Serum lipid profiles, total tract nutrient digestibility, and gastrointestinal tolerance by dogs of α-cyclodextrin

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ABSTRACT: The objectives were to quantify gastrointestinal tolerance, total tract nutrient digestibility, and serum lipid profiles of dogs as affected by α-cyclodextrin (ACD) supplementation and to validate the accuracy of fat analyses techniques using novel ACD–fat complexes. The ACD was hydrolyzed and free sugars and hydrolyzed monosaccharides were quantified using high performance liquid chromatography. Known amount of fats were complexed with ACD, and fat content of complexes were determined using the ether extraction and acid-hydrolyzed fat methods. Nine mixed-breed hounds were used in a crossover design with 3 periods of 10 d each, including 6 d for diet adaptation and 4 d for fecal collection. Dogs were fed twice daily a diet with poultry byproduct meal and brewer’s rice as the main ingredients, and chromic oxide (0.2%) was included as a digestion marker. Dogs were supplemented with either 0, 3, or 6 g of ACD diluted in 15 mL of water twice per day for a total of 0, 6, and 12 g ACD per day. The ACD had a very low free sugar concentration and, once hydrolyzed, released only glucose, as expected. Average daily food intake, fecal output (DM basis), and fecal scores were not significantly different among treatments. Body weight and condition score and serum triglycerides and cholesterol concentrations remained unaltered throughout the duration of the experiment. Dry matter, OM, and fat digestibility coefficients were lower (P < 0.05) for both treatment groups compared to the control. The acid-hydrolyzed fat method was valid to measure fat that was bound to ACD. Intake of ACD lowered fat digestibility somewhat but not to the extent previously reported, without affecting serum lipid concentrations or outcomes related to tolerance. Therefore, ACD supplementation resulted in a small decrease in fat digestibility, but ACD supplementation might have potential in modifying serum lipid profiles.

Key words: canine, cholesterol, dietary fiber, nutrient digestibility, tolerance


INTRODUCTION

It has been estimated that approximately 53% of the canine population of the United States is either overweight or obese, resulting in 36 million dogs with increased risk for weight-related disorders (Calabash, 2012). The increased incidence of obesity has led to a search for safe and practical ways to either lose or maintain BW, which includes exercise, dietary energy intake restriction, and nutritional supplements.

Alpha-cyclodextrin (ACD) is a dietary fiber with the unique ability to bind 9 times its own weight in fat (Artiss et al., 2006). Alpha-cyclodextrin is a nonreducing cyclic oligosaccharide comprising 6 glucopyranose units linked by α-1,4 bonds, with hydrophilic sites on the exterior and hydrophobic sites on the interior of its molecule (Szejtli, 1998). The structure of ACD makes it both soluble in water and capable of including other apolar molecules (Szejtli, 2004).

It has been hypothesized that ACD is able to form a very stable complex with dietary fat that is not absorbed in the gastrointestinal tract (Artiss et al., 2006). Animal feeding studies failed to show an increase in fecal fat, hypothesizing that conventional methods of fat extraction might be unable to extract the fat bound to the ACD (Gallaher et al., 2007). Because ACD is not digested and absorbed in the small intestine, with
up to 98% reaching the large bowel (Andersen et al., 1963), gastrointestinal tolerance might be a concern. The latter is often related to increased osmotic pressure and gas production, loose stools, and nausea.

Further study of the effect of ACD supplementation and validation of current fat analysis techniques will help to determine the effectiveness of ACD in blocking fat absorption. The objectives of this study were to measure chemical composition of ACD as well as its effect on gastrointestinal tolerance, total tract nutrient digestibility, and serum lipid profiles of dogs and to validate fat analysis techniques using novel ACD–fat complexes as substrates.

MATERIALS AND METHODS

Test Substrate

Commercial food-grade ACD (purity 99.4%) manufactured by Wacker Fine Chemicals, Adrian, MI (batch number 60F212), was obtained from Abbott Nutrition, Columbus, OH.

Compositional Analyses and Validation of Fat Analysis Techniques

Alpha-cyclodextrin was analyzed for DM and OM according to Horwitz (2002) methods 934.01 and 942.05, respectively. Acid-hydrolyzed fat (AHF) concentrations were determined using acid hydrolysis according to American Association of Cereal Chemists (2000) method 30-14.01 followed by ether extraction (Budde, 1952).

The ACD was hydrolyzed using the procedure of Hoebler et al. (1989). Hydrolyzed monosaccharides and free sugars were quantified using a Dionex DX500 HPLC system (Dionex Corporation, Sunnyvale, CA.) Standards for quantification included glucose, inositol, fucose, arabinose, rhamnose, galactose, xylose, and mannose. Free monosaccharides were injected at a volume of 25 μL. All assays were conducted using a CarboPac PA-1 column and guard column (Dionex Corporation, Sunnyvale, CA) following methods cited by Smiricky et al. (2002).

Tubes with ACD solution were prepared using 1 g of ACD and 6 mL of water in each tube. Tubes were vortexed for 10 sec. Graded amounts of corn oil and lard (2, 4, 6, 8, 10, and 12 g) were placed in quadruplicate tubes containing the ACD solution and vortexed for 10 s. After freeze-drying, fat was extracted from a duplicate set of tubes containing each concentration of corn oil or lard using the AHF method previously described, whereas the other duplicate set was extracted using the ether extraction method alone, as previously described.

Tolerance and Total Tract Nutrient Digestibility

Animals. Nine mixed-breed healthy adult hounds with an average age of 2.7 yr and an average starting BW of 21.3 kg (SD 2.4) were used in this experiment. The University of Illinois Institutional Animal Care and Use Committee approved all animal care procedures before initiation of the experiment. Dogs were individually housed in indoor pens (approximately 1.2 by 1.5 m) in an environmentally controlled facility with a 12:12 h light:dark cycle. Dogs were weighed and BCS was assessed using a 9-point scale (Laflamme, 1997) throughout the experiment.

Diets and Treatments. One experimental diet was formulated to meet or exceed the NRC (2006) nutrient profiles for adult dogs at maintenance. The diet consisted of poultry byproduct meal and brewer’s rice as the main ingredients and Solka-Floc (International Fiber Corporation, North Tonawanda, NY) as the fiber source. Chromic oxide was included as a digestion marker at 0.2% of the diet (Table 1). The diet was prepared in extruded, dry kibble form at Kansas State University Department of Grain Science and Industry (Manhattan, KS) under the supervision of Pet Food & Ingredient Technology, Inc. (Topeka, KS).

Dogs were fed 150 g of food twice per day for a total of 300 g of food per day. Food refusals from the previous feeding were collected and weighed. Dogs had ad libitum access to fresh water. Dogs were supplemented immediately after feeding with either 0, 3, or 6 g of ACD diluted in 15 mL of water twice per day for a total of 0, 6 (daily amount of ACD recommended by manufacturers), and 12 g (200% recommended amount) of ACD per day. Dogs were orally dosed with the ACD solution using a 60-mL syringe (without needle).

Experimental Design. The experimental design was a crossover design with 3 treatments and 3 periods. Each period consisted of 2 phases: 6 d for diet adaptation and 4 d for tolerance evaluation and fecal collection. Dogs were weighed, BCS was assessed, and blood was collected at the beginning and at the end of each period after a 12-h fast.

Sampling Procedures. A food sample of approximately 500 g was taken from each bag of diet used in this experiment. Samples were composited, and a 500-g subsample was removed, ground in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) through a 2-mm screen, and stored at 4°C until analysis.

During the 4-d collection phase, all voided feces were collected from the floor of the pen and weighed. Fecal samples were collected 3 times per day when available (0700, 1300, and 1900 h). Feces were scored using a 5-point scale, by only 1 researcher, with 1 being dry, hard pellets; 2 being dry, well-formed stool; 3 being soft, moist, formed stool; 4 being unformed stool; and
Tolerance by dogs of α-cyclodextrin

5 being watery liquid that can be poured. Feces were stored at –20°C until composited, dried in a forced-air oven at 55°C, and ground in a Wiley mill (model 4; Thomas Scientific) through a 2-mm screen for analysis.

Tolerance to ACD supplementation was assessed based on food intake data, fecal scores, and observance of emesis, retching, and/or nonproductive emesis.

Before d 1 and after d 10 of each period, 5 mL of blood were collected via jugular venipuncture. At 1900 h on the evening before each blood sampling, any remaining food was removed, and dogs were fasted overnight (12 h), during which time they consumed only water. Because periods were consecutive, blood sample metabolite concentrations for the end of one period also were used as representative values for the start of the next period. Blood was drawn into vacutainer serum separator tubes before feeding the dogs. Tubes were kept at room temperature for 30 min and centrifuged at 1,240 × g at 4°C for 10 min. Serum supernatant was collected and stored at −20°C for analyses.

### Chemical Analysis

Ground fecal and diet samples were analyzed for DM, OM, and AHF following procedures previously described. Crude protein concentrations were calculated using LECO (nitrogen analyzer model FP-2000; LECO Corporation, St. Joseph, MI) N values (N × 6.25; Horwitz, 2002). Total dietary fiber concentration of the diet was measured according to Prosky et al. (1985). Gross energy concentrations of ACD and diet were measured using oxygen bomb calorimeter (model 1261; Parr Instruments, Moline, IL). Food and fecal samples were prepared for chromium analysis according to the method of Williams et al. (1962), and chromium concentrations were measured using an atomic absorption spectrophotometer (model 3100; PerkinElmer, Waltham, MA).

Serum total cholesterol (TC) and triglyceride (TG) concentrations were measured on a Hitachi 917 analyzer (Roche Diagnostica, Indianapolis, IN) using enzymatic kits (catalog numbers 2016630 and 2016648, respectively; Roche Diagnostics). Concentrations of serum TC and TG correspond to samples of d 10 for each period. Changes in serum TC and TG concentrations were calculated by the difference in serum concentrations on the last day of the period minus serum concentrations on the first day of the period.

### Calculations

Apparent total tract DM digestibilities were calculated as 100 – [100 × marker concentration in the feed (%)/marker concentration in the feces (%)]. Apparent total tract nutrient digestibilities were calculated as 100 – 100 [marker concentration in the feed (%) × nutrient concentration in feces (%)]/[marker concentration in feces (%) × nutrient concentration in the feed (%)].

### Statistical Analyses

Data were analyzed as a crossover design using the Mixed Models procedure of SAS/STAT software, version 9.2 for Windows (SAS Inst. Inc., Cary, NC). The statistical model included the fixed effect of dietary treatment and the random effects of period and dog. Normal distribution of residuals and homogeneity of variances were tested and assumptions for ANOVA were fulfilled. Treatment least squares means are reported and were compared using a Bonferroni adjustment to ensure the overall protection level. Standard error of the mean values are associated with least squares means as calculated in the Mixed Models procedure. Differences among means with a $P < 0.05$ were considered significant, and $P < 0.10$ were considered trends.

### Table 1. Ingredient (% as-fed basis) and chemical (% DM basis) composition of the experimental diet

<table>
<thead>
<tr>
<th>Item</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-ash poultry byproduct meal</td>
<td>39.00</td>
</tr>
<tr>
<td>Poultry fat</td>
<td>14.00</td>
</tr>
<tr>
<td>Ground yellow corn</td>
<td>10.00</td>
</tr>
<tr>
<td>Brewer’s rice</td>
<td>28.85</td>
</tr>
<tr>
<td>Solka-Floc1</td>
<td>6.50</td>
</tr>
<tr>
<td>Mineral premix2</td>
<td>0.10</td>
</tr>
<tr>
<td>Vitamin premix3</td>
<td>0.10</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.10</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.65</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.50</td>
</tr>
<tr>
<td>Chrome oxide</td>
<td>0.20</td>
</tr>
<tr>
<td>Analyzed chemical composition</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>92.60</td>
</tr>
<tr>
<td>OM</td>
<td>91.70</td>
</tr>
<tr>
<td>CP</td>
<td>30.60</td>
</tr>
<tr>
<td>Acid-hydrolyzed fat</td>
<td>20.16</td>
</tr>
<tr>
<td>Total dietary fiber</td>
<td>7.34</td>
</tr>
<tr>
<td>GE, kcal/kg</td>
<td>5,215.01</td>
</tr>
</tbody>
</table>

1International Fiber Corporation, North Tonawanda, NY.

2Provided per kilogram of diet: 120 mg iron (FeSO4), 66 mg manganese (MnO), 18 mg copper (CuSO4), 1.8 mg iodine (C2H6N2·2HI), 240 μg selenium (Na2SeO3), and 240 mg zinc (ZnO).

3Provided per kilogram of diet: 10,560 IU vitamin A (vitamin A acetate), 1,056 IU vitamin D (vitamin D3), 105 IU vitamin E (α-tocopherol), 0.53 mg vitamin K (menadione sodium bisulfate complex), 2.64 mg thiamine (thiamine mononitrate), 23.76 mg niacin (niacin supplement), 3.43 mg riboflavin (riboflavin supplement), 13.2 mg pantothenic acid (d-calcium pantothenate), 66 μg vitamin B12 (vitamin B12 supplement), 2.11 mg pyridoxine (pyridoxine hydrochloride), 79 μg biotin (D-biotin supplement), and 264 μg folic acid (folic acid supplement).
RESULTS

Compositional Analyses and Validation of Fat Analyses Techniques

Percentage DM and OM were 98.2 and 100%, respectively, for ACD (Table 2). Mannose was the only free sugar detectable in the ACD (55.7 μg/g), whereas the only monosaccharide released after hydrolysis was glucose (1,217 mg/g; Table 2). An average of 99.9% of the fat in the tubes was recovered using the AHF method, whereas an average of 95.5% of the fat was recovered using the ether extract method (data not shown).

Tolerance and Total Tract Nutrient Digestibility

Some dogs experienced emesis and nonproductive emesis throughout the experiment. However, this was not consistent for a specific treatment or individual, with some dogs in the control treatment experiencing these signs as well. In some cases, dogs that saw other dogs being dosed experienced nonproductive emesis before receiving the ACD supplementation.

Body weight and BCS were not different among treatments (data not shown). Average daily food intake values were similar (P > 0.05) among treatments throughout the study, with most of the dogs ingesting all the food they were provided (Table 3). Apparent DM digestibility was greater for the control treatment (P < 0.05) than the ACD treatments. Apparent OM and AHF digestibility coefficients followed the same trend as DM.

Fecal output expressed on a DM basis was similar among treatments (Table 3). However, fecal output expressed on an as-in basis for the 12 g ACD treatment was greater (P < 0.05) than for the control treatment and not different from the 6 g ACD treatment (P > 0.05), which was also not different from the control treatment. Fecal output (as-is basis) per gram DM consumed was greater (P < 0.05) for both ACD treatments compared to the control. Conversely, fecal DM concentration for dogs fed the ACD treatments was lower (P < 0.05) than for the control treatment. Despite the fact that both ACD treatments generated feces with a greater amount of water, fecal scores for dogs fed the control treatment did not differ (P > 0.05) from the 6 g ACD treatment and only tended to be different (P = 0.07) from those on the 12 g ACD treatment.

Serum TC and TG concentrations were not different among treatments (Table 4). However, there was a numerical decrease in serum TC concentration in 3 dogs when they were fed both ACD treatments. These 3 dogs had an initial concentration of serum TC that was greater than the reference values (2.8–8.2 mmol TC/dL serum) and had a numerical reduction of 1.89, 2.76, and 6.08 mmol TC/dL serum, respectively, after receiving the ACD treatments. After receiving the control treatment, 2 of the 3 dogs presented an increase in serum TC concentrations (3.0 and 2.17 mmol TC/dL serum), returning to the values similar to those measured at the start of the experiment.

DISCUSSION

The high DM and OM concentration is a result of the purification and drying processes used in the manufacture of ACD. These values coincide with the information in the inspection certificate of the manufacturer (Wacker Fine Chemicals, Adrian, MI). The presence of a low amount of mannose might be indicative of bacterial contamination of the sample or contamination of the enzyme used in the synthesis of ACD, which is of bacterial origin (Biwer et al., 2002). The release of only glucose
after acid hydrolysis was expected as ACD is composed of 6 glucose molecules exclusively (Del Valle, 2004). The release of more than 1,000 mg glucose/g ACD can be attributed, in part, to the fact that during hydrolysis, water is used to break polysaccharide chains into smaller chains or into simple carbohydrates. The water added to the glucose molecules during ACD hydrolysis accounts for approximately 111 mg additional ACD/g, whereas the remaining difference can be attributed to analytical error due to the multiple steps this procedure involves (Hoebler et al., 1989).

The use of ether extraction alone was insufficient to extract all the fat present in the tubes. It has been reported that ACD can form complexes with fats that are bound to the hydrophobic cavity of ACD (Szejtli, 2004). This complex formation is so strong that traditional extraction methods are not able to recover all the complexed fat (Artiss et al., 2006; Gallaher et al., 2007). The use of acid hydrolysis before the ether extraction process hydrolyzes the ACD, freeing the fat, and therefore, practically all the fat in the tubes was recovered when the AHF procedure was used. Consequently, the AHF method is valid for measuring fat bound to ACD.

The only sign of intolerance observed were emesis and nonproductive emesis by some of the dogs. Intake of ACD has been previously reported to cause emesis. Spears et al. (2005b) evaluated meal tolerance of ACD, β-cyclodextrin (BCD), and γ-cyclodextrin (GCD). Dogs were offered 25 g of cyclodextrin in approximately 240 mL distilled, deionized water using a 60-mL syringe without needle. Dogs regurgitated within 30 to 60 min of cyclodextrin consumption, with ACD being regurgitated more quickly than BCD and GCD. On the other hand, the same authors reported feeding 63.0 g of GCD in an enteral diet without regurgitation or diarrhea. Other studies involving intake of larger amounts of ACD by dogs (up to 110 g/d) and rats for 13 wk demonstrated tolerance, with transient diarrhea as the only adverse effect (Lina and Bar, 2004a,b). However, in those experiments with no tolerance problems, cyclodextrins were included in a diet matrix, so perhaps this is the reason for better tolerance of ACD by dogs. In our study, as in the cited study, ACD was provided as a solution in water using a syringe. The physical effect of putting the syringe in the mouth of the dogs or the taste of the solution might have provoked the emesis. Spears et al. (2005b) also hypothesized that the high molarity of the solution may have affected electrolyte balance or water binding, resulting in regurgitation.

Intake of ACD has been reported to result in a decrease in BW gain of rats (Artiss et al., 2006; Kishino et al., 2009) and humans (Grunberger et al., 2007), which was attributed to a decreased fat absorption. In these studies, authors observed a decrease in BW gain and not a BW decrease. On the contrary, Comerford et al. (2011), in a double-blind crossover study with 28 healthy, overweight humans, reported a decrease of 0.41 kg BW when 6 g of ACD were supplemented to their regular diets for 30 d.

Body weight and BCS of the dogs in our study remained unaltered during the duration of the experiment. The reason for this difference might be attributed to the fact that the dogs were fed an amount of food calculated to meet their energy requirements and maintain their BW, whereas rats and humans were fed ad libitum. The lack of BW loss in our study also might be attributed to the shorter period of ACD supplementation.

Apparent DM, OM, and AHF digestibility coefficients were high and comparable to previously reported digestibility coefficients for diets with similar ingredient matrices (Middlebos et al., 2007; Faber et al., 2011). Acid-hydrolyzed fat digestibility was reduced in a linear fashion and might be due to ACD intake. Other studies have reported lower apparent fat digestibility due to ACD intake by rats (Gallaher et al., 2007) and GCD intake by dogs (Spears et al., 2005a). It has been hypothesized that ACD and fats form a complex in the stomach that remains bound through the gastrointestinal tract, preventing absorption in the small intestine, and therefore, fat digestibility is decreased in a ratio of 9 g of fat per gram of ACD (Artiss et al., 2006; Grunberger et al., 2007). However, in these studies, authors failed to detect differences in fat excretion and calculated this ratio based on the difference in BW gain among treatments. Intake of ACD by the dogs decreased fat digestibility by approximately 1 %, which represents a reduction of absorption of approximately 0.5 g fat for a mean ACD intake of 9 g/(dog·d). Therefore, this effect appears to be not as strong as previously reported.

Fecal DM and fecal output expressed on an as-is basis were affected by ACD intake; ACD is not degradable by hydrolytic–enzymatic digestion but is partially fermentable in the large intestine (Del Valle, 2004). Fermentation of ACD produces short-chain fatty acids.

### Table 4. Serum cholesterol and triglyceride concentrations and changes in concentration for dogs supplemented with α-cyclodextrin (ACD)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>6 g ACD</th>
<th>12 g ACD</th>
<th>SEM²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cholesterol, mmol/dL</td>
<td>6.65</td>
<td>6.26</td>
<td>6.57</td>
<td>1.09</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/dL</td>
<td>0.54</td>
<td>0.47</td>
<td>0.56</td>
<td>0.07</td>
</tr>
<tr>
<td>Δ Serum cholesterol, mmol/dL</td>
<td>0.43</td>
<td>-0.74</td>
<td>-0.46</td>
<td>0.54</td>
</tr>
<tr>
<td>Δ Serum triglycerides, mmol/dL</td>
<td>-0.01</td>
<td>-0.04</td>
<td>0.02</td>
<td>0.07</td>
</tr>
</tbody>
</table>

¹Concentrations were measured on d –10 of each period. Change was measured between d –1 and 10 of each period.

²Pooled SEM.
that influence wet fecal output (Sunvold et al., 1995). Even though there were differences in fecal DM content, fecal scores were not different. This is not unexpected, as several studies have reported a lack of correlation between fecal DM and fecal score (Grieshop et al., 2002; Gajda et al., 2005; Spears et al., 2005a).

Due to the amphiphilic nature of cyclodextrins, they have been used to bind cholesterol in a variety of substrates such as milk, butter, sperm, and experimental diets (Choi and Toyoda, 1998; Somogyi et al., 2006; Alonso et al., 2009). More specifically, the hypocholesterolemic effect of ACD in different species has been demonstrated (Artiss et al., 2006; Grunberger et al., 2007; Wagner et al., 2007, 2008; Comerford et al., 2011). In this study, 3 dogs were hypercholesterolemic at the beginning of the experiment. This situation was not planned and the experiment was not designed with that information in mind. It was evident that the numerical trend for decreased fasting serum TC concentrations occurred when the hypercholesterolemic dogs received ACD supplementation. However, the remaining 6 dogs that had normal fasting serum TC concentrations throughout the experiment that were not affected by ACD intake. Therefore, the reduction in fasting serum TC failed to reach statistical significance. Differences in fasting serum TC concentration responses also can be attributed to unique features of cholesterol metabolism in the dog. Cholesterol ester transfer protein (CETP) facilitates the transfer of cholesterol in exchange for TG from high-density lipoprotein (HDL) to low-density lipoprotein and very-low-density lipoprotein. The dog lacks CETP activity, which results in high concentrations of HDL cholesterol, facilitating the redirection of cholesterol to the liver for clearance (Bailhache et al., 2004). This lack of CETP activity makes the dog very efficient in metabolizing cholesterol. Furthermore, from the data gathered in this study together with previous findings reported in the literature using humans and rodent models, it appears that beneficial effects of ACD consumption are most effective in hyperlipidemic models, which may further explain the lack of response to ACD supplementation of the healthy adult dog model used in this study.

In conclusion, although ether extraction alone is not a valid method to measure fat content in ACD–fat complexes, the AHF procedure is valid for measuring fat bound to ACD. Intake of ACD appears to be well tolerated by dogs, resulting in approximately 1% unit decrease in DM, OM, and AHF total tract apparent digestibilities. The effect of reduction in fat digestibility by ACD intake appears to be not as strong as previously reported. Fecal characteristics of dogs consuming ACD were not drastically affected, with only a slight, but significant, increase in water content and a trend for higher fecal scores. Intake of ACD numerically decreased serum TC concentrations in hypercholesterolemic dogs but failed to reduce serum TC concentrations in normcholesterolemic dogs, probably due to a self-limiting mechanism that prevents cholesterol concentration from dropping too low. Supplementation with ACD might have potential in reducing serum TC concentrations, but this effect warrants further investigation.

**LITERATURE CITED**


