Evaluation of graded levels of *Bacillus coagulans* (GBI-30, 6086) on apparent nutrient digestibility, stool quality, and intestinal health indicators in healthy adult dogs

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Introduction

- Functional pet foods, such as those containing probiotics, are considered to offer health benefits that extend beyond providing essential nutrients (Di Cerbo et al., 2017). Probiotics are live microorganisms associated with improved gastrointestinal health in animals (Markowiak et al. 2018); however, limited data are available for assessing the differences between conventional extruded dog foods and those containing direct-fed microbials.
- Of particular interest to pet food applications is the patented bacterial strain *Bacillus coagulans* (GBI-30, 6086), a commercially available spore-forming non-toxigenic microorganism approved for use in pet foods with high resiliency to stresses associated with commercial manufacturing.

Objectives

- The objectives of this research were to determine the effects of application method and graded doses of *Bacillus coagulans* on stool quality parameters, apparent total tract nutrient digestibility, and microbial fermentation indicators (fecal pH, NH_3, SCFA, and BCFA) in healthy adult dogs.

Materials and Methods

- The study was designed as 5 x 5 replicated Latin square with 16-d adaptation followed by 5-d total fecal collection for each 21-d period. High-protein (35% crude protein), grain-free extruded diets (major ingredients: 34% chicken meal, 20% peas, 20% sweet potatoes, 8.5% chicken fat, 5% tapioca starch) with graded levels of probiotic were fed to ten individually housed adult Beagle dogs (7 castrated males, 3 spayed females) of similar age (5.75 ± 0.23 years).
- Food-grade titanium dioxide (TiO_2) was added to all diets at a level of 0.4% to serve as an indigestible marker for digestibility calculations.
- Proximate analysis was carried out using AOAC official methods; NH_3 by colorimetric method (Chaney & Marbach, 1962); and SCFA by gas chromatography (Jounay, 1982).

Materials and Methods (Cont.)

Table 1. Application method and concentration of *B. coagulans* in five experimental diets

Dietary Treatment	CON	PEX[1]	PCL[2]	PCM[2]	PCH[2]
Application	None	Base Ration	Coating	Coating	Coating
Formula[3], %	0.00	0.03	0.0002	0.002	0.02
Total CFU g^{-1} Diet	0.00	1.06×10^4	5.92×10^4	6.86×10^5	6.84×10^6
Total CFU dog^{-1} d^{-1} [4]	0.00	2.12×10^6	1.18×10^7	1.37×10^8	1.37×10^9

[1] Diet PEX contained *B. coagulans* applied in the dry base ration before extrusion and drying.
[2] Diets PCL, PCM, and PCH were coated simultaneously with *B. coagulans* chicken fat and digest flavoring on the exterior of the kibble after drying.
[3] *B. coagulans* in powdered form with 15 billion colony-forming units (CFU) g^{-1}.
[4] Based on an average daily food intake of 200 g/dog/day.

- Nutrient Digestibility was calculated as:

$$1 - \frac{\%\ Nutrient\ in\ Feces\ x\ \%\ TiO_2\ in\ Food}{\%\ Nutrient\ in\ Food\ x\ \%\ TiO_2\ in\ Feces}\ x\ \%\ Nutrient\ in\ Food$$

- Data were analyzed using a mixed model (SAS version 9.3, SAS Institute, Inc., Cary, NC) with treatment and period as fixed effects and dog as a random effect. The differences among treatment differences were considered significant at P <0.05, and a trend at P <0.10.

Results

- Food intake (range 189 -197 g d^{-1}), wet fecal output (range 113 - 127 g d^{-1}), fecal moisture (range 69 – 70%), defecation frequency (range 1.9 – 2.2 stools d^{-1}), and fecal score (range 3.7 – 3.8 on a 5-pt. scale where 1 = liquid stool and 5 = hard, dry stool) were similar among treatments.
- Fecal pH (range 5.3 – 5.5), fecal ammonia (range 94 – 107 µmol g^{-1} DM), total short chain fatty acids (acetate + propionate + butyrate) (range 171 – 197 µmol g^{-1} DM), and total branched chain fatty acids (isovalerate + isobutyrate + valerate) (range 9 – 12 µmol g^{-1} DM), were similar among treatments.

Results (Cont.)

- Apparent total tract organic matter (OM), crude protein (CP), crude fat (CF), and gross energy (GE) digestibilities were greatest for dogs fed the 9-log_{10} dose treatment with increases ($P < 0.05$) observed in GE and OM digestibility compared to the negative control. CP digestibility tended ($P < 0.10$) to increase as probiotic dose increased (Table 2).

Table 2. Apparent total tract digestibility (ATTD) of dogs fed diets with differing levels of B. coagulans using titanium dioxide (TiO_2) as a fecal marker.

ATTD	CON	PEX	PCL	PCM	PCH	SEM	*P*-Value[1]
DM, %	79.04^b	79.45^b	78.65^b	78.75^b	81.77^a	0.60	0.0044
OM, %	83.67^b	84.36^b	83.51^b	84.01^b	85.79^a	0.47	0.0122
CP, %	81.64^b	81.95^b	81.70^b	81.77^b	83.60^a	0.57	0.0743
CF, %	91.69	90.28	90.96	90.85	91.69	0.49	0.1981
Ash, %	34.94^b	36.23^b	33.76^{bc}	31.06^c	46.31^a	2.09	<0.0001
GE, %	81.94^b	81.66^b	82.03^{abc}	80.08^{bc}	83.96^a	0.52	0.0003

[1] *P*-value represents Type 3 Test of Fixed Effects for Diet
[abc] Means with shared superscripts within rows are not different (P <0.05).

Conclusions

- These results suggest that *Bacillus coagulans (GBI-30, 6086)* has a favorable impact on apparent total tract DM, OM, and GE digestibility, and no apparent adverse effects to stool quality parameters when added to extruded diets at a daily intake level of up to 9-log_{10} CFU in healthy adult dogs.

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References Available Upon Request