Safety and Tolerance of Dietary Supplementation With a Canine-Derived Probiotic (*Bifidobacterium animalis* Strain AHC7) Fed to Growing Dogs*

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CLINICAL RELEVANCE

Although probiotics are generally considered to be safe, their increasingly widespread use warrants better understanding of their risks in companion animals. This study evaluated the safety and tolerance of dietary supplementation with a canine-derived probiotic, *Bifidobacterium animalis* strain AHC7 (Prostora, Procter & Gamble Pet Care), fed to growing beagles beginning at approximately 6 months of age (11 males; 9 females). Probiotic *B. animalis* AHC7 administered orally once per day at a dose of up to 5×10^{10} colony-forming units for at least 12 consecutive weeks was well tolerated with no safety concerns.

INTRODUCTION

Probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts. Combinations of microorganisms, particularly species of lactic acid bacteria, including *Lactobacillus* and *Lactococcus*, have traditionally been used in fermented dairy products to promote human health through their influence on the microbial ecology of the host and the incidence of diarrhea.[1] Probiotics have also been used for many years in animal husbandry and have been demonstrated to be effective in improving gastrointestinal health. However, in the case of companion animals, particularly dogs and cats, less is known about the potential applications of probiotics.

Despite the fact that few studies have been conducted examining the efficacy and safety of probiotic supplementation in companion ani-

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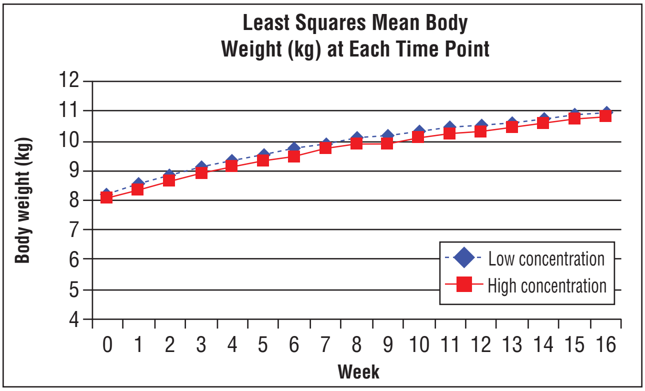

Figure 1. *Body weight increased gradually over the course of the study in both supplement groups as expected, and no statistically significant differences were noted between the groups at any time point.*

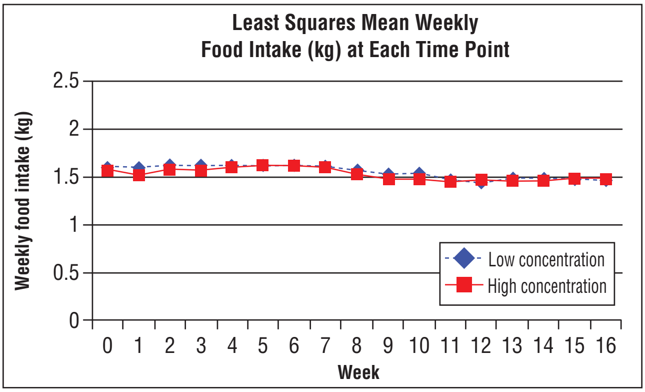

Figure 2. *Weekly food intake did not change over the course of the study in either supplement group, and no statistically significant differences were noted between the groups at any time point.*

mals, the number of commercially available probiotic products has proliferated rapidly. This has led to concern about the quality, labeling, and verification of claims attributed to some of these products.[2] Although probiotics are generally considered to be safe in humans,[3–6] it has been recommended that their use in severely compromised human patients be avoided.[7] Given their increasingly widespread use for clinical management in unhealthy pets, a better understanding of their risks in companion animals is warranted.

A strain of *Bifidobacterium* (*Bifidobacterium animalis* AHC7) was recently isolated from healthy canine gastrointestinal tissue and tested in adult dogs as a potential probiotic.[8] In this study, dogs received 1.5 × 10^9 colony-forming units (CFU)/day of *B. animalis* AHC7 for 6 weeks with no health concerns. A subsequent study found that supplementation with *B. animalis* AHC7 was effective in reducing the time to resolution of acute idiopathic diarrhea when fed at 2 × 10^{10} CFU/day for a maximum of 2 weeks with no health concerns.[9] The purpose of the present study was to evaluate the safety and tolerance of *B. animalis* AHC7 when administered at a dose of up to 5 × 10^{10} CFU/day for a longer duration (at least 12 consecutive weeks) to growing dogs.

MATERIALS AND METHODS

Twenty beagles from four litters were enrolled in the study at the Procter & Gamble Pet Health Nutrition Center (PHNC; Lewisburg, OH). The average age of the dogs at the time of enrollment was approximately 6 months. Before the study began, all dogs received a complete physical examination by a veterinarian, and blood was collected for evaluation of hematologic and serum chemistry parameters. All dogs were found to be in good health with no medical conditions that would adversely affect the study. All animal procedures met US Department of Agriculture procedures for laboratory care and were approved by the Procter & Gamble Pet Care Institutional Animal Care and Use Committee.

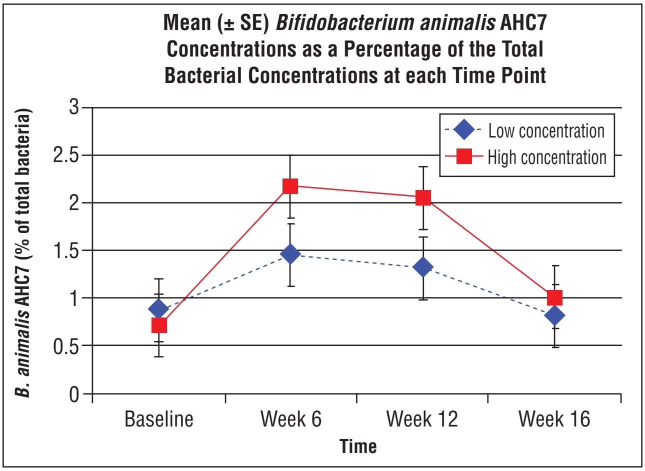

Figure 3. *Supplementation with* Bifidobacterium animalis *AHC7 significantly increased the percentage of* B. animalis *AHC7 present in fecal samples at weeks 6 and 12 in the high-concentration group and tended to increase the percentage in the low-concentration group.*

The study began with a 25-day prefeeding period in which all dogs were fed a commercial dry diet (Eukanuba Medium Breed Puppy, Procter & Gamble Pet Care) twice daily without active or placebo supplements, followed by a 7-day acclimation period in which all dogs received placebo supplements (0 CFU) in addition to daily rations of the commercial diet. Active and placebo supplements were provided in the form of cocoa butter treats. Each meal contained half of the estimated caloric need, with daily caloric intake adjusted to help maintain an optimal body condition. At the end of the acclimation period (baseline, week 0), dogs were weighed, rated for body condition score (BCS), and randomly assigned within each litter to one of two active supplements containing *B. animalis* AHC7 (high or low concentration) after balancing for sex, weight, and BCS by two PHNC personnel trained in the scoring procedure. The BCS was rated on a 5-point scale with half-points in between (1 = thin; 2 = underweight; 3 = ideal; 4 = overweight; 5 = obese). Active supplementation began the next day and continued throughout the following 12-week period (days 1 through 84).

Active supplements were given once daily with the morning meal for the duration of the 12-week supplementation period. Ten dogs (6 males; 4 females) received a low concentration of *B. animalis* AHC7 (1×10^9 CFU; overall average: $1.43 \pm 0.44 \times 10^9$ CFU), and 10 (5 males; 5 females) received a high concentration of *B. animalis* AHC7 (5×10^{10} CFU; overall average: $4.93 \pm 0.70 \times 10^{10}$ CFU). The supplementation period was followed by a 4-week observation period (days 85 through 112) in which all dogs received placebo supplements (0 CFU) in addition to daily rations of the commercial diet.

Analyses were performed on supplements every 4 weeks during the study to ensure stable levels of *B. animalis* strain AHC7. All *B. animalis* strain AHC7 microbial populations were determined in triplicate based on CFU plate counts using selective growth media for *Bifidobacterium* spp (de Man, Rogosa, Sharpe [MRS] agar; Oxoid, Hampshire, UK).

Dogs were monitored daily for stool scores, food intake, and daily activity. Stool scores were rated on a five-point scale (1 = liquid with or without particle matter; 2 = soft, shapeless; 3 = soft, with shape; 4 = firm, well formed; 5 = extremely dry). The primary fecal score from the first defecation of the day was analyzed. The dogs were double-housed (cohabitating dogs were in the same supplementation group);

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TABLE 1. Hematology Parameters of Dogs Randomly Assigned to Either a Low or a High Concentration of Probiotic *Bifidobacterium animalis* AHC7[a]

Parameter	***ANTECH Adult Reference Range 2009***	***Iams 18-Week-Old Puppy Reference Range 2006***	***Baseline (Week 0)***		***Week 6***		***Week 12***		***Week 16***	
			Low Concentration	*High Concentration*	*Low Concentration*	*High Concentration*	*Low Concentration*	*High Concentration*	*Low Concentration*	*High Concentration*
WBC (1000/µL)	4.0–15.5	3.35–70	8.49 ± 0.53	8.67 ± 0.53	7.88 ± 0.53	7.77 ± 0.53	7.61 ± 0.53	7.95 ± 0.53	7.84 ± 0.53	7.62 ± 0.53
% Neut	60–77	28–89	61.98 ± 1.93	63.85 ± 1.93	59.82 ± 1.93	64.57 ± 1.93	61.94 ± 1.93	64.43 ± 1.93	63.42 ± 1.93	66.44 ± 1.93
% Lymp	12–30	0–55	25.45 ± 2.11	26.08 ± 2.11	30.17 ± 2.11	27.60 ± 2.11	29.40 ± 2.11	26.32 ± 2.11	27.74 ± 2.11	25.21 ± 2.11
% Monos	3–10	0–13	8.51 ± 0.77	9.43 ± 0.77	8.75 ± 0.77	**7.35 ± 0.77**^{b}	7.71 ± 0.77	8.49 ± 0.77	8.18 ± 0.77	**7.41 ± 0.77**^{b}
% Eos	2–10	0–14	0.96 ± 0.30	0.62 ± 0.30	0.83 ± 0.30	0.24 ± 0.30	0.89 ± 0.30	0.48 ± 0.30	0.48 ± 0.30	0.74 ± 0.30
% Baso	0–1	0–1	0.11 ± 0.06	0.02 ± 0.06	**0.43 ± 0.06**^{b,c}	**0.24 ± 0.06**^{b,c}	**0.07 ± 0.06**^{c}	**0.27 ± 0.06**^{b,c}	0.18 ± 0.06	**0.20 ± 0.06**^{b}
% Ban	0–3	0–1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RBC (10^{6}/µL)	4.8–9.3	4.85–6.55	6.35 ± 0.12	6.46 ± 0.12	**6.99 ± 0.12**^{b}	**6.89 ± 0.12**^{b}	**6.89 ± 0.12**^{b}	**7.04 ± 0.12**^{b}	**7.19 ± 0.12**^{b}	**7.35 ± 0.12**^{b}
Hgb (g/dL)	12.1–20.3	11.3–15.1	14.39 ± 0.29	14.66 ± 0.29	**15.48 ± 0.29**^{b}	**15.46 ± 0.29**^{b}	**16.15 ± 0.29**^{b}	**16.57 ± 0.29**^{b}	**16.82 ± 0.29**^{b}	**17.29 ± 0.29**^{b}
HCT (%)	36–60	35.2–52.2	44.31 ± 0.85	44.78 ± 0.85	**48.31 ± 0.85**^{b}	**47.60 ± 0.85**^{b}	**49.25 ± 0.85**^{b}	**50.47 ± 0.85**^{b}	**51.31 ± 0.85**^{b}	**52.58 ± 0.85**^{b}
MCV (fL)	58–79	61–83.6	69.90 ± 0.53	69.43 ± 0.53	**69.07 ± 0.53**^{b}	69.11 ± 0.53	**71.51 ± 0.53**^{b}	**71.63 ± 0.53**^{b}	**71.39 ± 0.53**^{b}	**71.62**^{b} **± 0.53**^{b}

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TABLE 1. Hematology Parameters of Dogs Randomly Assigned to Either a Low or a High Concentration of Probiotic *Bifidobacterium animalis* AHC7[a] (cont.)

Parameter	*ANTECH Adult Reference Range 2009*	*Iams 18-Week-Old Puppy Reference Range 2006*	*Baseline (Week 0)*		*Week 6*		*Week 12*		*Week 16*	
			Low Concentration	*High Concentration*	*Low Concentration*	*High Concentration*	*Low Concentration*	*High Concentration*	*Low Concentration*	*High Concentration*
MCH (pg)	19–28	21.7–25	22.69 ± 0.20	22.74 ± 0.20	**22.15 ± 0.20**[b]	**22.45 ± 0.20**[b]	**23.47 ± 0.20**[b]	**23.53 ± 0.20**[b]	**23.42 ± 0.20**[b]	**23.55 ± 0.20**[b]
MCHC (g/dL)	30–38	21.6–33.9	**32.45 ± 0.09**[c]	**32.73 ± 0.09**[c]	**32.08 ± 0.09**[b,c]	**32.48 ± 0.09**[b,c]	**32.83 ± 0.09**[b]	32.85 ± 0.09	**32.77 ± 0.09**[b]	32.88 ± 0.09
PLT (1000/μL)	170–400	130–429	296.60 ± 17.96	330.10 ± 17.96	287.50 ± 17.96	**292.00 ± 17.96**[b]	301.90 ± 17.96	311.50 ± 17.96	312.60 ± 17.96	310.80 ± 17.9
NeutAb (1000/μL)	2.06–10.60	1.91–19.6	5.30 ± 0.43	5.57 ± 0.43	4.75 ± 0.43	5.06 ± 0.43	4.74 ± 0.43	5.14 ± 0.43	4.99 ± 0.43	5.10 ± 0.43
LymAb (1000/μL)	0.69–4.50	0–7.26	2.20 ± 0.20	2.24 ± 0.20	2.35 ± 0.20	2.09 ± 0.20	2.25 ± 0.20	2.07 ± 0.20	2.17 ± 0.20	1.89 ± 0.20
MonoAb (1000/μL)	0–0.84	0–1.4	0.71 ± 0.07	0.79 ± 0.07	0.69 ± 0.07	0.58 ± 0.07[b]	0.56 ± 0.07	0.68 ± 0.07	0.63 ± 0.07	**0.56 ± 0.07**[b]
EosAb (1000/μL)	0–1.20	0–1.4	0.08 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	0.02 ± 0.02	0.06 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.05 ± 0.02
BasoAb (1000/μL)	0–0.15	0	0.01 ± 0.01	0.00 ± 0.01	**0.03 ± 0.01**[b,c]	**0.02 ± 0.01**[c]	0.01 ± 0.01	**0.02 ± 0.01**[b]	0.01 ± 0.01	0.02 ± 0.01
BanAb (1000/μL)	0–0.30	0–0.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

[a] Data shown are least squares mean ± SE.

[b] Statistically significant ($P < .05$) change from baseline within the supplement group based on a repeated measure, mixed model method with time point and supplement level as the fixed factors.

[c] Statistically significant ($P < .05$) difference between the supplement groups based on a repeated measure, mixed model method with time point and supplement level as the fixed factors.

TABLE 2. Serum Chemistry Profile Parameters of Dogs Randomly Assigned to Either a Low or a High Concentration of Probiotic *Bifidobacterium animalis* AHC7[a]

Parameter	*ANTECH Adult Reference Range 2009*	*Iams 18-Week-Old Puppy Reference Range 2006*	*Baseline (Week 0)*		*Week 6*		*Week 12*		*Week 16*	
			Low Concentration	*High Concentration*	*Low Concentration*	*High Concentration*	*Low Concentration*	*High Concentration*	*Low Concentration*	*High Concentration*
Chol (mg/dL)	92–324	166–316	216.20 ± 9.04	206.10 ± 9.04	**230.20 ± 6.85**^{b}	**223.40 ± 6.85**^{b}	**259.60 ± 8.75**^{b}	**251.80 ± 8.75**^{b}	**240.20 ± 8.24**^{b}	**234.40 ± 8.24**^{b}
Na (mmol/L)	139–154	139–153	148.50 ± 0.40	149.10 ± 0.40	**150.30 ± 0.85**^{b}	**151.50 ± 0.85**^{b}	**155.00 ± 0.64**^{b}	**155.50 ± 0.64**^{b}	148.00 ± 0.91	149.70 ± 0.91
K (mmol/L)	3.6–5.5	4–6.2	4.32 ± 0.09	4.41 ± 0.09	4.45 ± 0.09	4.34 ± 0.09	4.50 ± 0.10	4.48 ± 0.10	4.29 ± 0.08	4.20 ± 0.08
Cl (mmol/L)	102–120	106–117	113.00 ± 0.46	113.30 ± 0.46	**115.50 ± 0.62**^{b}	**116.00 ± 0.62**^{b}	**118.70 ± 0.53**^{b}	**119.20 ± 0.53**^{b}	**113.30 ± 0.43**^{c}	**114.90 ± 0.43**^{b}
TB (mg/dL)	0.1–0.3	0.1–0.2	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.14 ± 0.02	**0.14 ± 0.02**^{b}	0.11 ± 0.02	0.12 ± 0.02
GGT (U/L)	1–12	3–14	1.50 ± 0.00	1.50 ± 0.00	1.95 ± 0.72	2.25 ± 1.30	2.20 ± 0.95	1.80 ± 0.63	2.20 ± 0.95	2.00 ± 1.15
ALT (U/L)	12–118	24–338	41.40 ± 3.93	40.10 ± 3.93	**57.40 ± 8.91**^{b}	53.60 ± 8.91	42.30 ± 3.74	43.20 ± 3.74	41.10 ± 3.99	44.20 ± 3.99
AST (U/L)	15–66	17–105	36.40 ± 2.43	38.90 ± 2.43	48.70 ± 6.24^b	51.80 ± 6.24	37.50 ± 3.51	**44.40 ± 3.51**^{b}	36.80 ± 4.13	40.30 ± 4.13
LDH (U/L)	20–500	30–685	122.40 ± 21.13	126.70 ± 21.13	93.40 ± 9.18	**95.10 ± 9.18**^{b}	129.10 ± 21.32	152.00 ± 21.32	122.40 ± 18.62	108.30 ± 18.62
Mg (mEq/L)	1.5–2.5	0.3–3.2	2.25 ± 0.07	2.34 ± 0.07	2.32 ± 0.04	2.29 ± 0.04	2.29 ± 0.04	2.23 ± 0.04	2.20 ± 0.06	**2.17 ± 0.06**^{b}
ALP (U/L)	5–131	107–215	112.80 ± 10.15	117.70 ± 10.15	**85.10 ± 7.24**^{b}	**96.50 ± 7.24**^{b}	**82.20 ± 7.56**^{b}	**90.40 ± 7.56**^{b}	**73.20 ± 5.73**^{b}	**67.10 ± 5.73**^{b}
CK (U/L)	59–895	195–708	234.40 ± 21.68	242.50 ± 21.68	**190.10 ± 28.46**^{b}	258.70 ± 28.46	199.20 ± 61.45	**374.10 ± 61.45**^{b}	213.80 ± 62.42	312.40 ± 62.42
TGs (mg/dL)	29–291	35–114	33.00 ± 3.66	32.70 ± 3.66	**39.30 ± 3.11**^{b}	36.30 ± 3.11	**41.30 ± 4.49**^{b}	**40.90 ± 4.49**^{b}	36.20 ± 3.39	**38.80 ± 3.39**^{b}

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TABLE 2. Serum Chemistry Profile Parameters of Dogs Randomly Assigned to Either a Low or a High Concentration of Probiotic *Bifidobacterium animalis* AHC7[a] (cont.)

Parameter	***ANTECH Adult Reference Range 2009***	***Iams 18-Week-Old Puppy Reference Range 2006***	***Baseline (Week 0)***		***Week 6***		***Week 12***		***Week 16***	
			Low Concentration	*High Concentration*	*Low Concentration*	*High Concentration*	*Low Concentration*	*High Concentration*	*Low Concentration*	*High Concentration*
UA (mg/dL)	—	0.2–0.3	0.12 ± 0.02	0.14 ± 0.02	0.12 ± 0.01	0.10 ± 0.01	0.17 ± 0.03	0.20 ± 0.03	0.15 ± 0.02	0.12 ± 0.02
Cr (mg/dL)	0.5–1.6	0.7–1.3	0.79 ± 0.08	0.99 ± 0.08	0.81 ± 0.02	0.83 ± 0.02	**1.00** **± 0.04**^{b}	1.06 ± 0.04	**0.97** **± 0.04**^{b}	1.00 ± 0.04
Gluc (mg/dL)	70–138	32–134	108.40 ± 2.15	108.20 ± 2.15	106.60 ± 2.15	108.30 ± 2.15	**101.70** **± 2.62**^{b}	**98.30** **± 2.62**^{b}	**97.30** **± 1.48**^{b}	**97.90** **± 1.48**^{b}
BUN (mg/dL)	—	8–111	16.11 ± 1.59	19.94 ± 1.59	**15.23** **± 0.92**^{c}	**18.22** **± 0.92**^{c}	**19.87** **± 1.07**^{b}	21.47 ± 1.07	17.98 ± 0.68	19.19 ± 0.68
TP (g/dL)	5.0–7.4	4.4–5.5	5.20 ± 0.07	5.20 ± 0.07	**5.37** **± 0.06**^{b}	**5.36** **± 0.06**^{b}	**5.74** **± 0.09**^{b}	**5.77** **± 0.09**^{b}	5.35 ± 0.07	5.34 ± 0.07
ALB (g/dL)	2.7–4.4	3.0–3.8	3.15 ± 0.05	3.18 ± 0.05	**3.42** **± 0.05**^{b}	**3.45** **± 0.05**^{b}	**3.50** **± 0.06**^{b}	**3.54** **± 0.06**^{b}	**3.37** **± 0.04**^{b}	**3.38** **± 0.04**^{b}
Ca (mg/dL)	8.9–11.4	9.4–12.1	11.37 ± 0.08	11.46 ± 0.08	**10.82** **± 0.06**^{b}	**10.86** **± 0.06**^{b}	11.46 0.09	11.51 ± 0.09	**11.04** **± 0.11**^{b}	**10.99** **± 0.11**^{b}
P (mg/dL)	2.5–6.0	7.9–10.6	6.31 ± 0.18	6.57 ± 0.18	**5.70** **± 0.15**^{b}	**5.55** **± 0.15**^{b}	**5.83** **± 0.25**^{b}	**5.99** **± 0.25**^{b}	**4.69** **± 0.25**^{b}	**4.73** **± 0.25**^{b}
Glob (g/dL)	1.6–3.6	1.1–2.1	2.05 ± 0.07	2.02 ± 0.07	1.95 ± 0.05	1.91 ± 0.05	**2.24** **± 0.05**^{b}	**2.23** **± 0.05**^{b}	1.77 ± 0.15	1.96 ± 0.15
A/G ratio	0.8–2.0	1.48–3.0	1.56 ± 0.07	1.58 ± 0.07	**1.76** **± 0.06**^{b}	**1.82** **± 0.06**^{b}	1.57 ± 0.04	1.57 ± 0.04	1.53 ± 0.12	1.71 ± 0.12
B/C ratio	4–27	10–123.33	20.38 ± 0.69	20.36 ± 0.69	18.83 ± 1.34	22.28 ± 1.34	19.93 ± 0.68	20.29 ± 0.68	**18.61** **± 0.69**^{b}	19.31 ± 0.69

[a] Data shown are least squares mean ± SE.

[b] Statistically significant ($P < .05$) change from baseline within the supplement group based on a repeated measure, mixed model method with time point and supplement level as the fixed factors.

[c] Statistically significant ($P < .05$) difference between the supplement groups based on a repeated measure, mixed model method with time point and supplement level as the fixed factors.

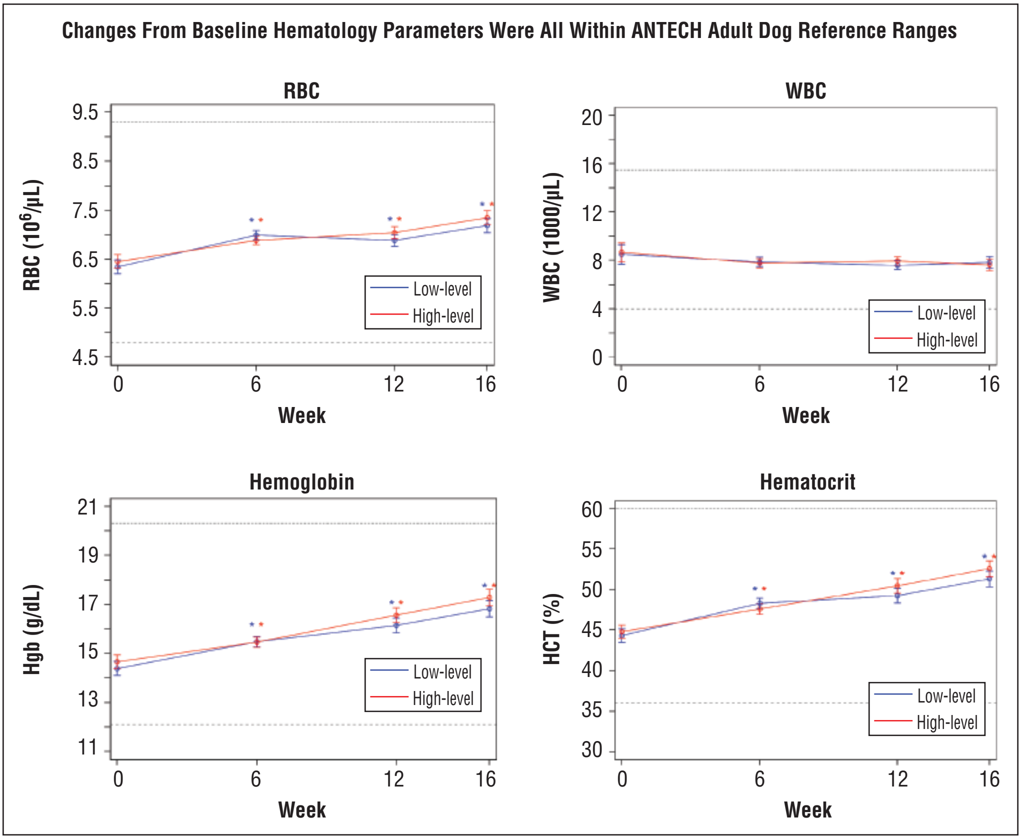


Figure 4. *Changes in selected hematology parameters from baseline. All data are represented as least squares mean (± SE) over time. Dotted lines represent ANTECH Diagnostics adult dog reference ranges (2009). * = Statistically significant (*P *<.05) change from baseline within the supplement group based on a repeated measure, mixed model method with time point and supplement level as the fixed factors.*

therefore, it was not always possible to score stool samples for individual dogs. If a dog was observed defecating, its score was recorded; otherwise, a single score was recorded for the cohabitating dogs. Primary fecal scores were also converted to a binary rating system by denoting all scores >2 as "acceptable" (coded as 1) and all scores ≤2 as "unacceptable" (coded as 0).

The dogs were monitored weekly for body weight and every 3 weeks for BCS. In addition, they received medical examinations (including a complete physical examination, hematology, and serum chemistry profile) at weeks 0, 6, 12, and 16 to evaluate general health. Hematology and serum chemistry profiles were evaluated in relation to reference ranges provided by ANTECH Diagnostics (Los Angeles, CA) for adult dogs and those established by the PHNC Clinical Laboratory (Lewisburg, OH) for rapidly growing puppies (Iams 18-week-old puppy reference range).

Fecal samples were collected in sterile containers at weeks 0, 6, 12, and 16 and submitted to the PHNC Microbiology Laboratory

(Lewisburg, OH) for enumeration of fecal bacteria. *B. animalis* AHC7 populations were enumerated using reverse-transcription polymerase chain reaction[10,11] and the following primers: universal, 5’ 6-FAM-CGTATTACC GCGGCTGCTGGCAC-3’-TAMRA; forward, 5’-TCCTACGGGAGGCAGCAGT-3’; and reverse, 5’-GGACTACCAGGGTATC TAATCCTGTT-3’ (all Applied Biosystems, Inc., Foster City, CA); *B. animalis* AHC7, 5’ 6FAM-CGGGTGGTGTCCCTTGCTG GCT-3’-MGBNFQ; forward, 5’-GCTTC CTTTCCTGGCCGT-3’; and reverse, 5’-ACACCACAAGGGCGCAGG-3’ (all developed internally by Procter & Gamble Pet Care based on 16s ribosomal sequence and produced by Applied Biosystems, Inc.).

Changes from baseline (week 0) within supplement group and differences between supplement groups were analyzed using a repeated measure, mixed model method with time point and supplement level as the fixed factors. The analyses were conducted using PC SAS 9.2 (v8.2 SAS Institute, Inc, Cary, NC). All data are reported as mean or least squares mean ± standard error. A *P* value <.05 was declared significant.

Body weight and feed intake were analyzed with general linear models (GLM) procedures of SAS to determine differences between treatments. The model accounted for study week and treatment group. Analysis of the microbial population data was performed with GLM procedures of SAS using repeated measures to determine population changes over time. The model accounted for sample timing as well as individual animal within a treatment group. All personnel involved in collection and analysis of data were blind to treatment grouping.

RESULTS

All dogs enrolled (11 males and 9 females) completed all aspects of the study.

Baseline (Week 0)

No clinically or statistically significant differences were observed between the supplement groups at baseline (week 0) for the following parameters: mean BCS (3.0 for both groups; data not shown), mean body weight (8.24 and 8.08 kg for the low- and high-concentration groups, respectively; Figure 1), mean fecal scores (3.87 and 3.94 for the low- and high-concentration groups, respectively; data not shown), mean weekly food intake (1.60 and 1.57 kg/wk/dog for the low- and high-concentration groups, respectively; Figure 2), and mean fecal bacteria population (Figure 3).

No clinically significant differences were observed between the supplement groups at baseline (week 0) for mean hematology and serum chemistry profile parameters (Tables 1 and 2). A statistically significant difference between the supplement groups was observed at baseline for MCHC (32.45 and 32.73 g/dL for the low- and high-concentration groups, respectively [*P* <.05]), but the mean baseline MCHC values were within the adult and 18-week-old puppy reference ranges. In addition, no clinically significant abnormalities were seen during physical examinations in either group at baseline.

Supplementation and Observation Periods

Physical Examinations

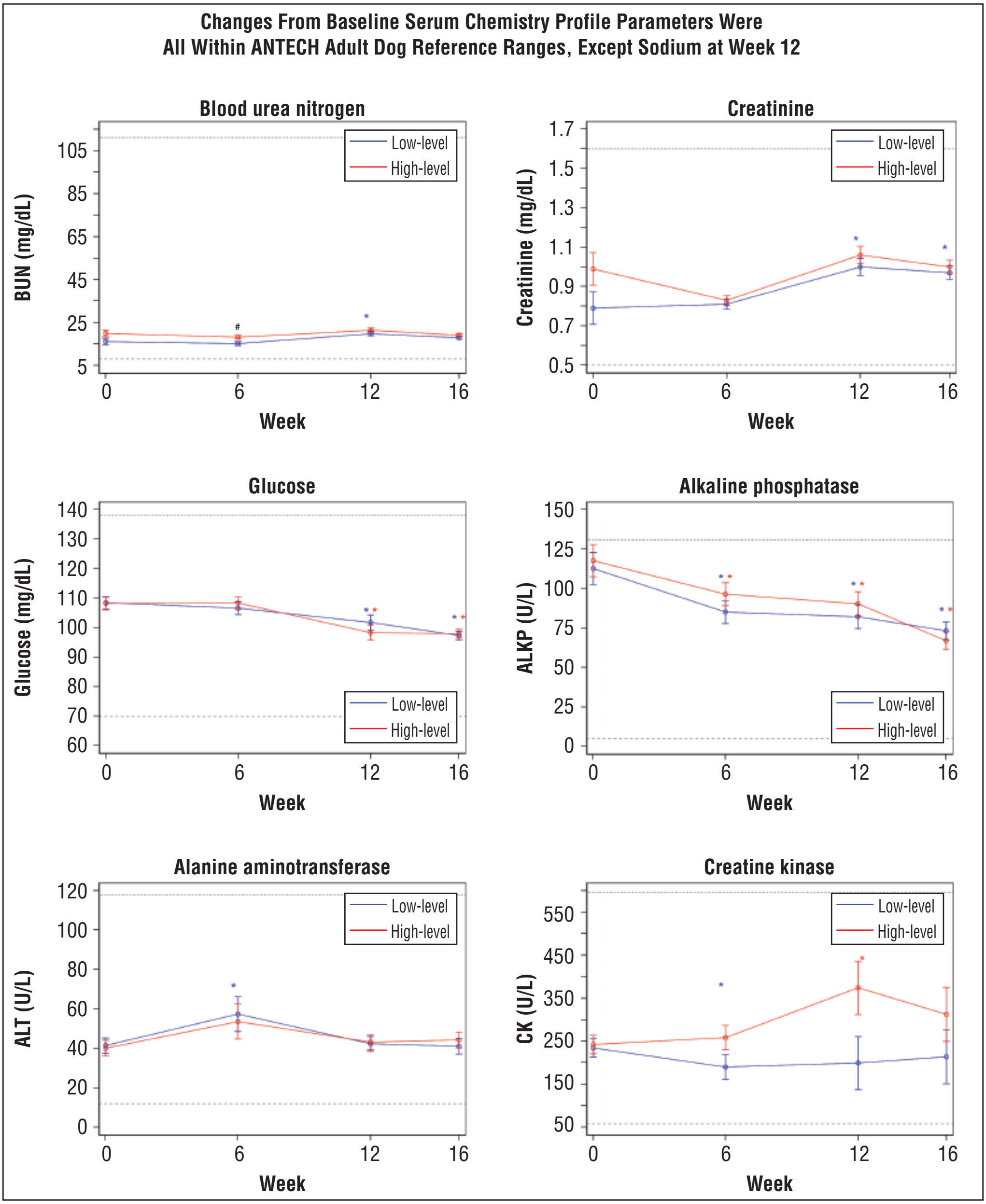
Over the course of the study, no clinically significant abnormalities were observed during physical examinations. No clinically or statistically significant changes in heart rate, body temperature, or respiration rate (data not shown) were seen in either group over the course of the study.

Hematology and Serum Chemistry Profiles

Complete Blood Count With Differential Leukocyte Cell Count

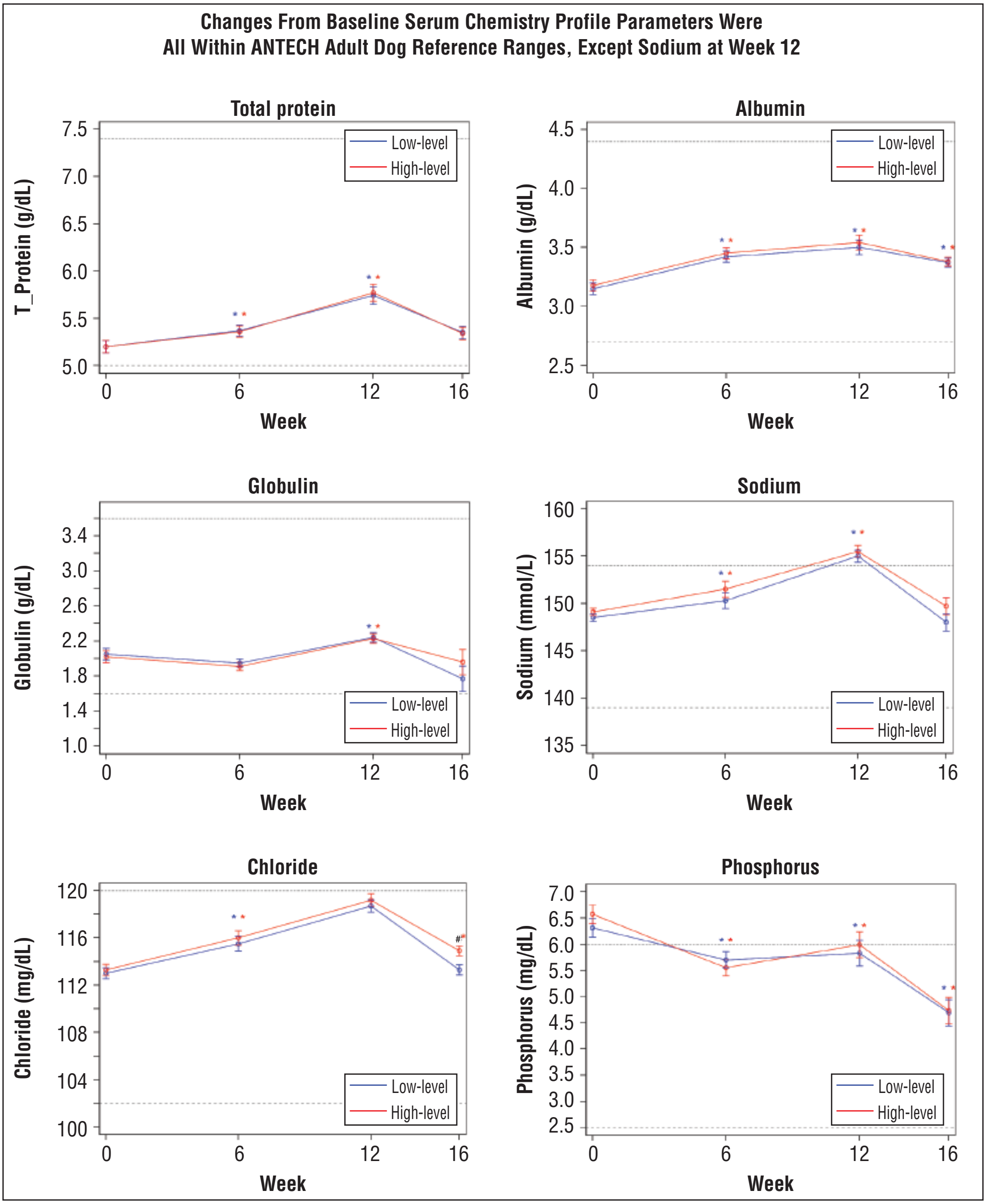
No clinically significant abnormalities were observed in hematology results over the course

(text continues on page E12)



Changes From Baseline Serum Chemistry Profile Parameters Were All Within ANTECH Adult Dog Reference Ranges, Except Sodium at Week 12

Figure 5. *Changes in selected serum chemistry profile parameters from baseline. All data are represented as least squares mean (± SE) over time. Dotted lines represent ANTECH Diagnostics adult dog reference ranges (2009). * = Statistically significant (*P *<.05) change from baseline within the supplement group based on a repeated measure, mixed model method with time point and supplement level as the fixed factors. # = Statistically significant (*P *<.05) difference between the supplement groups based on a repeated measure, mixed model method with time point and supplement level as the fixed factors. (Figure continues on next page.)*



Changes From Baseline Serum Chemistry Profile Parameters Were All Within ANTECH Adult Dog Reference Ranges, Except Sodium at Week 12

Figure 5. (cont.)

of the study, although some changes from baseline were detected statistically (Table 1; Figure 4). Statistically significant mean changes from baseline within each supplement group were observed over the course of the study for red blood cell count and hemoglobin; these values were within the adult reference ranges but above the 18-week-old puppy reference ranges. In addition, a statistically significant mean change from baseline was found at week 16 in hematocrit for the high-concentration group; this value was within the adult reference range but slightly above the 18-week-old puppy reference range. All other statistically significant mean changes from baseline within supplement group or mean differences between supplement groups for hematology parameters were within both reference ranges.

Serum Chemistry Profile

No clinically significant abnormalities were observed in serum chemistry results over the course of the study, although some changes from baseline were detected statistically (Table 2; Figure 5). A statistically significant mean change from baseline was observed within each supplement group at week 12 for sodium that was above both adult and puppy reference ranges. Statistically significant mean changes from baseline within each supplement group were observed over the course of the study for alkaline phosphatase and phosphorus; these changes were within the adult reference ranges but below the 18-week-old puppy reference ranges. Statistically significant mean changes from baseline within the supplement groups were observed at some time points for chloride, creatine kinase, and total protein; these changes were also within the adult reference ranges but outside of the 18-week-old puppy reference ranges. All other statistically significant mean changes from baseline within supplement group or mean differences between supplement groups for serum chemistry profile parameters were within both reference ranges.

Body Condition Scores

The mean BCS was 3.0 in both supplement groups at baseline and remained near this ideal score in both groups for the duration of the study. Mean scores ranged from 3.10 to 3.30 in the low-concentration group and 3.30 to 3.47 in the high-concentration group during the supplementation and observation periods.

Body Weight and Food Intake

Body weight increased gradually over the course of the study in both supplement groups, as expected (Figure 1). Weekly food intake was similar in both groups (Figure 2). No statistically significant differences were noted between the groups at any time point for either of these parameters.

Fecal Scores

Mean fecal scores were maintained at approximately 4 (firm, well-formed stool) throughout the study in both groups, although statistically significant differences between the low- and high-concentration groups were seen at weeks 2 (3.85 and 3.99, respectively [*P* <.01]), 3 (3.79 and 3.97, respectively [*P* <.0001]), 4 (3.81 and 3.95, respectively [*P* <.01]), and 9 (3.85 and 3.97, respectively [*P* <.05]) of the supplementation period and at week 15 (3.85 and 3.95, respectively [*P* <.05]) of the observation period. All primary fecal scores converted to a binary rating system (acceptable versus unacceptable) were denoted "acceptable" for both probiotic supplement groups.

Fecal Bacteria Populations

As expected, supplementation with *B. animalis* AHC7 resulted in statistically significant (*P* ≤.05) increases in the overall percentage of *B. animalis* AHC7 present in fecal samples in the high-concentration group during the supplementation period (weeks 6 and 12), with percentages returning to baseline during the observation period (Figure 3). In the low-concentration group, *B. animalis* AHC7 increased numerically during the supplementation period but had no statistical difference from baseline levels at 6 weeks (*P* <.11) and 12 weeks (*P* <.13), with levels at the end of the observation period (16 weeks) being similar to baseline levels.

Total bacterial concentrations were not affected by either low- or high-concentration supplementation with *B. animalis* AHC7. The percentage of total *Lactobacillus* was not affected by *B. animalis* AHC7 supplementation, although overall levels were slightly reduced numerically at weeks 12 and 16 (data not shown).

DISCUSSION

Commercially available probiotics for pets are becoming more common, even though little information exists regarding their safety. Although probiotic supplementation is generally considered harmless, some safety concerns have been documented in humans.[7] The present study concentrated primarily on evaluating the safety and tolerance of dietary supplementation with the probiotic *B. animalis* AHC7 fed to growing dogs, a group most likely to exhibit problems if any were to occur, at a higher dose and longer duration than tested in previous studies.[8,9]

No clinically significant abnormalities were observed during physical examinations of the dogs fed up to 5×10^{10} CFU/day for at least 12 consecutive weeks. Although statistically significant changes from baseline that were outside the reference ranges for some hematology and clinical chemistry parameters were seen in both supplement groups, none of these changes were considered to be clinically significant. Hematology and clinical chemistry values are known to change as healthy puppies mature, and puppy values may lie outside the established normal ranges for adults. In puppies younger than 8 months, serum alkaline phosphatase of bone origin is commonly up to twice the adult level,[12] and packed cell volume increases to reach a normal, mature level between 2 and 6 months of age.[13] The statistically significant differences from baseline observed in this study generally followed expected age-related trends.

The serum sodium concentration was increased in both supplement groups at 6 and 12 weeks, but it did not reach clinically significant hypernatremia (>160 mEq/L for dogs).[14] Baseline serum sodium levels in the dogs in this study were at the higher end of the reference range, so the increases were relatively modest. Although neither this nor other studies have revealed a cause-and-effect association of probiotic supplementation and increased serum sodium concentration, probiotics have been reported to enhance absorption of other minerals.[15,16]

Mean BCS was maintained at approximately 3.0 (ideal body condition) throughout the study in both supplement groups. As expected, these young dogs continued to grow during the study period as evidenced by increasing body weight over time. The growth rate observed in this study was similar to that seen with growing beagles of similar age.[17] Statistically significant differences between the low- and high-concentration groups were observed at some time points for mean fecal scores. However, it is unlikely that fractional differences of the magnitude observed for the group means would be detected for individual subjects. Mean fecal scores were maintained at approximately 4 (firm, well-formed stool) throughout the study in both groups, and noted statistical differences were not considered to be clinically significant.

As expected, supplementation with *B. animalis* AHC7 resulted in statistically significant ($P \leq .05$) increases in the overall percentage of *B. animalis* AHC7 present in fecal samples during the 12-week supplementation period in the high-concentration group, as well as an increasing trend in the low-concentration group. Having a larger number of dogs might have overcome the high variability in fecal bacterial population at the low concentration of supplementation to enable detection of significant increases in both groups during supplementation. As expected, percentages returned to baseline during the 4-week observation period in both groups.

■ CONCLUSION

This study demonstrated that oral administration of canine-derived *B. animalis* AHC7 once per day at a dose of up to 5×10^{10} CFU for at least 12 consecutive weeks was well tolerated by growing dogs with no safety concerns.

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