrait of a canine probiotic Bifidobacterium – from gut to gut. *Vet Microbiol* 2009;139:106-112.
18. Kelley RL, Park JS, O'Mahony LO. Safety and tolerance of dietary supplementation with a canine-derived probiotic *Bifidobacterium animalis* strain AHC7 fed to growing dogs. *Vet Ther* 2010;11:E1-E14.
19. Kelley RL, Minikhiem D, Kiely B, et al. Clinical benefits of probiotic canine-derived Bifidobacterium animals strain AHC7 in dogs with acute idiopathic diarrhea. *Vet Ther* 2009;10:121-130.
20. Suchodolski JS, Ruaux CG, Steiner JM, et al. Application of molecular fingerprinting for qualitative assessment of small-intestinal bacterial diversity in dogs. *J Clin Microbiol* 2004;42:4702-4708.
21. Malinen E, Rinttila T, Kajander K, et al. Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. *Am J Gastroenterol* 2005;100:373-382.
22. Hiby EF, Rooney NJ, Bradshaw JWS. Behavioural and physiological responses of dogs entering rehoming kennels. *Physiol Behav* 2006;89:385-391.
23. Sales G, Hubrecht R, Peyvandi A, et al. Noise in dog kenneling: Is barking a welfare problem for dogs? *Appl Anim Behav Sci* 1997;52:321-329.
24. Hennessy MB, Williams MT, Miller DD, et al. Influence of male and female petters on plasma cortisol and behavior: can human interaction reduce the stress of dogs in a public animal shelter? *Appl Anim Behav Sci* 1998;61:63-77.
25. Coppola CL, Enns RM, GrandinT. Noise in the Animal Shelter Environment: Building Design and the Effects of Daily Noise Exposure. *J Appl Anim Welfare Sci* 2006;9:1-7.
26. Oliver J, Saker K, Lenier CB, et al. Probiotic compared to metronidazole for treatment of acute stress colitis in an adult canine shelter population. Abstract 11th Annual AAVN Clinical Nutrition & Research Symposium, June 15, 2011, Denver Colorado.
27. Santos J, Yang PC, Soderholm JD, et al. Role of mast cells in chronic stress induced colonic epithelial barrier dysfunction in the rat. *Gut* 2001;48:630-636.
28. Soderholm, JD and Perdue MH. Stress and gastrointestinal tract. II. Stress and intestinal barrier function. *Am J Physiol Gastrointest Liver Physiol* 2001;280:G7-G13.
29. Meddings JB, Swain MG. Environmental stress induces a generalized permeability mediated by endogenous glucocorticoids in the rat. *Gastroenterol* 2000;119:1019-1028.
30. Lutgendorff F, Akkermans LMA, Soderholm JD. The role of microbiota and probiotics in stress-induced gastrointestinal damage. *Curr Mol Med*

2008;8:282-298.

31. Sauter SN, Allenspach K, Gaschen F, et al. Cytokine expression in an ex vivo culture system of duodenal samples from dogs with chronic enteropathies: modulation by probiotic bacteria. *Domest Anim Endocrinol* 2005;29:605-622.
32. Sauter SN, Benyacoub J, Allenspach K, et al. Effects of probiotic bacteria in dogs with food responsive diarrhea treated with an elimination diet. *J Anim Physiol Anim Nutr* 2006;90:269-277.
33. Pascher M, Hellweg P, Khol-Prisnini A, Zentek J. Effects of a probiotic *Lactobacillus acidophilus* strain on feed tolerance in dogs with non-specific dietary sensitivity. *Arch Anim Nutr* 2880;62:107-116.
34. Ley RE, Lozupone CA, Hamady M, et al. Worlds within worlds: evolution of the vertebrate gut microbiota. Nat Rev Microbiol 2008;6:776-788.
35. McCoy S, Gilliland SW. Isolation and characterization of *Lactobacillus* species having potential for use as probiotic cultures for dogs. *J Food Sci* 2007;72:M94-M97.
36. Manninen TJK, Rinkinen ML, Beasley SS, Saris PEJ. Alteration of the canine small-intestinal lactic acid bacterium microbiota by feeding potential probiotics. *Appl Environ Microbio* 2006;72:6539-6543.l
37. Salimen S, von Wright A, Morellis L, et al. Demonstration on safety of probiotics – A review. *Internat J Food Microbiol* 1998;44:93-106.
38. Weese JS, Anderson MEC. Preliminary evaluation of *Lactobacillus rhamnosus* strain GG, a potential probiotic in dogs. *Can Vet J* 2002;43:771-774.
39. Weese JS, Anderson MEC, Lowe A, et al. Preliminary investigation of the probiotic potential of *Lactobacillus rhamnosus* strain GG in horses: fecal recovery following oral administration and safety. *Can Vet J* 2003;44:299-302.
40. Goldin BR, Gorgach SL, Saxelin M, et al. Survival of *Lactobacillus* species (Strain GG) in human gastrointestinal tract. *Dig Dis Sci* 1992;37:121-128.
41. Marcinakova M, Simonova M, Strompfova V, Laukova A. Oral application of *Enterococcus faecium* strain EE3 in healthy dogs. *Folia Microbiol* 2006;51:239-242.
42. Sunvold GD, Fahey GC, Merchen NR, et al. Dietary fiber for dogs: IV. In vitro fermentation of selected fiber sources by dog fecal inoculums and in vivo digestion and metabolism of fiber supplemented diets. *J Anim Sci* 1995;73:1099-1109.
43. Nery JV, Biourge C, Tournier V, et al. Influence of dietary protein content and source on fecal quality, electrolyte concentrations, and osmolarity and digestibility in dogs differing in body size. *J Anim Sci* 2010;88:159-169.