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In vitro digestibility of expanded pork skin and rawhide chews, and digestion and metabolic characteristics of expanded pork skin chews in healthy adult dogs¹

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ABSTRACT: Chews are an important part of the pet product industry, with many having potential to decrease plaque or calculus formation. However, their digestion characteristics and gut transit time are virtually unknown. Two experiments were conducted to determine in vitro DM digestibility of expanded pork skin chews and rawhide chews, and apparent total tract digestibility (ATTD), gastrointestinal transit time, and blood metabolite measurements in healthy adult dogs fed a weight-control commercial diet and expanded pork skin chews. In Exp. 1, an in vitro method that simulated gastric and small intestinal digestion was used to determine DM digestibility of expanded pork skin chews and rawhide chews. In Exp. 2, after a 22-d baseline phase, 10 purpose-bred, intact female dogs (5 to 5.5 yr of age; 18.9 to 23.1 kg BW) were fed the diet plus an expanded pork skin chew (~45 g) each day for 22 d. In vitro gastric digestibility of expanded pork skin chews increased with time, with chews being 54.7%, 58.6%, 76.4%, and 86.4% digestible after 6, 12, 18, and 24 h of gastric digestion, respectively. By contrast, gastric digestibility of rawhide chews was 7.6% at 6 h, slowly increased over time, and reached a

maximum of 41.6% at 18 h. In vitro gastric plus small intestinal digestibility results indicated near complete digestibility of expanded pork skin chews at all times, whereas rawhide chews were 50 to 85% digestible. In vivo ATTD of DM, OM, and N were greater ($P < 0.05$) when dogs were fed expanded pork skin chews along with the basal diet, compared with the basal diet alone. However, chew intake did not change transit time measured with a wireless motility device. By contrast, motility index and contraction pattern of the colon were altered ($P < 0.05$) during chew feeding relative to control. Blood urea N concentrations were greater ($P < 0.05$) in dogs fed expanded pork skin chews, compared with baseline; this was not surprising, given the increased N intake and absorption from the chews. Intake of expanded pork skin chews resulted in reduced blood cholesterol concentrations ($P < 0.05$) and tended to decrease blood triglyceride concentrations ($P < 0.10$). Expanded pork skin had a greater DM digestibility than rawhide chews. In addition, expanded pork skin decreased blood cholesterol and triglyceride concentrations, which may justify further research in this area.

Key words: canine, cholesterol, transit time, triglycerides

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INTRODUCTION

Treats are an important contributor to the U.S. pet product industry, with \$2.1 billion in annual sales. Natural chews are promoted to improve oral health in pets because chewing helps reduce plaque (Hennet et al., 2006) and calculus formation (Hennet et al., 2006; Stookey, 2009). Production of beef-derived rawhides and pork skins, which are made from collagenous hy-

podermic interstitial tissue (Bowes et al., 1955), starts with the washing and cleaning of dried skin. The moist skin is then cut to size, pressed, shaped, and dried (typically 65 to 85°C for 24 to 48 h). Rawhide and pork skin chews are made of collagen, but differences in processing (i.e., expansion technology in expanded pork skin chews) may impact digestibility. The effects of rawhide and pork skin chews on gut transit time and nutrient digestibility are largely unknown.

A wireless motility device that is indigestible and measures gastrointestinal pH, pressure, and temperature in real time, and transmits this information to a receiver can be used to calculate transit time. In addition to whole gut transit time (WGTT), the device will provide gastric

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emptying time (**GET**), small bowel transit time (**SBTT**), small and large bowel transit time (**SLBTT**), and colon transit time (**CTT**). Data from such a device are comparable to data from standard measurement methods, such as scintigraphy (Cassilly et al., 2008; Boillat et al., 2010a). This technology has been used to diagnose gut motility disorders (Kuo et al., 2008; Rao et al., 2009) in human nutritional studies (Timm et al., 2011; Willis et al., 2011) and is as repeatable as scintigraphy in dogs (Boillat et al., 2010a).

An *in vitro* experiment was conducted to determine the DM digestibility of expanded pork skin and rawhide chews. A second experiment was conducted to test the effects of expanded pork skin chews on apparent total tract digestibility (**ATTD**), gastrointestinal transit time, and blood metabolite concentrations in healthy adult dogs.

MATERIALS AND METHODS

All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee.

In vitro Digestibility Experiment

In vitro DM digestibility was analyzed using the modified method of Boisen and Eggum (1991), which was developed to be similar to *in vivo* digestibility data of nonruminants. Briefly, 250 mL of phosphate buffer and 100 mL of HCl-pepsin solution was added to containers with whole expanded pork skin and rawhide chews. After addition of 5 mL of chloramphenicol solution, containers were sealed and incubated at 39°C for 6, 12, 18, and 24 h for gastric digestibility. Then, samples were filtered through polyester fabric, rinsed, and dried at 57°C. Small intestinal digestibility was estimated after further addition of a pancreatin-phosphate buffer mixture with incubation at 39°C for 18 h.

Animals and Diets

Ten purpose-bred, intact female healthy dogs (5 to 5.5 y of age; 18.9 to 23.1 kg BW; Butler Farms, Clyde, NY) with hound bloodlines were used in the *in vivo* study. Dogs were individually housed (2.3- × 1.1-m pens) in climate-controlled rooms (Edward R. Madigan Laboratory, University of Illinois, Urbana). Pens allowed for nose-to-nose contact between dogs in adjacent runs and visual contact with all dogs in the room. A 16-h light:8-h dark cycle was used. All dogs were fed a commercially available dry dog food (Iams Weight Control; Procter & Gamble, Cincinnati, OH) to maintain BW throughout the study. The commercial dog food was formulated to meet all nutrient requirements of adult dogs (AAFCO, 2009). Food intake was recorded daily. Fresh water was offered for *ad libitum* consumption.

Experimental Design

After a 22-d baseline phase, during which only the dry commercial diet was fed, diet plus an expanded pork skin chew (45 to 55 g) was fed each day during a 22-d treatment period. On d 8 to 11 of the baseline and treatment periods, total fecal collection was conducted to determine ATTD. A wireless motility device (SmartPill Corp., Buffalo, NY) was used to determine gut motility outcomes during the last 10 d (d 12 to 21; 2 dogs measured each day) of each period. A 24-h food-restricted blood sample was collected on the last day (d 22) of the baseline and treatment phases for serum chemistry measurements.

Fecal and Blood Sample Collections

During the 4-d fecal collection phase, total feces excreted were collected from the bottom of the cage, weighed, scored, and frozen at -20°C until further analyses. The fecal samples were scored according to the following system: 1 = hard, dry pellets, which are small hard mass; 2 = hard, formed and dry stool, which remains firm and soft; 3 = soft, formed and moist stool, which retains shape; 4 = soft, unformed stool, which assumes shape of container; and 5 = watery, liquid, which can be poured. A fresh fecal sample (within 15 min after defecation) was used to determine pH (AP10 pH Meter; Denver Instrument, Bohemia, NY). This instrument was equipped with an electrode (Beckman Instruments, Inc., Fullerton, CA). On blood sampling days, 5 mL of blood was collected for serum metabolite measurements via jugular puncture. Samples were immediately transferred to appropriate Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ), allowed to clot for at least 30 min, and then centrifuged (2,000 × g for 15 min at 4°C) for serum collection. Serum samples were analyzed at the University of Illinois Veterinary Medicine Diagnostics Laboratory, using a clinical chemistry analyzer (Roche-Hitachi 911; Roche Diagnostics, Indianapolis, IN).

Chemical Analyses

Diet and fecal samples were ground through a 2-mm screen in a Wiley Mill (Model 4, Thomas Scientific, Swedesboro, NJ) and then analyzed according to procedures for DM and OM composition (Methods 934.01, 942.05; AOAC, 2006). Total N was analyzed using Leco technology (Method 992.15; AOAC, 2006). Total lipid content (acid hydrolyzed fat) of the diets and feces were determined according to the methods of AACC (1983) and Budde (1952). Gross energy of diet samples was measured using an oxygen bomb calorimeter (Model 1261; Parr Instruments, Moline, IL).

Gut Motility Analysis

Gut motility analysis was conducted, again, using a wireless motility technology (SmartPill Corp.), according to manufacturer instructions. Briefly, the process included: 1) just before use, the capsule (27 × 12 mm) was activated using a strong magnet; 2) the capsule was given orally to dogs after they finished eating their food; 3) once the capsule had entered the stomach (indicated by decrease in pH to <3 on data receiver display), the data receiver was placed on the cage of the dog; 4) pH, temperature, and pressure data were transmitted to the receiver during transit; 5) once the capsule was passed in the feces, the data were transferred from the data receiver to the computer. SmartPill software was used to calculate gut transit and motility characteristics. All gastric emptying and transit times (GET, SBTT, CTT, SLBTT, and WGTT) were calculated on the basis of pH data. The GET is calculated as time between capsule ingestion (indicated by temperature increase) and an abrupt rise in pH from the low pH of the stomach to an alkaline duodenal pH. The SBTT is the time between duodenal and cecum entry. Cecal entry is the time when the first sustained drop in pH of more than 1 unit is noticed. The CTT is the difference between entry into the cecum and body exit (indicated by abrupt temperature decrease). The SLBTT is the sum of SBTT and CTT. The WGTT is the time between wireless motility device ingestion and exit from the body.

Statistical Analysis

Data for continuous variables were analyzed with the MIXED procedure and data for discontinuous variables were analyzed with the GLIMMIX procedure (SAS Inst. Inc., Cary, NC), using dog as an experimental unit. The statistical model included fixed effects of diet. Results were reported as least squares means with $P < 0.05$ defined as significant and $P \geq 0.05$ and < 0.10 as trends.

RESULTS

In vitro Digestibility

In vitro gastric digestibility of expanded pork skin chews was 54.7%, 58.6%, 76.4%, and 86.4%, after 6, 12, 18, and 24 h, respectively (Table 1). By contrast, gastric digestibility for rawhide chews was 7.6% at 6 h and reached a maximum of 41.6% at 18 h. *In vitro* gastric + small intestinal digestion results indicated near complete digestibility of expanded pork skin chews at all times (Table 2). Rawhide chews were only 70% digested after 6 h gastric + 18 h small intestinal digestion. Gastric digestion at 12 and 18 h did not result in further

Table 1. *In vitro* gastric (HCl/pepsin) digestibility results for expanded pork skin and rawhide chews (n = 2 or 3)

Item	Expanded pork skin chews	Rawhide chews
6 h, gastric digestion		
Initial DM weight, g	47.26	69.24
Final DM weight, g	21.55	64.04
DM digestibility, %	54.7	7.6
12 h, gastric digestion		
Initial DM weight, g	48.43	59.88
Final DM weight, g	20.72	48.68
DM digestibility, %	58.6	19.1
18 h, gastric digestion		
Initial DM weight, g	42.01	63.21
Final DM weight, g	9.89	37.62
DM digestibility, %	76.4	41.6
24 h, gastric digestion		
Initial DM weight, g	37.35	67.85
Final DM weight, g	5.10	52.12
DM digestibility, %	86.4	23.1

improvement in rawhide digestibility. At 24 h gastric + 18 h small intestinal digestion of rawhide chews, DM digestibility increased to 85%.

Food Intake, Fecal Output, Fecal pH, and Fecal Score

All dogs remained healthy throughout the experiment. Two dogs refused to eat chews consistently and were removed from the study. Nutrient composition of the control diet and chews is presented in Table 3. Organic matter content was slightly greater and ash content was less in expanded pork skin chews than the basal diet. The nitrogen content was greater (16.1%) in expanded pork skin chews. By contrast, acid hydrolyzed fat content was less in expanded pork skin chews than

Table 2. *In vitro* gastric and small intestinal digestibility results for expanded pork skin and rawhide chews (n = 2 or 3)

Item	Expanded pork skin chews	Rawhide chews
6 h gastric + 18 h small intestinal digestion		
Initial DM weight, g	46.09	53.04
Final DM weight, g	0.43	16.27
DM digestibility, %	99.0	70.1
12 h gastric + 18 h small intestinal digestion		
Initial DM weight, g	45.11	52.68
Final DM weight, g	0.00	22.49
DM digestibility, %	100.0	59.3
18 h gastric + 18 h small intestinal digestion		
Initial DM weight, g	38.60	65.91
Final DM weight, g	0.04	32.99
DM digestibility, %	99.9	52.9
24 h gastric + 18 h small intestinal digestion		
Initial DM weight, g	40.61	63.93
Final DM weight, g	0.03	9.98
DM digestibility, %	99.9	85.9

Table 3. Chemical composition of the commercial weight-control baseline diet and expanded pork skin chews fed to healthy adult dogs

Item	Control diet	Expanded pork skin chews
DM content, %	93.3	91.1
Content, DM basis		
OM, %	93.2	96.8
Ash, %	6.8	3.2
N, %	3.6	16.1
Acid hydrolyzed fat, %	10.6	6.7
GE, kcal/kg	4,419	4,781

the control diet. Expanded pork skin chews had greater GE content than the control diet.

Intake of the control diet alone tended to be greater ($P = 0.089$) during the baseline period (Table 4). However, total food intake (basal diet + chew) did not change between baseline and expanded pork skin chew intake periods. Fecal output (DM basis) tended to be less ($P < 0.07$) for dogs eating expanded pork skin chews, compared with the control diet alone. Fecal pH and fecal scores for dogs during the feeding of expanded pork skin chews were not different from the control diet alone.

Apparent Total Tract Nutrient Digestibility

Apparent total tract DM, OM, and N digestibility were greater ($P < 0.05$) for dogs consuming expanded pork skin chew, compared with the control diet alone (Table 5). Expanded pork skin chew consumption did not affect the apparent digestibility of fat and ash.

Transit Time

Transit time outcomes did not differ for dogs fed expanded pork skin chews, compared with the control diet (Table 6). Similarly, the motility index, contractions per minute, and pH of stomach, antrum, duodenum, and small bowel did not differ between dogs fed expanded pork skin chews and control diet (Table 7). However, the motility index and number of contractions per minute for the colon were less ($P < 0.05$) when dogs were fed expanded pork

Table 5. Apparent total tract digestibility in dogs ($n = 8$) fed a commercial weight-control diet and diet + expanded pork skin chews (%)

Item	Control	Control + expanded pork skin chews	<i>P</i> -value
DM	82.1 ± 0.5	84.4 ± 0.5	0.005
OM	85.4 ± 0.4	87.5 ± 0.4	0.003
N	81.8 ± 0.8	88.6 ± 0.8	<0.001
Fat	88.6 ± 0.4	89.5 ± 0.4	0.151
Ash	37.0 ± 1.9	37.6 ± 1.9	0.811

Table 4. Food intake, fecal output, fecal pH, and fecal scores in dogs ($n = 8$) fed a commercial weight-control diet and diet + expanded pork skin chews

Item	Control	Control + expanded pork skin chews	<i>P</i> -value
Control diet intake (as-fed basis), g/d	287.5 ± 10.7	260.0 ± 10.7	0.089
Control diet intake (DM basis), g/d	268.3 ± 9.9	242.6 ± 9.9	0.089
Chew intake (as-fed basis), g/d	0.0	45.1 ± 3.2	-
Chew intake (DM basis), g/d	0.0	41.0 ± 2.9	-
Total intake (as-fed basis), g/d	287.5 ± 11.0	305.0 ± 11.0	0.279
Total intake (DM basis), g/d	268.3 ± 10.3	283.6 ± 10.3	0.308
Fecal output (as-fed basis), g/d	152.2 ± 8.0	137.7 ± 8.0	0.208
Fecal output (DM basis), g/d	47.9 ± 1.5	43.6 ± 1.5	0.067
Fecal pH	6.4 ± 0.2	6.1 ± 0.2	0.285
Fecal score ¹	2.6 ± 0.1	2.6 ± 0.1	0.605

¹Fecal score based on the following scale: 1 = hard, dry pellets; 2 = dry, well-formed stool; 3 = soft, moist, formed stool; 4 = soft, unformed stool; 5 = watery, liquid that can be poured.

skin chews, compared with control diet alone.

Serum Chemistry

All of the serum metabolite concentrations were within the normal range for adult dogs (Table 8). Blood urea N concentrations were greater ($P < 0.05$) for dogs fed expanded pork skin chews, compared with control diet alone. By contrast, blood cholesterol concentrations were less ($P < 0.05$) for dogs fed chews, as compared with control diet alone. Blood triglyceride concentrations tended to be less ($P = 0.062$) after intake of expanded pork skin chews, compared with control diet alone. No other serum metabolites were affected by expanded pork skin chew consumption.

Table 6. Gastrointestinal transit time for dogs ($n = 8$) fed a commercial weight-control diet and diet + expanded pork skin chews

Item ¹	Control	Control + expanded pork skin chews	<i>P</i> -value
GET, min	1,357 ± 223	1,842 ± 238	0.161
SBTT, min	145 ± 14	131 ± 15	0.489
CTT, min	1,076 ± 117	1,145 ± 138	0.707
SLBTT, min	1,226 ± 127	1,284 ± 150	0.772
WGTT, min	2,610 ± 261	3,130 ± 309	0.228

¹GET = gastric emptying time; SBTT = small bowel transit time; CTT = colon transit time; SLBTT = small and large bowel transit time; and WGTT = whole gut transit time.

Table 7. Gastrointestinal motility index, contractions/minute, and pH for dogs (n = 8) fed a commercial weight-control diet and diet + expanded pork skin chews

Item	Control	Control + expanded pork skin chews	P-value
Motility index ¹			
Stomach	231.8 ± 70.5	251.1 ± 75.4	0.854
Antrum	198.3 ± 104.0	258.9 ± 111.2	0.697
Duodenum	1,505.5 ± 461.3	876.5 ± 493.2	0.369
Small bowel	1,168.8 ± 229.1	682.1 ± 244.9	0.170
Colon	243.9 ± 23.7	148.0 ± 28.0	0.026
Contractions/min			
Stomach	2.1 ± 0.2	1.7 ± 0.2	0.264
Antrum	1.5 ± 0.6	2.5 ± 0.7	0.307
Duodenum	11.2 ± 1.0	11.2 ± 1.1	0.979
Small bowel	14.5 ± 1.0	11.6 ± 1.1	0.070
Colon	1.9 ± 0.2	0.9 ± 0.2	0.006
pH			
Stomach	1.3 ± 0.1	1.4 ± 0.1	0.787
Antrum	1.4 ± 0.2	1.4 ± 0.2	0.925
Duodenum	6.8 ± 0.1	7.0 ± 0.2	0.192
Small bowel	7.7 ± 0.1	7.9 ± 0.1	0.371
Colon	6.2 ± 0.3	5.9 ± 0.3	0.469

¹Calculated by software as sum of pressure amplitude × no. contractions + 1. Indication of gastrointestinal motility defined by movements of the digestive system and transit of contents within it.

DISCUSSION

To our knowledge, this is the first study to report in vitro DM digestibility of whole rawhide vs. expanded pork skin chews. This study indicated a high gastric and small intestinal in vitro DM digestibility of expanded pork skin chews, compared with rawhide chews. Although chews are moistened and chewed by dogs, large pieces may be consumed. Our digestibility results indicated high values (54.7%, 58.6%, 76.4%, and 86.4% after 6, 12, 18, and 24 h of gastric digestion and 99 to 100% after gastric + small intestinal digestion) and, thus, safety of expanded pork skin chews if ingested as large chunks.

In vitro results on expanded pork skin chews were supported in the in vivo study that demonstrated a high digestibility and unaltered gut transit time. The nutrient digestibility of the control diet + expanded pork skin chew was compared with that of the control diet alone. Dry matter, OM, and N digestibilities were greater as a result of expanded pork skin chew consumption, which may be interpreted as greater digestibility of chews. In the current study, a weight-control diet was fed. It is unknown how nutrient digestibility may have been affected by expanded pork skin chew consumption if a diet with greater digestibility had been fed. A previous study in rats indicated that the digestibility of collagen was near 100% (Whitemore et al., 1975). This also might be true in dogs, but collagen digestibility in dogs has not

Table 8. Blood metabolite concentrations for dogs (n = 8) fed a commercial weight-control diet and diet + expanded pork skin chews

Item	Control	Control + expanded pork skin chews	P-value
Creatinine, mg/dL	0.8 ± 0.0	0.8 ± 0.0	0.438
Urea nitrogen, mg/dL	9.8 ± 1.3	17.8 ± 1.3	<0.001
Total protein, g/dL	5.7 ± 0.1	5.9 ± 0.1	0.254
Albumin, g/dL	3.2 ± 0.1	3.2 ± 0.1	0.632
Calcium, mg/dL	9.8 ± 0.1	9.6 ± 0.1	0.085
Phosphorus, mg/dL	3.3 ± 0.1	3.3 ± 0.1	0.885
Sodium, mmol/L	145.9 ± 0.5	144.3 ± 0.5	0.051
Potassium, mmol/L	4.1 ± 0.1	4.3 ± 0.1	0.373
Chloride, mmol/L	113.0 ± 0.6	111.4 ± 0.6	0.088
Glucose, mg/dL	83.4 ± 2.2	84.8 ± 2.2	0.659
Alkaline phosphatase, U/L	51.3 ± 4.8	39.5 ± 4.8	0.105
Corticosteroid-induced alkaline phosphatase, U/L	5.8 ± 2.1	4.6 ± 2.1	0.626
Alanine aminotransferase, U/L	55.1 ± 10.3	49.4 ± 10.3	0.698
Gamma-glutamyl transferase, U/L	4.1 ± 0.3	3.4 ± 0.3	0.109
Total bilirubin, mg/dL	0.2 ± 0.0	0.2 ± 0.0	0.090
Cholesterol, mg/dL	244.8 ± 17.7	175.4 ± 17.7	0.015
Triglycerides, mg/dL	45.8 ± 3.4	35.9 ± 3.4	0.062
Bicarbonate, mmol/L	21.1 ± 0.5	22.4 ± 0.5	0.114

been assessed. Furthermore, there was no effect on fat or ash digestibility, indicating that expanded pork skin chews did not negatively affect ATTD of any nutrients measured. In the future, it would be informative to compare the nutrient digestibility of pork skin vs. rawhide chews to determine whether dogs respond similarly to both products.

Because dog owners sometimes are concerned about the risk of choking or gastrointestinal blockage when chews are fed, gut transit time data for dogs fed expanded pork skin chews was collected and compared with data obtained with dogs fed the control diet alone. This is the first study to use wireless motility technology in a canine nutritional intervention study. In the current study, there was a numerical increase in GET when dogs were fed expanded pork skin chews and the control diet, as compared with feeding the control diet alone, but this was due to high variability among dogs and data were not statistically different. Increased GET may not be surprising because the meal must be broken down to small particles in the stomach before passing into the duodenum. We observed greater GET values than those reported in the literature (22 to 30 h in the current study vs. 6.5 to 15.0 h observed in Boillat et al., 2010a,b), even with dogs of similar BW and use of the same technology. Gastric emptying may be controlled and affected by many factors, including diet type (e.g., extruded vs. canned), viscosity, or macronutrient composition (e.g., fat, protein, or carbohydrate), which may have contributed to differences among studies (Ehrlein

and Prove, 1982; Meyer et al., 1985; Clegg and Shafat, 2010). In contrast to the current study, in which dogs were fed 100% of their daily meal before dosing with the wireless motility device, dogs in those studies were fed only 25% and 30% (Boillat et al., 2010a,b) of their daily food intake, which likely contributed to the differences. Because none of the mean gastrointestinal transit times measured in expanded pork skin chews-fed dogs were altered, compared with the control, our data indicate that addition of pork chews did not adversely affect gastrointestinal motility. The large size of the wireless motility pill means that it is retained in the stomach until the end of the meal. Even though this may not allow an estimation of initial emptying, it should serve as a valid method of estimating when the meal was completely moved from the stomach. Because some dogs should not be fed highly digestible, high protein-containing foods (e.g., renal disease patients), feeding must be considered on a case-by-case basis. The likelihood of choking was not tested in this study.

Serum chemistry measurements were also performed to assess the safety of expanded pork skin chew consumption. Blood urea nitrogen was greater during expanded pork skin chew consumption, but still within the reference range (<30 mg/dL), a response that was likely due to the intake of a highly digestible N source in the form of a chew. In addition, normal creatinine concentrations indicated that expanded pork skin chew consumption for 22 d did not affect kidney function. An interesting observation of this study was the lesser serum cholesterol and trend toward lesser serum triglyceride concentrations after expanded pork skin chew consumption. In humans, high protein diets have been promoted to increase BW loss and improve blood triglyceride concentrations in overweight and obese individuals (Papakonstantinou et al., 2010; Skov et al., 1999). In the current study, we observed decreased cholesterol and a tendency for decreased triglycerides in healthy, lean adult dogs. These decreases may have been due to altered hepatic lipid metabolism or increased energy expenditure from increased protein intake, because ATP is required for the initial steps of metabolism, storage, and oxidation, including urea synthesis. In comparison to the diet alone, which provided approximately 22%, 25%, and 53% of ME from protein, fat, and digestible carbohydrates, respectively, the diet + pork skin chews provided approximately 31%, 23.5%, and 45.5% of ME from protein, fat, and digestible carbohydrates, respectively. More research is necessary to identify mechanisms by which these effects may have occurred. Although our dogs had normal blood triglyceride and cholesterol concentrations, and none were obese, this interesting observation needs further research in overweight or obese dogs. The ability to positively affect blood triglyceride and chole-

sterol concentrations may be another benefit to expanded pork skin chew consumption.

In conclusion, our *in vitro* results indicated a high DM digestibility of expanded pork skin chews, compared with rawhide chews. Our *in vivo* experiment confirmed the high DM digestibility of expanded pork skin chews and demonstrated no differences in gastrointestinal transit outcomes. The decreased cholesterol and trend for decreased blood triglyceride concentrations observed with expanded pork skin chew consumption highlights another possible benefit and justifies further research in this area. The feeding of expanded pork skin chews seemed to be safe because none of the other serum chemistry metabolites, fecal characteristics, and general behavior of dogs was altered during expanded pork skin chew feeding.

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