Diet and Age Affect Intestinal Morphology and Large Bowel Fermentative End-Product Concentrations in Senior and Young Adult Dogs[1,2]

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ABSTRACT The objective of this study was to determine the effects of age and diet on intestinal morphology and large bowel fermentative end-product concentrations in healthy dogs. Small intestinal villus width, height, and area, and small intestinal and colonic crypt depth were measured. Large bowel digesta samples were analyzed for ammonia, SCFAs, and branched-chain fatty acids (BCFAs). SCFAs are considered to be beneficial fermentative end-products in the intestine because they exert trophic effects on intestinal cells. Twelve senior (age = 11.1 y ± 0.6 at baseline; 6 male, 6 female) and 12 young adult (age = 8 wk old at baseline; 6 male, 6 female) beagles were randomly assigned to 1 of 2 dietary treatments, an animal product–based diet (APB) and a plant product–based diet (PPB). Diets were fed for 12 mo. Jejunal ($P = 0.03$) and ileal ($P = 0.02$) villus height, and duodenal ($P = 0.04$) villus width were greater for dogs consuming the PPB diet. Young dogs had greater ($P = 0.04$) jejunal villus height, whereas senior dogs had greater ($P < 0.001$) colonic crypt depth. Ammonia concentrations decreased ($P = 0.03$) from proximal to distal colon and were higher in dogs consuming APB ($P = 0.03$). Age and treatment affected butyrate concentrations, with senior dogs ($P = 0.04$) and dogs consuming APB ($P = 0.04$) having higher concentrations. Both diet and age affected small and large intestinal morphology, and colonic fermentative end-product concentrations in dogs. J. Nutr. 135: 1940–1945, 2005.

KEY WORDS: • *canine* • *dietary fiber* • *fermentative end-products* • *intestinal morphology*

The concept of "gut health" is complex and broadly defined. According to Conway (1), 3 major components of "gut health" exist, namely, diet, intestinal mucosa, and intestinal microbiota. Intestinal morphology changes with nutritional variations, stress, aging, and(or) disease and affects the physiology of the intestine, specifically nutrient absorption and metabolism. Because the absorptive functions of the intestine are related to its morphology, alterations in morphology may predispose the intestine to functional disorders.

Villus height and crypt depth are direct representations of the intestinal environment and may be used as indicators of intestinal health. A harsh environment, including low pH and the presence of select bacterial end-products, may lead to abnormal changes in these morphometric indices. A decrease in either villus height or crypt depth may lead to a reduction in nutrient absorption.

In vitro and in vivo studies show that end-products of fermentation produced by colonic bacteria depend largely on the chemical composition of the digesta reaching the large bowel. The primary fermentative end-products produced from dietary fiber are SCFAs, predominantly acetate, propionate, and butyrate. The SCFAs produced are rapidly absorbed from the intestinal lumen, with 95–99% being absorbed before reaching the distal colon (2). Individual SCFAs occur in varying ratios depending on substrate and microbial populations, and have specific roles in host metabolism. For example, resistant starch fermentation yields high concentrations of butyrate, whereas pectin results in high concentrations of acetate (3). Butyrate is used primarily by the colonocytes as an energy source and stimulates the development and growth of the large and small intestines by stimulating epithelial cell proliferation (4).

Microbial fermentation of undigested amino acids results in the production of several putrefactive compounds (5). These include ammonia, which results from the deamination of amino acids, phenols, indoles (products of aromatic amine decarboxylation), and branched-chain fatty acids (BCFAs),[4] derived from branched-chain amino acid catabolism. Protein

[1] Presented in part at Experimental Biology 04, April 17–21, 2004, Washington, DC [Kuzmuk, K. N., Swanson, K. S., Schook, L. B. & Fahey, G. C., Jr. (2004) Diet and age affect fermentative end-product concentrations in the proximal, middle, and distal regions of the dog colon. FASEB J. 18: #269.6 (abs.)] and at the annual meeting of the American Society of Animal Science, July 25–29, 2004, St. Louis, MO [Kuzmuk, K. N., Swanson, K. S., Schook, L. B. & Fahey, G. C., Jr. 2004 Diet and age affect canine small intestinal and colonic gut morphology. J Anim. Sci. 82 (suppl. 1): 244: # 374 (abs.)].

[2] Funded in part by Pyxis Genomics Incorporated, Chicago, IL 60612.

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[4] Abbreviations used: AAFCO, American Association of Feed Control Officials; APB, animal product–based; BCFA, branched-chain fatty acid; BW, body

Manuscript received 23 March 2005. Initial review completed 20 April 2005. Revision accepted 4 May 2005.

catabolites not only result in fecal odor, but are toxic at high concentrations (6).

Bacterial populations and fermentative end-product concentrations are altered in the aging gastrointestinal tract. Recent studies suggest that age affects the intestinal microflora, with a decrease in anaerobes and bifidobacteria and an increase in enterobacteria in elderly humans (7). Research in dogs demonstrated that the number of *Bacteroides* was lower in older dogs than in younger dogs, whereas lactobacilli and bifidobacteria numbers were not influenced by age (8). A study by Andrieux et al. (9) demonstrated that fecal samples collected from elderly humans (69–89 y old) had higher concentrations of metabolites from protein fermentation (ammonia, valerate, isobutyrate, and isovalerate) compared with samples collected from younger adults (30–46 y old) and children (3–15 y old).

As veterinary care and diet quality increase, dogs continue to live longer lives. Therefore, the effects of age and diet on intestinal morphology and large bowel health are of importance. The objective of this study was to evaluate the effects of age and diet on intestinal morphology and large bowel fermentative end-product concentrations in healthy young adult and geriatric dogs.

MATERIALS AND METHODS

Animals and diets. Senior (mean age = 11.1 y ± 0.6 at baseline; 6 males and 6 females) and weanling (8 wk old at baseline; 6 males and 6 females) beagles (Marshall Farms) were used in this experiment. Three dogs of each gender and age were assigned to 1 of 2 dietary treatments. The animal product–based (APB) diet (**Table 1**) was composed primarily of highly digestible, animal-derived ingredients and formulated to contain 30% crude protein (CP) and 20% fat. The plant product–based (PPB) diet (Table 1) was composed primarily of plant-derived ingredients and was formulated to meet CP (22%) and fat (8%) recommendations for growth according to American Association of Feed Control Officials (AAFCO) (10). For formulation purposes, meat and bone meal were included as 10% of the PPB diet. Both diets were formulated to meet or exceed all nutrient requirements for growth according to AAFCO (10) and represent 2 distinct types of dog food currently on the market. Because diets were fed for 12 mo, mean ages of dogs were 12 y (seniors) and 1 y (young adults) when they were killed and samples collected. The amount of food offered initially was calculated by using standard equations for determining energy requirements of active adult dogs [ME requirement (kcal) = $132 \times BW_{kg}^{0.67}$; where ME = metabolizable energy, BW = body weight] and small breed puppies [ME requirement (kcal) = $375 \times BW_{kg}^{0.67}$] (11). The amount of food offered was adjusted to maintain the initial BW in seniors and to allow ad libitum consumption in weanlings throughout the experiment. Food refusals were weighed daily and food intake calculated. Dogs were housed individually in kennels (1.1 × 0.9 m) in temperature-controlled rooms with a 12-h light:dark cycle at the Edward R. Madigan Laboratory on the University of Illinois campus. The Institutional Animal Care and Use Committee approved all animal care procedures before initiation of the study.

Sample collection and handling. After 12 mo of experimental feeding, the dogs were food deprived for 12 h and then given a lethal i.v. dose (130 mg/kg BW) of sodium pentobarbital (Euthasol®, Virbac) into the left forearm. Death was confirmed by lack of respiration and a corneal reflex, and absence of a heartbeat detected with a stethoscope placed under the left elbow.

Intestinal sections were collected from the duodenum (10 cm distal to the pyloric sphincter), jejunum (10 cm distal to ligament of trites), ileum (10 cm proximal to the ileocecal junction), and colon (midpoint), and placed in phosphate-buffered formalin for preservation. All samples were collected within 20 min of the time of death. Tissues were embedded in paraffin and sliced into 3-μm sections using a microtome. Samples then were placed on glass slides followed by staining with hematoxylin and eosin. Digital images of tissues were taken using a Nikon Optiphot-2 microscope (Nikon). Height and width measurements of small intestinal villi were taken using Image Pro Plus® software (Universal Imaging). From this, cross-sectional villus area was determined by multiplying width measurements by height; 15 villus height and width measurements were attempted per sample. However, in 16 of 95 samples, only 7–14 villus height and width measurements could be taken. Crypt depth measurements, a minimum of 15/section, were taken from both small intestinal and colonic tissue samples.

Digesta were collected from the proximal, middle, and distal regions of the colon and stored at −20°C until further analyses. Samples were acidified using 10 mL of HCl (2 mol/L) to maintain pH before storage.

Chemical analyses. Diets were analyzed for dry matter (DM) and ash using methods of the Association of Official Analytical Chemists (12). CP was calculated using Leco total N values (13). Total lipid

TABLE 1

Ingredient and chemical composition of the APB and PPB diets fed to weanling and senior dogs for 12 mo

Ingredient	APB[1]	PPB[2]
	%, as-is	
Corn		45.00
Brewers rice	44.23	
Poultry byproduct meal	32.91	
Soybean meal		19.96
Poultry fat	14.99	3.97
Wheat middlings		13.20
Meat and bone meal		10.00
Beet pulp	4.00	4.00
Dehydrated egg	2.20	2.20
Sodium chloride	0.65	0.65
Potassium chloride	0.65	0.65
Choline chloride	0.13	0.13
Vitamin premix[3]	0.12	0.12
Mineral premix[3]	0.12	0.12
Analyzed composition		
Dry matter, %	93.8	94.3
	% of DM	
Organic matter	92.8	92.3
Ash	7.2	7.7
Crude protein	28.0	25.5
Acid hydrolyzed fat	22.6	11.2
Total dietary fiber	4.8	15.2
Gross energy, *kJ/g*	22.51	19.87

[1] Provided per kg of APB diet: choline, 2654 mg; retinyl acetate, 15.2 KIU; cholecalciferol, 0.9 KIU; α-tocopherol, 62.5 IU; menadione sodium bisulfite complex (source of vitamin K), 0.6 mg; thiamin, 13.1 mg; riboflavin, 14.0 mg; pantothenic acid, 25.3 mg; niacin, 70.0 mg; pyridoxine, 13.56 mg; biotin, 0.11 mg; folic acid, 949 μg; vitamin B-12, 129 μg; manganese (as $MnSO_4$), 19.6 mg; iron (as $FeSO_4$), 253.9 mg; copper (as $CuSO_4$), 17.8 mg; cobalt (as $CoSO_4$), 2.4 mg; zinc (as $ZnSO_4$), 166.9 mg; iodine (as KI), 6.3 mg; and selenium (as Na_2SeO_3), 0.32 mg.

[2] Provided per kg of PPB diet: choline, 2457 mg; retinyl acetate, 16.3 KIU; cholecalciferol, 0.9 KIU; α-tocopherol, 74.1 IU; menadione sodium bisulfite complex (source of vitamin K), 1.2 mg; thiamin, 14.4 mg; riboflavin, 11.5 mg; pantothenic acid, 23.9 mg; niacin, 79.3 mg; pyridoxine, 15.8 mg; biotin, 0.24 mg; folic acid, 1024 μg; vitamin B-12, 33.3 μg; manganese (as $MnSO_4$), 24.0 mg; iron (as $FeSO_4$), 214.6 mg; copper (as $CuSO_4$), 23.1 mg; cobalt (as $CoSO_4$), 2.4 mg; zinc (as $ZnSO_4$), 144.3 mg; iodine (as KI), 24.0 mg; selenium (as Na_2SeO_3), 0.27 mg.

[3] Trouw Nutrition USA, LLC, Highland, IL.

weight; CP, crude protein; DM, dry matter; GLP, glucagon-like peptide; ME, metabolizable energy; PPB, plant product–based; TDF, total dietary fiber.

content was determined by acid hydrolysis followed by ether extraction according to the American Association of Cereal Chemists (14) and Budde (15). The total dietary fiber (TDF) concentration was determined according to Prosky et al. (16,17).

Digesta samples were analyzed for ammonia concentration according to the method of Chaney and Marbach (18). SCFA and BCFA concentrations were determined via GC according to Erwin et al. (19). Concentrations of acetate, butyrate, propionate, valerate, isovalerate, and isobutyrate were determined in the supernatant of acidified colonic aliquots using a Hewlett-Packard 5890A Series II GC and a glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco). Nitrogen was the carrier gas with a flow rate of 75 mL/min. Oven temperature, detector temperature, and injector temperature were 125, 175, and 180°C, respectively.

Statistical analyses. A 2 × 2 factorial arrangement of treatments (age and diet) in a completely randomized design was used. Fermentative end-product data were analyzed using the PROC MIXED procedure of SAS (SAS Institute). The influence of age, diet, and intestinal region (proximal, middle, and distal small intestine, and colon) was examined. Intestinal morphology data were analyzed using the PROC GLM procedure of SAS. Probability values <0.05 were considered significant, and P-values <0.10 were considered trends.

RESULTS AND DISCUSSION

Preamble. The data presented herein represent one of several manuscripts generated from a large canine nutritional genomics experiment we performed examining the effects of nutrition and age on metabolic characteristics and gene expression profiles. To our knowledge, this experiment is the first of its kind in dogs. Our overall goal is to correlate metabolic indices with gene expression profiles, identifying gene-metabolite relations important in understanding canine metabolism. Food intake, nutrient digestibility, serum biochemistry, and hematology from this experiment were published previously (20). The current dataset is focused on the effects of age and diet on the gastrointestinal tract, including intestinal morphology and colonic fermentative end-product concentrations. The reported findings are novel because collection of intestinal tissues and digesta in dogs is rarely practiced. Thus, these data will be very useful to companion animal researchers and pet food professionals.

Because very little is known about the effects of diet and age on gene expression profiles, we chose to study 2 very different age groups and diets for comparison. Because the aging population has an increased susceptibility to gastrointestinal dysfunction, we chose to study geriatric (11 y old at baseline) and weanling dogs (8 wk old at baseline). Given the length of the experiment, these dogs were 12 y old and 1 y old (young adults) at time of tissue and digesta collection. We chose to formulate diets that were representative of 2 distinct types of dog foods: *1*) a highly digestible, animal protein–based diet with high concentrations of protein and fat and a low concentration of dietary fiber; and *2*) a plant protein–based diet with moderate protein and fat concentrations and a high concentration of dietary fiber. Given the increase in dietary fiber, a lower digestibility was expected with this diet. Because diets varied in concentration and source of nutrients, the effects cannot be attributed to any one nutrient in particular.

Intestinal morphology. Age affected intestinal morphology because young dogs tended to have a greater duodenal villus area ($P = 0.09$), jejunal villus height ($P = 0.04$), and jejunal villus:crypt ratio ($P = 0.03$) (**Table 2**). In agreement with the results of our study, previous research by Altman et al. (21) demonstrated that both weanling (16–18 d) and young (36–39 d) rats had greater jejunal villus height compared with

TABLE 2

Gut morphology measurements in senior and weanling beagles fed APB or PPB diets for 12 mo[1]

Item	Treatment				Pooled SEM	*P*-value[2]	
	Senior APB	Senior PPB	Weanling APB	Weanling PPB		Age	Diet
			μm				
Duodenum							
Villus height	737	737	871	768	81.8	0.33	0.54
Villus width	175	199	187	209	10.5	0.31	0.04
Villus area[3]	122,985	147,388	161,858	162,014	14,886.6	0.09	0.43
Crypt depth	171	164	143	132	27.3	0.19	0.68
Villus:crypt[4]	4.6	5.4	6.4	5.8	0.8	0.18	0.90
Jejunum							
Villus height	626	649	639	825	43.9	0.04	0.03
Villus width	208	183	194	166	13.6	0.27	0.07
Villus area[3]	130,158	117,503	123,048	137,292	10,270.3	0.54	0.94
Crypt depth	171	207	124	204	38.3	0.42	0.07
Villus:crypt[4]	3.8	3.3	6.8	5.1	1.0	0.03	0.34
Ileum							
Villus height	507	579	457	590	38.8	0.62	0.02
Villus width	179	161	151	154	9.4	0.08	0.44
Villus area[3]	90,548	93,355	71,118	91,861	8716.0	0.24	0.19
Crypt depth	133	153	102	133	39.6	0.29	0.28
Villus:crypt[4]	4.5	4.1	4.8	5.4	0.7	0.30	0.91
Colon							
Crypt depth	493	459	331	310	46.7	<0.01	0.47

[1] Values are means, $n = 6$.
[2] Age = main effect of age; Diet = main effect of dietary treatment.
[3] Villus area is represented as μm^2.
[4] Ratio of villus height to crypt depth.

adult (85–90 d) rats. A trend was observed for increased ($P = 0.08$) ileal villus width in senior dogs. Also, senior dogs had greater ($P < 0.01$) colonic crypt depth.

The villus:crypt ratio is an indicator of the likely digestive capacity of the small intestine. An increase in this ratio corresponds to an increase in digestion and absorption (4). This suggests that young adult dogs had increased ($P = 0.03$) digestive and absorptive capacity in the jejunum.

Studies performed with rats (22) and humans (23,24) reported reduced villus height and surface area of the proximal small intestine with increasing age. Investigators hypothesized that this could lead to decreased absorption in old age. In contrast, Lipski et al. (25) reported no effect of age on proximal small intestinal morphology in humans. However, the subjects studied varied widely in age, and subjects with conditions associated with abnormal proximal small bowel mucosa were excluded. This is not always the case for studies examining human intestinal morphology. It is nearly impossible to obtain small bowel biopsy specimens from normal healthy elderly subjects with no past or present symptoms of gastrointestinal disease. Most studies examine subjects undergoing rehabilitation; thus, tissues examined are considered "diseased," skewing the data.

Overall, the structure, motility, and absorptive functions of the small intestine remain intact in healthy elderly individuals (26). Corazza et al. (27) demonstrated that there were no age-related alterations in small intestinal anatomy. Enterocyte height and intraepithelial lymphocyte counts were unchanged. They also reported that the small intestine maintains normal absorptive function in the case of carbohydrates, fats, and vitamin B-12 with aging.

The effects of age on indices associated with intestinal health have been poorly studied in dogs due to constraints associated with terminal experiments and access to tissues. However, the experiments that have been performed demonstrate that there are no age-related changes in absorption of nutrients. In a previous publication, we reported that total tract nutrient digestibility did not differ between the senior and young dogs of this experiment (20). Balance studies performed by Sheffy et al. (28) showed no changes in apparent digestibility and retention of macronutrients (carbohydrates, protein, and fat), minerals (Ca, P, Mg, Zn, Cu, Fe, K, and Na), and vitamins (vitamins C and E) due to advanced age. These results suggest that the aged canine gastrointestinal tract has the ability to compensate for any decreases occurring in absorptive efficiency.

Duodenal villus width ($P = 0.04$) and jejunal ($P = 0.03$) and ileal ($P = 0.02$) villus height were greater for dogs consuming the PPB diet (Table 2). This response was likely due to the differences in dietary fiber content between the APB (4.8%) and PPB (15.2%) diets. Dietary fiber is resistant to digestion by the normal secretory and digestive mechanisms present in the gut, and is the main substrate for bacterial fermentation. Increased dietary fiber consumption may increase both villus height and crypt depth, thereby increasing the surface area available for nutrient absorption (29). Prolonged ingestion of dietary fiber is associated with changes in the structure of the small intestine, including changes in villus height. Both cellulose, a nonfermentable fiber component, and pectin, a viscous, fermentable component, increased villus height and width in rats (30). Intestinal cells use the metabolic products of fiber digestion, SCFAs, especially butyrate, as an energy source and as a substrate for metabolism. Cell proliferation in the intestinal epithelium in vivo was shown to be stimulated by SCFAs via increased glucagon-like peptide (GLP)-2 concentrations, a 33-amino acid peptide responsible for inducing intestinal cell proliferation (31). Jin et al. (32) demonstrated that a high (10% wheat straw) dietary fiber concentration altered the rate of intestinal cell turnover as well as intestinal morphology in growing pigs. The width of intestinal villi and the rate of cell proliferation tended to increase ($P < 0.10$) in pigs consuming the high-fiber diet compared with pigs consuming a diet containing no fiber.

It was proposed that small changes in the percentage of total dietary lipid may influence active and passive intestinal transport processes in rats (33). Therefore, an increase in jejunal villus width may be indicative of increased lipid transport in this section of the small intestine. This coincides with the observation that dogs consuming the APB diet, which had higher fat concentrations, exhibited an increase in jejunal villus width.

There was both an age effect ($P = 0.04$) and a diet effect ($P = 0.03$) for jejunal villus height, resulting in a trend for an interaction ($P = 0.08$) between the 2 main effects. This suggests that age and diet resulted in additive effects on jejunal villus height. Young dogs consuming the PPB diet exhibited an enhanced villus height that was greater than that for all other individual treatment groups. This resulted perhaps from the increased fiber concentration in the PPB diet that stimulated intestinal growth in the young dogs.

There was a general trend for decreased villus height, width, and area from the proximal to the distal regions of the small intestine. This is in agreement with the observations made by Paulsen et al. (34) in dogs that reported greater circumference and surface area in the proximal small intestine compared with the middle and distal regions of the small intestine. Altmann et al. (21) demonstrated in rats that there was nearly a 50% decrease in villus height from duodenum to terminal ileum, with adult rats having marginally greater villus height in the duodenum, and with height decreasing toward the distal regions of the small intestine.

Our study demonstrated that aging canines had increased ($P < 0.01$) colonic crypt depth. This may be due to the increase ($P = 0.02$) in colonic butyrate concentrations found in senior dogs (**Table 3**), which would be expected to stimulate cell proliferation via production of GLP-2. Drucker et al. (31) demonstrated that GLP-2 stimulated crypt cell proliferation in mice. Tappenden et al. (35) reported that systemic SCFA administration rapidly upregulates the expression of proglucagon and early response genes, with previous studies demonstrating the involvement of these genes in cellular proliferation and differentiation (35–38). The epithelial cells in the deeper portions of the colonic crypts proliferate and migrate up toward the lumen. The newly formed cells replace the old ones, allowing for continuous renewal of the intestinal epithelium (20). This implies that the increased butyrate found in the senior dogs may help maintain the aging intestine via increased cell renewal, which is demonstrated by the deeper crypts. Because no significant ($P < 0.05$) diet × age interactions on gut morphology were observed, these *P*-values are not reported.

Fermentative end-product concentrations. Ammonia concentrations (Table 3) decreased ($P = 0.03$) from proximal to distal colon, and were greater ($P = 0.03$) in those dogs consuming the APB diet. In contrast to SCFAs that originate from carbohydrate catabolism, putrefactive compounds are formed as a result of protein catabolism and can be harmful to the host. Ammonia is a putrefactive compound that induces faster turnover of epithelial cells and is toxic to colonocytes (39). Increasing dietary carbohydrate concentrations may reduce ammonia concentrations by stimulating carbohydrate fermentation, which in turn stimulates bacterial protein syn-

TABLE 3

Colonic ammonia, SCFA, and BCFA concentrations in senior and weanling beagles fed APB or PPB diets for 12 mo[1]

Item	Treatment				Pooled SEM	*P*-value[2]		
	Senior APB	Senior PPB	Weanling APB	Weanling PPB		Region	Age	Diet
	μmol/g dry matter							
Ammonia								
Proximal	228	168	165	84	44.2	0.03	0.10	0.03
Middle	188	91	132	67	44.5			
Distal	125	78	89	33	41.1			
Acetate								
Proximal	445	496	364	378	114.6	0.99	0.48	0.71
Middle	447	392	392	442	115.8			
Distal	449	463	302	429	120.3			
Propionate								
Proximal	172	108	94	106	37.4	0.06	0.22	0.99
Middle	198	147	152	145	37.8			
Distal	162	214	101	155	38.8			
Butyrate								
Proximal	63	41	31	23	10.9	0.59	0.02	0.04
Middle	68	38	50	24	11.0			
Distal	53	42	34	26	11.5			
Σ SCFA[3]								
Proximal	678	644	489	506	151.8	0.88	0.35	0.93
Middle	713	561	594	611	153.4			
Distal	664	713	439	610	158.5			
Valerate								
Proximal	2	4	1	1	1.15	0.11	0.05	0.31
Middle	1	2	2	1	1.16			
Distal	4	5	1	2	1.21			
Isovalerate								
Proximal	14	6	5	6	2.81	0.92	0.04	<0.01
Middle	14	5	10	4	2.84			
Distal	16	6	8	5	2.98			
Isobutyrate								
Proximal	14	26	11	23	4.8	0.34	0.93	0.02
Middle	12	12	9	22	4.85			
Distal	16	18	10	24	5.07			
Σ BCFA[4]								
Proximal	29	36	17	30	6.88	0.55	0.22	0.32
Middle	26	20	22	27	6.95			
Distal	36	30	18	31	7.29			

[1] Values are means, $n = 6$.
[2] Region = main effect of region; Age = main effect of age; Diet = main effect of dietary treatment.
[3] Total SCFA = acetate + propionate + butyrate.
[4] Total BCFA = valerate + isovalerate + isobutyrate.

thesis, making use of the ammonia produced in the process. Because no significant ($P < 0.05$) interactions among region, age, and diet were observed for the SCFA, BCFA, and ammonia data, only *P*-values for the main effects are reported.

Although total SCFA concentrations did not differ among treatments, differences were observed in certain individual SCFAs (Table 3). There were differences in SCFA concentrations noted among colonic regions, with propionate concentration increasing ($P = 0.06$) from the proximal to the distal regions of the colon. This is likely due to the change in substrate available in the different regions of the colon. SCFA concentrations are highest in the proximal large intestine, likely due to greater carbohydrate availability. The chemical composition and amount of substrate available affect bacterial fermentation reactions, which also are dependent on the types and numbers of colonic bacteria present (40). The higher concentration of propionate in the distal region of the colon may be the result of either decreased propionate absorption from this site or a change in microbial composition, resulting in production of greater concentrations of this particular SCFA.

Both age and diet affected butyrate concentrations, with both senior dogs ($P = 0.02$) and dogs consuming APB ($P = 0.04$) having higher concentrations. Butyrate is the preferred energy source of colonocytes. In fact, 70–90% of butyrate is metabolized to energy during transit through the colonocytes and is available directly for tissue use (2). SCFAs, especially butyrate, play central metabolic roles in maintaining the intestinal mucosal barrier. The main source of SCFAs is dietary fiber and other fermentable carbohydrates such as oligosaccharides and resistant starch. A lack of butyrate production or absorption may be the cause of ulcerative colitis and other inflammatory conditions, due to lack of energy to the intestinal enterocytes. Data suggest that increasing dietary fiber to increase luminal butyrate concentrations may be an appropriate means of ameliorating symptoms of inflammatory bowel diseases (41).

Age did not affect total BCFA concentrations, but geriatric

dogs had increased concentrations of valerate ($P = 0.05$), an end-product of isoleucine catabolism, and of isovalerate ($P < 0.01$), an end-product of leucine catabolism. Potential reasons for differences in individual BCFA concentrations due to age would be changes in colonic microbial composition or changes in host absorptive capacity of amino acids in the small intestine, which would leave a larger amount of substrate entering the colon for microbial degradation. However, after 10 mo of experimental feeding, DM and CP digestibilities did not differ due to age (20), and microbial populations were not quantified.

Isobutyrate, which is derived from the breakdown of valine, was increased ($P = 0.02$) in dogs fed the PPB diet. Isovalerate was increased ($P < 0.01$) in dogs fed the APB diet. The main source of protein in the APB diet was poultry by-product meal, and low concentrations of meat and bone meal (10%) were included in the PPB diet. Previous research showed that the digestibility of amino acids in animal meals such as poultry by-product meal and meat and bone meal varies greatly (42,43). Variation in protein quality and amino acid availability between the APB and PPB diets may account for the differences noted in BCFA concentrations.

In summary, differences in villus height and width were observed due to dietary treatment, but there were no significant ($P < 0.05$) changes in villus area. Because absorption occurs in the upper one third of intestinal epithelial cells, height may affect nutrient absorption more than width. Young dogs tended to have a greater duodenal villus area, which may be indicative of increased absorptive capacity in this region of the small intestine. Dogs consuming the PPB diet exhibited an increase in duodenal villus width, and jejunal and ileal villus height, supporting the idea that dietary factors influence intestinal morphology.

Dietary factors also can affect intestinal metabolic function, with dogs consuming a diet largely composed of animal products and containing higher concentrations of protein having higher concentrations of putrefactive compounds. On the other hand, the dogs consuming the APB diet also had elevated butyrate concentrations, which were shown to play a central role in maintaining the intestinal mucosal barrier.

ACKNOWLEDGMENT

The authors thank the Wenger Manufacturing Company, Sabetha, KS, for diet preparation.

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