

## Oral Administration of Arabinogalactan Affects Immune Status and Fecal Microbial Populations in Dogs

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**ABSTRACT** Seven ileally cannulated dogs were randomly assigned to a control or arabinogalactan (AG) treatments in a 7 × 7 Latin square design to evaluate effects of oral AG administration on nutritional and immunological characteristics. Arabinogalactan treatments included a high (1.65 g/d) and low (0.55 g/d) dose of AG100, AG1000 or AG3000 provided via gelatin capsules. Arabinogalactan forms differed in purification procedures. Each period consisted of a 6-d adaptation followed by a 4-d collection. Blood and fresh fecal samples were collected on d 10 of each period. Fecal score increased ( $P < 0.02$ ) in dogs supplemented with the low dose of AG1000. Ileal and total tract dry matter (DM) and organic matter (OM) digestibilities were not affected by treatment. Dogs supplemented with the high dose of AG1000 tended ( $P = 0.15$ ) to have a higher concentration of total aerobic fecal bacteria than control dogs. Dogs supplemented with the low dose of AG1000 and the high dose of AG3000 had higher concentrations of fecal lactobacilli ( $P = 0.04$ ) and tended to have higher concentrations of fecal bifidobacteria ( $P \leq 0.16$ ) compared with control dogs. Dogs fed the low dose of AG3000 tended ( $P = 0.10$ ) to have a lower concentration of fecal *Clostridium perfringens* compared with control dogs. Arabinogalactan treatments did not affect ( $P > 0.05$ ) serum immunoglobulin G, M or A concentrations. Specific forms and doses of AG increased white blood cell, neutrophil and eosinophil concentrations. Arabinogalactan is a unique dietary fiber that affects the digestive physiology and immunological characteristics of dogs. J. Nutr. 132: 478–482, 2002.

**KEY WORDS:** • arabinogalactan • dogs • immune system • digestion • microflora

Arabinogalactans (AG)<sup>2</sup> are long, densely branched polysaccharides with molecular weights ranging from 10,000 to 120,000. Many plants are rich sources of AG, including carrots, radishes, pears and leek seeds. The major commercial source of AG is the larch tree, either Western Larch (*Larix occidentalis*) or Mongolian Larch (*Larix dahurica*). Arabinogalactan is extracted from the larch tree via a countercurrent extraction process. High grade larch AG can contain >98% AG. Larch AG is composed of galactose and arabinose units in a 6:2 ratio, with a trace amount of uronic acid (1). Unique properties of larch AG are its complete solubility and stability over a wide range of concentrations, pHs and temperatures.

Larch AG has a stimulatory effect on the immune system of both humans and animals. Cultures of human peripheral blood mononuclear cells, pre-separated peripheral nonadherent cells, and monocytes showed enhancement of natural killer cytotoxicity against K562 tumor cells when pretreated with AG from *Larix occidentalis* for 48 to 72 h (2). An AG-induced increase in interferon  $\gamma$  was involved in the enhancement of natural killer cytotoxicity (2).

In addition to affecting the immune system, it was hypothesized that AG could also affect the digestive physiology of the animal. The potential benefits of numerous ingredients, such as AG, on efficiency of digestion and overall health of com-

panion animals are currently being investigated. The objective of this experiment was to determine the effects of AG on digestive events and immune status of healthy, adult dogs. Discovery of ingredients that have positive effects on digestion and immune status of dogs would be beneficial to the pet food industry.

### MATERIALS AND METHODS

**Animals and diets.** Seven purpose-bred adult female dogs (Butler Farms USA, Clyde, NY) with hound bloodlines and an average weight of 24.3 kg were utilized in this experiment. All dogs were surgically fitted with a permanent "T" type cannula ~11 cm proximal from the ileocecal junction according to the method described by Walker and co-workers (3). Dogs were housed in individual 1.2 × 3.1 m solid floor pens in an environmentally controlled room and had ad libitum access to water. All animal care procedures for this experiment were conducted under a research protocol approved by the Campus Laboratory Animal Care Advisory Committee, University of Illinois at Urbana-Champaign.

All dogs were fed 250 g (as-fed basis) of a common diet at 0800 and 2000 h daily for a total of 500 g/d. The ingredient composition of the diet is presented in Table 1. The diet's primary protein source was chicken protein. The mixed fat source consisted of both plant and animal sources. Feed refusals from previous feedings were collected and weighed before each feeding.

**Experimental treatments.** Three forms of AG were provided by Larex, (Cohasset, MN); raw AG (AG100), Laraceutical (AG1000) and Fiberaid (AG3000). All forms of AG contained the same hemicellulosic compound extracted by water from nondelignified *Larix* (larch) trees. The initial larch AG extract, AG100, was obtained by

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<sup>2</sup> Abbreviations used: AG, arabinogalactans; DM, dry matter; Ig, immunoglobulin; OM, organic matter; SCFA, short-chain fatty acids; TDF, total dietary fiber.

TABLE 1

*Ingredient composition of experimental diet fed to ileally cannulated dogs*

Ingredient	% of dry matter
Chicken protein	37.2
Ground corn	19.5
Brewers rice	19.5
Mixed fat source (64% animal:36% plant)	13.0
Dried beet pulp	4.0
Liquid digest <sup>1</sup>	3.0
Minerals <sup>2</sup>	1.9
Brewers dried yeast	1.0
Vitamins <sup>3</sup>	0.4
Potassium chloride	0.3
Choline chloride	0.2

<sup>1</sup> Liquid digest is the material that results from chemical and/or enzymatic hydrolysis of clean and undecomposed animal tissues.

<sup>2</sup> Provided the following per kg of diet: 600 mg Mg; 124 mg Fe; 58.4 mg Mn; 233 mg Zn; 42.2 mg Cu; 2.6 mg I; and 0.34 mg Se.

<sup>3</sup> Provided the following per kg of diet: 27,900 IU vitamin A; 139.1 IU vitamin E; 1,826 IU vitamin D-3; 17.7 mg thiamine; 41.0 mg riboflavin; 69.5 mg niacin; 35.3 mg D-pantothenic acid; 10.0 mg pyridoxine; 0.57 mg biotin; 1.93 mg folic acid; 2416 mg choline; and 0.28 mg vitamin B-12.

wood chip water and steam soaking followed by pressing, screening and concentration. This produced a dark colored but clear, thin, viscous solution (50–54% solids). This larch AG solution in water still contained its natural flavonoids. It was spray-dried to produce a powder, which then was encapsulated for this study. Removal of the maple sugar-type color from the AG100 product by standard food unit operations yielded AG1000, the second product evaluated in this study. Additional processing of the material beyond decolorization to include flavor and odor improvements, again by approved food processes commonly understood to improve natural product sensory profiles, yielded the third AG product tested in this evaluation, AG3000. All of these larch AG products were based upon and standardized to pure AG, each product's major component.

Each form of AG was fed at two levels (1.65 and 0.55 g/d) using a 7 × 7 Latin square design. Daily AG supplementation was provided as gelatin capsules given in two equal doses before each feeding. Dogs also were dosed orally twice daily with a gelatin capsule containing 500 mg of chromic oxide used as a digestion marker.

**Feeding and sampling procedures.** Each treatment period consisted of a 6-d adaptation phase followed by a 4-d sample collection phase. Ileal and fecal sample collections were conducted on d 7–10 of each treatment period. Ileal effluent was collected for 1-h periods at 4-h intervals, three times per day. Initial sampling time was delayed for 1 h on each subsequent day of the period. Before ileal sample collection, the cannula barrel was cleaned and any existing ileal effluent was discarded. Ileal samples were collected by attaching a Whirlpak bag (Pioneer Container, Cedarburg, WI) to the cannula hose clamp with a rubber band. During collections, the dogs were encouraged to move around freely. A Bite-Not collar (Bite-Not Products, San Francisco, CA) was placed on individual dogs only when it was necessary to deter them from removing the collection bag.

All feces voided during the 4-d collection period were collected from the floor of the pen, scored, weighed and frozen for subsequent analyses. Fecal scores were based on the following scale: 1 = hard, dry pellets; 2 = dry, well formed, stool; 3 = soft, moist, formed stool that retains shape; 4 = soft, unformed stool, assumes shape of container, pudding-like; 5 = watery, liquid that can be poured. On d 10 of each period, a freshly voided fecal sample was collected within 15 min of defecation. A subsample was immediately placed into preweighed Carey-Blair transport media containers (Meridian Diagnostics, Cincinnati, OH) for subsequent bacterial enumeration.

Blood samples were collected via jugular venipuncture into sterile

vacutainer tubes on d 10 of each period. Whole blood was collected in EDTA-containing tubes for white blood cell distribution analysis, whereas serum samples were collected in tubes containing no anti-coagulant for immunoglobulin (Ig) analyses.

**Sample handling.** Ileal effluent and fecal samples were individually frozen at –4°C immediately postcollection. At the end of each period, ileal effluent and fecal samples for each dog were composited and freeze-dried in a Tri-Philizer MP microprocessor-controlled lyophilizer (FTS Systems, Stone Ridge, NY). After drying, samples were ground through a 2-mm screen in a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ).

**Chemical analyses.** The diet was analyzed for dry matter (DM), organic matter (OM), ash (4), crude protein via Kjeldahl N determination (4), total lipid via acid hydrolysis followed by ether extraction (5,6) and total dietary fiber (TDF) (7). Ileal and fecal samples were analyzed for DM, OM, ash (4) and chromium (8) concentrations. In addition, the concentrations of arabinose and galactose in the ileal and fecal samples were determined by hydrolysis (9) and HPLC quantification (10) of the individual arabinose and galactose units. For all chemical analyses, samples were analyzed in duplicate. If a deviation > 5% occurred between duplicates, the analysis was repeated.

Total anaerobes, total aerobes, bifidobacteria, lactobacilli, *Clostridium perfringens* and *Escherichia coli* concentrations were determined in fresh fecal samples by serial dilution of anaerobic diluent before inoculation onto respective petri dishes of sterile agar (11). Total anaerobe and total aerobe agars were prepared according to the methods described by Bryant and Robinson (12) and Mackie and co-workers (13). *C. perfringens* agar was prepared according to the method of the Food and Drug Administration (14). The selective medium for bifidobacteria (BIM-25) was prepared using reinforced clostridial agar (BBL Microbiology Systems, Cockeysville, MD) according to the method described by Muñoa and Pares (15). Lactobacilli were cultured on Rogosa SL agar (Difco Laboratories, Detroit, MI). *E. coli* were cultured on EMB Agar (Difco Laboratories). All plates except *E. coli* and total aerobe plates were incubated anaerobically (95% CO<sub>2</sub>/5% H<sub>2</sub>) at 37°C. Colony-forming units were defined as being distinct colonies measuring at least 1 mm in diameter.

Whole blood and serum samples were sent to the University of Illinois, College of Veterinary Medicine Laboratories of Veterinary Diagnostic Medicine for complete blood count and Ig analyses. Serum Ig A, G and M were determined by radial immunodiffusion (ICN Biomedicals Aurora, OH). Whole-blood samples were sent to the Indiana Veterinary Diagnostic Laboratory (Fishers, IN) for analysis of the following lymphoid cell subset markers using the following antibodies: mouse anti-canine CD3, mouse anti-canine CD4, mouse anti-canine CD8a (Serotec, Raleigh, NC), mouse anti-human B cell CD19 and mouse anti-human natural killer cell CD56 (DAKO, Carpinteria, CA).

**Calculations.** Dry matter (g/d) recovered as ileal effluent or excreted as feces was calculated by dividing the Cr intake (mg/d) by ileal or fecal Cr concentrations (mg Cr/g ileal effluent or feces), respectively. Ileal and fecal nutrient flows were calculated by multiplying the DM flow by the concentration of the nutrient in the ileal or fecal DM. Ileal and total tract nutrient digestibilities were calculated as nutrient intake (g/d) minus the ileal or fecal nutrient flow (output, g/d), divided by nutrient intake (g/d).

**Statistical analysis.** Data were analyzed as a 7 × 7 Latin square design using the General Linear Models procedure of SAS (16). Sources of error included in the model were treatment, period and dog effects. Individual treatment means were compared with the control using nonorthogonal contrasts. Comparisons were considered different if the *P*-value was <0.05. Comparisons with a *P*-value of <0.20 were considered trends due to the variability associated with some of the criteria evaluated.

## RESULTS AND DISCUSSION

The crude protein, fat, and TDF concentrations of the diet were 31.5, 22.7 and 7.2 g/100 g dry matter, respectively. The gross energy content was 50.0 kcal/g (209.2 kJ/g) on a dry matter basis. Arabinogalactan treatments did not affect feed

intake, wet fecal weight or fecal DM for the dogs in this study (Table 2). Increased fecal weight is a common outcome of dietary fiber supplementation, especially when large amounts of insoluble fiber are fed. Conversely, AG is composed of 80–85% soluble fiber (17). Although both soluble and insoluble fibers can increase fecal output and fecal DM, this effect is more pronounced when insoluble fibers are fed. For example, Burkhalter and co-workers (18) showed that as the ratio of insoluble to soluble fiber increased in soybean hull-containing diets, fecal output of dogs increased in a linear manner. Stool weight increases that occur in response to feeding soluble fibers appear to be a result of the highly fermentable nature of these fibers. Soluble fiber provides available substrate for intestinal bacteria, causing rapid bacterial proliferation and, subsequently, increased excretion of bacterial cell mass in the feces and increased fecal weight (19).

Dogs supplemented with the low level of AG1000 had higher ( $P = 0.02$ ) fecal scores than control dogs, but fecal weight and dry matter were unaffected (Table 2). A higher fecal score is associated with increased fecal moisture. Although excessively hard and dry feces are not desirable, excessive amounts of water retained in the feces can lead to soft unformed stool or diarrhea. Increased fecal moisture content has been demonstrated in rats fed AG (20). Although the fecal scores of the dogs fed the low level of AG1000 increased, their scores remained in a range that is considered healthy and desirable (well formed, soft stools) and were not associated with excess moisture content or diarrhea.

Ileal DM and OM digestibilities were not significantly affected by AG supplementation (Table 2), although total tract DM digestibilities tended to decrease in dogs fed the high levels of AG100 and AG1000 ( $P = 0.16$  and  $0.12$ , respectively). Total tract OM digestibilities tended ( $P = 0.11$ ) to decrease in dogs fed the high level of AG100 and AG1000. Although excess intake of dietary fiber can negatively affect nutrient digestibility (21,22), the production of normal fecal volume and form in dogs fed AG, as well as these minimal

changes in digestibility coefficients, indicate that the amount of AG provided was not excessive and did not affect the dogs' ability to digest the diet.

Regardless of treatment, arabinose plus galactose (A + G) concentration was higher in ileal effluent than feces, demonstrating that bacterial fermentation had occurred in the large bowel of the dogs (Table 2). Possible origins of A + G in the ileal effluent and feces of control dogs include dietary AG and other fibrous components such as pectin and hemicellulose. Arabinose plus galactose concentration increased ( $P = 0.02$ ) in the ileal effluent of dogs supplemented with the high dose of AG100 and tended to increase ( $P = 0.12$ ) in dogs supplemented with the high dose of AG3000. In addition, A + G concentration increased ( $P \leq 0.01$ ) in the feces of dogs supplemented with the high dose of all forms of AG, and tended ( $P = 0.13$ ) to be higher in dogs supplemented with the low dose of AG100 and AG3000. Bacterial fermentation of AG results in production of short-chain fatty acids (SCFA). Although fecal SCFA concentrations were not measured in the present experiment, in other studies, *in vitro* incubation of AG with human feces resulted in increased SCFA production, particularly butyrate and propionate, and decreased ammonia production (23,24).

The results of the fecal microbial analyses are presented in Table 3. Arabinogalactan supplementation did not affect the concentration of total anaerobic fecal bacteria. However, dogs supplemented with the high dose of AG1000 tended ( $P = 0.15$ ) to have a higher concentration of total aerobic fecal bacteria compared with control dogs. Arabinogalactan supplementation also tended to increase the concentration of bifidobacteria in the feces of dogs supplemented with the low dose of AG1000 ( $P = 0.08$ ) and the high dose of AG3000 ( $P = 0.16$ ) compared with concentrations in control dogs. Similarly, fecal concentrations of lactobacilli increased ( $P = 0.04$ ) in dogs supplemented with the low dose of AG1000 and high dose of AG3000, and tended ( $P = 0.17$ ) to increase in the

TABLE 2

Feed intake, fecal characteristics, digestibility coefficients and arabinogalactan concentrations in digesta of dogs supplemented with arabinogalactan (AG)<sup>1</sup>

	Arabinogalactan treatment								Contrasts <sup>2</sup> : Control vs.						
	Form	Control		AG100		AG1000		AG3000	AG100		AG1000		AG3000		
	Dose, g/d	0	0.55	1.65	0.55	1.65	0.55	1.65	SEM	0.55	1.65	0.55	1.65	0.55	1.65
Intake, DMB, <sup>3</sup> g/d		332	376	323	370	310	345	324	19.9	0.13	NS	0.19	NS	NS	NS
Wet feces, g/d		176	181	172	202	159	192	164	13.4	NS	NS	0.19	NS	NS	NS
Fecal dry matter, %		29.8	29.9	30.0	28.9	29.8	29.5	30.8	0.6	NS	NS	NS	NS	NS	NS
Fecal score <sup>4</sup>		2.9	3.0	3.1	3.2	3.0	3.0	3.0	0.1	NS	0.07	0.02	NS	NS	NS
Ileal digestion, %															
Dry matter		72.3	70.0	71.2	79.2	69.4	68.3	73.1	5.1	NS	NS	NS	NS	NS	NS
Organic matter		77.3	75.6	76.5	82.9	74.4	74.5	78.1	4.2	NS	NS	NS	NS	NS	NS
Total tract digestion, %															
Dry matter		82.3	82.5	80.4	82.2	80.1	80.9	81.6	0.96	NS	0.16	NS	0.12	NS	NS
Organic matter		86.7	86.8	84.9	86.5	84.9	85.4	86.1	0.76	NS	0.11	NS	0.11	NS	NS
Arabinose plus galactose, mg/g															
Ileal effluent		70.3	71.2	94.2	75.9	82.4	69.7	84.6	6.5	NS	0.02	NS	NS	NS	0.12
Feces		38.0	42.6	52.2	40.6	48.5	42.6	48.6	2.1	0.13	0.01	NS	0.01	0.13	0.01

<sup>1</sup> Values are least-squares means,  $n = 7$ .

<sup>2</sup> NS, not significantly different ( $P > 0.20$ ).

<sup>3</sup> DMB, dry matter basis.

<sup>4</sup> Fecal scores were based on the following scale: 1 = hard, dry pellets; 2 = dry, well-formed stool; 3 = soft, formed, moist, softer than stool that retains shape; 4 = soft, unformed, stool assumes shape of container, pudding-like; 5 = watery, liquid that can be poured.



TABLE 3

Bacterial concentrations in feces of ileally cannulated dogs supplemented with arabinogalactan (AG)<sup>1</sup>

Form	Arabinogalactan treatment								Contrasts <sup>2</sup> : Control vs.					
	Control	AG100		AG1000		AG3000		SEM	AG100		AG1000		AG3000	
	Dose, g/d	0	0.55	1.65	0.55	1.65	0.55		1.65	0.55	1.65	0.55	1.65	0.55
Total anaerobes	10.92	11.02	10.96	10.87	10.95	10.91	10.84	0.13	NS	NS	NS	NS	NS	NS
Total aerobes	8.54	8.41	8.92	8.52	9.06	8.44	8.41	0.25	NS	NS	NS	0.15	NS	NS
Bifidobacteria	9.26	9.48	9.16	9.75	9.31	9.41	9.66	0.20	NS	NS	0.08	NS	NS	0.16
<i>Clostridium perfringens</i>	10.01	9.82	9.83	10.01	9.86	9.74	9.89	0.12	NS	NS	NS	NS	0.10	NS
<i>Escherichia coli</i>	7.96	7.95	7.95	7.66	8.25	7.81	7.69	0.25	NS	NS	NS	NS	NS	NS
Lactobacilli	8.63	8.98	9.14	9.40	8.88	9.03	9.39	0.26	NS	0.17	0.04	NS	NS	0.04

<sup>1</sup> Least-squares means are expressed as log<sub>10</sub> colony-forming units/g dry feces, *n* = 7.<sup>2</sup> NS, not significantly different (*P* > 0.20).

feces of dogs supplemented with the high dose of AG100, compared with concentrations in feces of control dogs.

Arabinogalactan is fermented in the colon by bacteria such as *Bifidobacterium* and *Bacteroides* (25,26). Both bifidobacteria and lactobacilli are beneficial bacterial populations. Increased concentrations of these organisms have been associated with decreased fecal concentrations of potentially pathogenic bacteria (27,28) and decreased levels of carcinogenic and putrefactive compounds in digesta (29–31).

Fecal concentrations of *C. perfringens* tended (*P* = 0.10) to decrease in dogs supplemented with the low dose of AG3000 compared with the control. Arabinogalactan supplementation did not affect concentrations of *E. coli* in the feces of any treatment groups. A reduction in the number of clostridia in the gut is beneficial to the dog because this organism is pathogenic and can exert harmful effects on the host (32). *E. coli* exhibits both harmful (i.e., production of carcinogens) and beneficial (i.e., stimulation of immune function) effects in the gut (32). Alterations in the numbers of these two organisms could, therefore, result in both positive and negative effects on the animal.

The effect of AG supplementation on immunological characteristics of dogs is presented in Table 4. Arabinogalactan supplementation did not significantly affect (*P* > 0.05) serum concentration of Ig A, M or G, although supplementation with the high dose of AG3000 tended (*P* = 0.15) to result in a higher serum concentration of Ig G. Supplementation with either level of AG1000 and AG3000 tended to increase (*P* < 0.10) total white blood cell concentrations compared with values in control dogs. White blood cell differentiation revealed that this increase was due to increases in neutrophil and eosinophil concentrations in AG-supplemented dogs compared with controls. Conversely, AG100 did not affect white blood cell count or individual immune cell concentrations.

To further assess the effects of AG supplementation on circulating immune cells, lymphoid cell markers were evaluated (Table 4). The CD3 surface cell marker was used as a general T-cell marker. Dogs supplemented with the low doses of AG100 and AG3000 had lower (*P* = 0.11 and *P* = 0.02, respectively) concentrations of cells expressing this cell marker. The CD4 and CD19 surface markers were utilized to identify helper T cells and B cells, respectively. Arabinogalac-

TABLE 4

Effects of arabinogalactan (AG) supplementation on immunological characteristics of ileally cannulated dogs<sup>1</sup>

Form	Arabinogalactan treatment								Contrasts <sup>2</sup> : Control vs.					
	Control	AG100		AG1000		AG3000		SEM	AG100		AG1000		AG3000	
	Dose, g/d	0	0.55	1.65	0.55	1.65	0.55		1.65	0.55	1.65	0.55	1.65	0.55
Serum immunoglobulins, g/L														
IgA	0.13	0.13	0.12	0.12	0.12	0.13	0.13	0.009	NS	NS	NS	NS	NS	NS
IgM	0.95	0.95	0.98	1.00	0.99	0.98	1.06	0.055	NS	NS	NS	NS	NS	0.15
IgG	20.00	20.50	20.84	19.77	18.94	21.49	20.57	1.13	NS	NS	NS	NS	NS	NS
White blood cells, cells × 10 <sup>3</sup> /μL	9.35	9.71	9.85	10.74	10.53	10.35	10.64	0.38	NS	NS	0.02	0.04	0.08	0.02
Neutrophils	6.00	6.26	6.21	6.82	6.81	6.63	7.05	0.33	NS	NS	0.09	0.09	0.19	0.03
Lymphocytes	1.83	1.71	2.06	2.11	2.04	2.05	1.93	0.15	NS	NS	0.19	NS	NS	NS
Monocytes	1.13	1.25	1.13	1.24	1.17	1.14	1.25	0.09	NS	NS	NS	NS	NS	NS
Eosinophils	0.38	0.47	0.42	0.50	0.50	0.50	0.41	0.04	0.13	NS	0.06	0.04	0.05	NS
Lymphoid cell subset markers, %														
CD3	74.29	68.00	72.43	70.14	71.00	65.00	72.00	2.71	0.11	NS	NS	NS	0.02	NS
CD4	10.43	8.43	9.71	9.14	10.86	9.29	10.71	1.25	NS	NS	NS	NS	NS	NS
CD8	26.71	21.57	27.00	28.00	28.14	24.86	26.71	1.57	0.03	NS	NS	NS	NS	NS
CD19	15.43	16.29	14.71	14.71	17.43	13.86	13.71	1.90	NS	NS	NS	NS	NS	NS
CD56	2.57	3.00	3.43	4.14	2.57	3.43	3.57	0.59	NS	NS	0.07	NS	NS	NS

<sup>1</sup> Values are least-squares means, *n* = 7.<sup>2</sup> NS, not significantly different (*P* > 0.20).

tan supplementation did not affect the concentration of lymphocytes expressing either of these surface markers. The CD8 and CD56 surface markers were utilized to identify cytotoxic T cells and natural killer cells. Dogs supplemented with the low dose of AG100 had lower ( $P = 0.03$ ) concentrations of lymphocytes expressing the CD8 cell marker compared with controls, whereas dogs supplemented with the low dose of AG1000 tended to have a higher ( $P = 0.07$ ) concentration of cells expressing the CD56 cell markers.

Hauer and Anderer (2) demonstrated that the mononuclear cells, pre-separated peripheral nonadherent cells and monocytes from cultures of human peripheral blood that had been pretreated with AG from *Larix occidentalis* for 48–72 h showed enhancement of natural killer cytotoxicity against tumor cells. The enhancement of cytotoxicity was not direct, but rather occurred through stimulation of various cytokines, particularly interferon  $\gamma$  (2). Luettig et al. (33) also found that acidic AG from plant cell cultures of *Echinacea purpurea* was effective at activating macrophages to cytotoxicity against tumor cells and microorganisms. Similar to the results of Hauer and Anderer (2), Luettig found that the acidic AG-induced macrophages produced the various cytokines.

In conclusion, as determined in this experiment, nutrient digestion, fecal microbial concentrations and serum lymphocyte concentrations all were affected by specific doses and forms of AG. Further investigation is required to determine the specific mechanisms of actions of each of these compounds.

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