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Evaluation of dried apple pomace on digestibility and palatability of diets for cats

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ABSTRACT - The objective of this study was to evaluate increasing levels of dried apple pomace on cat diets and its effects on the apparent total tract digestibility (ATTD) of nutrients and diet metabolizable energy (ME) and palatability. Fecal characteristics of cats were also evaluated. Four experimental diets were produced to contain 0, 30, 60, and 90 g kg^{-1} of dried apple pomace. Two experiments were carried out. In experiment 1, the diets were offered to twelve adult cats distributed in a completely randomized block design (n = 6). Two evaluation periods (blocks) of 11 days were used to analyze diet digestibility and fecal characteristics. In experiment 2, the palatability of diets containing 0 (control) vs. 90 g kg^{-1} of dried apple pomace was compared using 20 adult cats on two consecutive days (n = 40). Increasing dietary dried apple pomace levels (0-90 g kg^{-1}) linearly reduced the ATTD of crude protein (834.0-798.0 g kg^{-1} of dry matter) and ME (4290.1-4161.0 kcal). There was a quadratic effect of the increasing dried apple pomace levels on the ATTD of the other nutrients, as well as on fecal dry matter (fDM) content and output. The dietary inclusion of 90 g kg^{-1} of dried apple pomace promoted a greater intake ratio (0.61) in relation to the control diet. The inclusion of up to 90 g kg^{-1} of dried apple pomace reduces diet digestibility, ME, and fDM of cats. However, it improves diet palatability. These results indicate that the dried apple pomace is a palatable fibrous component and its inclusion can be used to reduce the energy content of cat foods.

Keywords: byproducts, cats, cellulose, fiber, fruits, intestinal health

1. Introduction

Although cats are considered obligate carnivores, there are reports of the benefits of dietary fiber inclusion in diets for these animals. The benefits include hairball control, maintenance of intestinal peristalsis, good functionality of the immunological system, and modulation of intestinal microbiota. Dieyary fiber may also regulate glycemia, reducing the incidence of diseases, such as obesity and diabetes mellitus (Rochus et al., 2014; Loureiro et al., 2017; Detweiler et al., 2019; Donadelli and Aldrich et al., 2020). Therefore, the physiological importance of fiber for cats is documented, attracting the interest of the pet food industry about dietary fiber sources.

Beet pulp and cellulose are typical fiber sources included in dog and cat foods. However, considering the increasing limitation of food resources, animal feeds should include fiber sources that are not used for human consumption. Byproducts from fruit processing for human consumption are highly available and may potentially be used as affordable and sustainable raw materials for pet foods, thereby contributing to the reduction of economic and environmental problems.

According to Swanson et al. (2000), fruit byproducts may potentially be used as fiber sources in dog and cat foods. Dried apple pomace is a residue of the dehydration process of fresh apples

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deemed unsuitable for human consumption. It contains bioactive compounds, such as flavonoids and carotenoids, which present several biological effects, including antioxidant, antimicrobial, and anti-inflammatory features (Ülger et al., 2018). Besides, it presents a good balance between insoluble and soluble fibers (around 2:1), promoting gastrointestinal health (Kröger et al., 2017). However, the different inclusion levels of apple pomace in pet foods need to be evaluated, mainly regarding its effects on fecal characteristics and diet digestibility and palatability.

Although studies evaluating the fermentative and nutritional characteristics of conventional fiber sources included in cat foods have been published in the literature (Sunvold et al., 1995a,b,c), only one work about the effects of dietary apple fiber inclusion for cats was found (Fekete et al., 2004). This demonstrates the importance of more studies. In this context, the objective of the present study was to evaluate the effects of increasing inclusion levels of dried apple pomace on apparent total tract digestibility (ATTD) of nutrients, metabolizable energy (ME) content, and palatability of diets, as well as on the fecal characteristics of cats.

2. Material and Methods

The experimental procedures were approved by the institutional Committee of Ethics on Animal Use (case no. 025/2015). The studies took place in Maringá, Paraná, Brazil (23°25'38" S, 51°56'15" W, average elevation of 551 m).

The research was divided into two experiments, being digestibility and fecal characteristics assay and palatability trial. All animals used in the study were previously subjected to clinical and physical examination, vaccinated, and de-wormed.

Four diets with increasing inclusion levels of dried apple pomace were evaluated: 0, 30, 60, and 90 g kg^{-1}. Dried apple pomace was included in the diets at the expense of cellulose and corn, to achieve similar total dietary fiber (TDF) levels in all diets. The diets (Table 1) were formulated to supply the nutritional maintenance requirements for cats, according to the FEDIAF (2016) recommendations. The dried apple pomace (Table 2) was obtained by grinding and drying whole apples (Fuji cultivar) deemed unsuitable for human consumption. After the ingredients were mixed, diets were ground into 1.0-mm particle size and extruded in a single-screw extruder, with a capacity of 4,000 kg/h (Ferraz, E-130; Ribeirão Preto, Brazil).

In experiment 1, we used twelve castrated adult (2.5±0.1 years old) cats (six males and six females) of undefined breed, with a mean body weight of 4.00±1.0 kg. During the digestibility assay, animals were housed in individual stainless-steel metabolic cages (0.9 × 0.8 × 0.9 m).

The animals were distributed according to a completely randomized block design, with two evaluation periods (blocks) and 12 animals per period. Four treatments, with six replicates per treatment (three cats were fed each diet in each period) were evaluated.

The experiment lasted 22 days, comprising two periods of 11 days each. Each period contained five days of adaptation followed by six days of total fecal collection. Cats were fed twice a day (between 08:00 and 10:00h and between 16:00 and 18:00 h) in sufficient amount to supply their metabolizable energy requirements (MER), calculated as MER (kcal/day) = 100 × body weight$^{0.67}$, according to the NRC (2006). Water was offered *ad libitum*.

Feces were collected at least twice daily, weighed, identified per period and per cat, and stored in a freezer at −14 ℃ in individual recipients. By the end of the collection periods, feces were thawed, homogenized, and dried in a forced-ventilation oven (320-SE, Fanem, São Paulo, Brazil) at 55 ℃ for 72 h, when constant weight was achieved.

Dry feces and diets were ground to 1-mm particle size in a hammer mill (Arthur H. Thomas Co., Philadelphia, PA) and analyzed for DM at 105 ℃, crude protein (CP, method 954.01), crude fiber (CF, method 962.10), acid hydrolyzed ether extract (AHEE, method 954.02), and ash (Ash, method 942.05) contents, according to the Association of Official Analytical Chemists (AOAC, 1995).

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Table 1 - Ingredients and analyzed chemical composition of diets containing increasing levels of dried apple pomace

Item	Level of dried apple pomace (g kg^{-1})			
	0	30	60	90
Ingredient (g kg^{-1} as fed)				
Corn	385	363	341	318
Poultry fat	100	100	100	100
Corn gluten 60	170	170	170	170
Poultry offal meal	260	260	260	260
Salt	5.0	5.0	5.0	5.0
Taurine	1.0	1.0	1.0	1.0
Liquid poultry offal hydrolysate	3.0	3.0	3.0	3.0
Powdered poultry offal hydrolysate	1.0	1.0	1.0	1.0
BHA	0.075	0.075	0.075	0.075
BHT	0.15	0.15	0.15	0.15
Citric acid	0.3	0.3	0.3	0.3
Calcium propionate	2.0	2.0	2.0	2.0
Choline chloride	4.0	4.0	4.0	4.0
Mineral-vitamin supplement[1]	3.0	3.0	3.0	3.0
Potassium chloride	5.4	5.4	5.4	5.4
Dried apple pomace	0.0	30	60	90
Cellulose	23.0	15.3	7.5	0.0
Analyzed chemical composition (g kg^{-1} dry matter)				
Dry matter	961.4	956.4	954.7	950.8
Crude protein	323.5	334.3	336.7	328.0
Acid hydrolyzed ether extract	164.2	166.7	157.9	160.4
Crude fiber	44.1	42.8	48.2	44.5
Total dietary fiber	132.2	130.8	136.8	129.6
Insoluble fiber (IF)	107.6	102.3	104.6	93.5
Soluble fiber (SF)	24.6	28.5	32.2	36.1
IF:SF ratio	4.4:1	3.6:1	3.2:1	2.6:1
Ash	66.6	84.7	71.4	80.6
Calcium	15.8	17.2	15.1	16.8
Phosphorus	11.1	12.2	11.5	12.1
Metabolizable energy (kcal kg^{-1})[2]	3879.0	3831.2	3797.8	3797.8

[1] Levels per kg feed: vitamin A, 20,000 IU; vitamin D3, 2000 IU; vitamin E, 480 IU; vitamin K3, 48 mg; vitamin B1, 4 mg; vitamin B2, 32 mg; B12, 0.2 mg; pantothenic acid, 16 mg; niacin, 56 mg; choline, 800 mg; zinc, 150 mg; iron, 100 mg; copper, 15 mg; iodine, 1.5 mg; manganese, 30 mg; selenium, 0.2 mg; antioxidant, 240 mg.
[2] Estimated according to the NRC (2006).

Table 2 - Chemical composition of the dried apple pomace

Item	Dry matter basis (g kg^{-1} as dry matter)
Dry matter	963.0
Crude protein	35.0
Acid hydrolyzed ether extract	20.7
Crude fiber	94.3
Ash	20.7
Total dietary fiber	232.3
Insoluble fiber (IF)	159.2
Soluble fiber (SF)	73.2
IF:SF ratio	2.2:1

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Total dietary fiber (TDF) and soluble (SF) and insoluble (IF) fiber fractions were determined according to Prosky et al. (1992). Gross energy (GE) content was determined in a bomb calorimeter (Ika® Werke Calorimeter System C 2000 basic C 2000 control). Original fecal dry matter content was calculated according to the equation:

$$\text{DM} = \text{dry matter content at 55 °C (DM55)} \times \text{dry matter content at 105 °C (DM105)}/100 \qquad (1)$$

Fecal dry matter content, fecal output (g feces/g DM intake/six days), fecal score, and sialic acid content were evaluated. Fecal score was evaluated always by the same researcher, using the following 1-5 scale: 1 = watery feces (can be poured from the container); 2 = soft and unshaped stools; 3 = soft, shaped, and moist stools; 4 = well-shaped and uniform stools; 5 = well-shaped, hard, and dry stools, as proposed by Carciofi et al. (2009). Feces were freeze-dried (Alpha 1-4 LO plus, Christ, Osterodeam Hans, Germany) to determine sialic acid content, according to Jourdian et al. (1971).

Based on the laboratory results obtained, the apparent total tract digestibility (ATTD) of DM, CP, AHEE, and ME content of the experimental diets were calculated as:

$$\text{ATTD} = [(\text{g of nutrient intake} – \text{g of nutrient excretion})/\text{g of nutrient intake}] \qquad (2)$$

Metabolizable energy was estimated according to the equation (AAFCO, 2016):

$$\text{ME (kcal.kg}^{-1}) = \{\text{kcal.g}^{-1}\text{ GE intake} – \text{kcal.g}^{-1}\text{ GE fecal excretion} – [(\text{g CP intake} – \text{g CP fecal excretion}) \times 0.86\text{ kcal.g}^{-1}]\}/\text{g of feed intake} \qquad (3)$$

Data were tested for normality (Shapiro-Wilk test) and when this assumption was accepted, data were subjected to analysis of variance considering the effects of block (period) and treatments. As there was no effect of block on any of the variables analyzed (P>0.05), data were subjected to regression analysis (P<0.05). Non-parametric data were analyzed by Kruskal-Wallis test (P<0.05).

In experiment 2, we used twenty castrated (10 males and 10 females) adult (2.5±0.1 years old) cats of undefined breed, with a mean body weight of 4.00±1.0 kg. The animals were housed in individual stainless-steel metabolic cages (0.9 × 0.8 × 0.9 m), and water was offered *ad libitum.*

Diet palatability was determined based on feed preference and first-choice tests. For the test, two diets were compared: 0 vs. 90 g kg⁻¹ of dryed apple pomace. The diets were formulated and processed as described above, and the feed allowance was 30% higher than the MER recommendations of the NRC (2006). The two diets to be compared were offered during 30 min in two different bowls twice a day, at 08:00 and 16:00 h. The amounts of feed offered and feed residues were quantified to calculate intake ratio. The first choice was determined by recording the first bowl the cat approached at the time the bowls were presented. The position of the bowls was changed on the second day of the test to prevent any conditioning to bowl positioning (Griffin, 2003). The palatability tests were performed on two consecutive days.

Experiment 2 was distributed in a completely randomized experimental design. Feed preference was calculated as the relative intake between the two diets, according to the equation:

$$\text{Intake ratio (IR)} = \text{g of diet A or B intake}/\text{g of total feed consumed (A + B)} \times 100 \qquad (4)$$

Feed preference results were analyzed by the Student's t-test, and first-choice results by the Chi-square test, both at 5% probability level, totaling 40 replicates.

3. Results

All animals completely consumed the diets offered, and we did not observe episodes of refusal, vomiting, and diarrhea during the experiments. Cats' body weight remained constant throughout the experiments.

Increasing dietary dried apple pomace levels linearly reduced the ATTD of CP and ME content, whereas the ATTD of AHEE, IF, SF, and TDF, as well fDM and fecal output presented a quadratic response (P<0.05) (Table 3). Regarding fecal characteristics, fecal sialic acid content and fecal score were not influenced by treatments, being similar to the control (P>0.05).

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The first choice was not influenced by dietary treatments (P>0.05). However, a greater intake ratio was obtained for the diet containing 90 g kg⁻¹ dried apple pomace relative to the diet this ingredient (P<0.05) (Table 4).

Table 3 - Apparent total tract digestibility (ATTD) of nutrients, dietary metabolizable energy content (ME, kcal kg⁻¹), and fecal characteristics of cats fed diets containing increasing levels of dried apple pomace

Item	Level of dried apple pomace (g kg⁻¹)				SEM	P-value	
	0	30	60	90		L	Q
ATTD							
Dry matter	0.793	0.803	0.787	0.790	0.003	0.472	0.652
Crude protein	0.834	0.831	0.801	0.798	0.005	0.003	0.989
AHEE	0.924	0.902	0.889	0.899	0.003	<0.001	<0.001
TDF	0.204	0.407	0.476	0.478	0.026	<0.001	0.002
Soluble fiber	0.612	0.790	0.708	0.681	0.021	0.460	0.012
Insoluble fiber	0.154	0.327	0.429	0.429	0.027	<0.001	0.014
ME (kcal kg⁻¹)	4290.1	4268.6	4134.7	4161.0	19.77	0.001	0.434
Fecal parameter							
Dry matter	0.339	0.307	0.277	0.295	0.006	0.002	0.023
Sialic acid (μmol/g sample)	0.902	1.011	0.906	1.063	0.004	0.250	0.950
Fecal output[1]	0.145	0.122	0.122	0.133	0.002	0.277	0.030
Fecal score[2]	3.00	3.00	3.00	3.00	-	-	-

SEM - standard error of the mean; L - linear effect; Q - quadratic effect; AHEE - acid hydrolyzed ether extract; TDF - total dietary fiber.
Regression equations: ATTD CP = −0.4648x + 83.695 (R^2 = 0.3343); ATTD AHEE = 0.0919x^2 − 1.114x + 92.488 (R^2 = 0.7096); ATTD TDF = −0.5568x^2 + 7.9803x + 20.776 (R^2 = 0.7528); ATTD SF = −0.5695x^2 + 5.5444x + 62.781 (R^2 = 0.256); ATTD IF = −0.4822x^2 + 7.4241x + 15.286 (R^2 = 0.7078); ME = −0.721x + 17.960 (R^2 = 0.3717); sialic acid = −0.0004x + 0.0422 (R^2 = 0.0437); fDM = 0.1368x^2 − 1.7651x + 34.143 (R^2 = 0.4723); fecal output = 0.001x^2 − 0.0098x + 0.145 (R^2 = 0.2624).
[1] g feces/g dry matter intake/d.
[2] Fecal score medians by the Kruskal-Wallis test (P = 0.151).

Table 4 - First choice and intake ratio of cats fed diets containing 0 or 90 (g kg⁻¹) dried apple pomace

Item	Level of dried apple pomace (g kg⁻¹)		P-value
	0	90	
First choice[1]	19	21	>0.05
Intake ratio[2]	0.39	0.61	<0.001

[1] First choice by the Chi-square test (P<0.05).
[2] Intake ratio by the Student's t test (P<0.05).

4. Discussion

Dietary inclusion of dried apple pomace caused a linear reduction in CP digestibility. This result could be due to the composition of apple fiber fractions. According to Swanson et al. (2000) in a study with dogs, apple byproducts (dried and wet apple pomace and dried apple pulp) contain different SF and IF proportions, and therefore, present different digestibility and palatability. The dried apple pomace used in the present study contained 232.3 g kg⁻¹ TDF, out of which 159.2 g kg⁻¹ corresponded to IF and 73.2 g kg⁻¹ to SF, which would explain the observed reduction on protein digestibility.

Considering that the experimental diets contained similar TDF content (132.4 g kg⁻¹, on average), the observed reduction in the ATTD of CP and AHEE and in dietary ME level may be attributed to the increasing SF contents of the experimental diets (24.6-36.1 g kg⁻¹). Insoluble fibers are non-viscous and slowly fermentable and in high levels may reduce nutrient digestibility by increasing the feed rate of passage, consequently reducing the contact of dietary nutrients with digestive enzymes (Kröger et al., 2017; Loureiro et al., 2017). On the other hand, SF is fermentable and negatively affects digestibility

by increasing the viscosity of the digesta, impairing the action of endogenous enzymes on nutrients (Godoy et al., 2013). In general, SF has a stronger negative effect on nutrient digestibility than IF, as demonstrated by Sabchuk et al. (2017), who reported lower nutrient digestibility of dog foods containing higher SF than of those containing high IF levels.

The dietary inclusion of fermentable fibers and their subsequent fermentation in the colon may increase protein fermentation or microbial nitrogen production as energy availability is increased (Loureiro et al., 2017). Thus, in the presence of energy, fermentable fibers promote microbial growth, contributing to the production of nitrogen compounds (Brambillasca et al., 2013; Kröger et al., 2017).

The results of the present study are consistent with those of Fekete et al. (2001), who observed a decrease in AHEE digestibility when dried apple pomace was added to cat food. Fiber intake may also reduce fat utilization, as soluble fibers create a viscous barrier, preventing the action of the digestive enzymes and reducing the resorption of bile and bile salts in the ileum (Sabchuk et al., 2017). Besides that, this fiber source has between 6.4 to 19.0% lignin (Sato et al., 2010), a compound with high affinity for binding bile acids, which may reduce bile acid reabsorption and fat absorption in cats (Loureiro et al., 2017; Donadelli and Aldrich et al., 2020).

The observed linear reduction in dietary ME content may be attributed to the decrease in nutrient digestibility as the inclusion levels of dried apple pomace increased. According to Godoy et al. (2013), fiber may dilute dietary energy density. Therefore, the inclusion of fiber-rich feedstuffs that considerably dilute the energy content of dog and cat foods may be particularly interesting for weight loss purposes (Sabchuk et al., 2017; Detweiler et al., 2019).

Fecal sialic acid content was measured in the present study as an indicator of changes in the intestinal mucosa that may be caused by dietary fiber. However, fecal sialic acid levels did not increase as a function of increasing the inclusion of dried apple pomace, as previously found by Sabchuk et al. (2017) in a study on different fiber levels in dog foods. However, our result is different from that of Larsen et al. (1993), who observed that fecal sialic acid levels increased with increasing fiber inclusion in rat diets. It is probable that only higher levels of dietary fiber increase mucus production in the intestine.

In addition to feed digestibility, fecal output and quality also need to be considered when testing new ingredients in cat food. Despite the high SF inclusion levels, and consequent reduction on diet digestibility, fecal scores were not influenced by the treatments: cats produced soft, shaped, and moist stools, which are considered optimal for this species (Carciofi et al., 2009). This result is consistent with those found by Falconi (2015) and Brambillasca et al. (2013), who evaluated the inclusion of apple pomace in dog food.

Fecal DM content was linearly reduced as dried apple pomace levels increased. This result is according to those observed by Brambillasca et al. (2013) in a study with dogs fed apple pomace (70 g kg^{-1}) and Donadelli and Aldrich (2020) in a study on beet pulp (100 g kg^{-1}) in diets for cats. According to Davidson and McDonald (1998), the high SF content of apples may increase the fermentation capacity of the colonic microbiota, increasing the production of acidic compounds and water retention in feces. However, no change in the fecal texture was observed in the present study, as mentioned above.

Case et al. (2000) mentioned that fiber intake may increase the digesta rate of passage in the large intestine, resulting in greater fecal output in dogs, and according to Pinto (2007), the fecal output is directly related to TDF intake. In the present study, however, fecal output decreased as dietary TDF increased. This result may be attributed to the fermentation of the high SF content of the evaluated dried apple pomace in the gut, because this fiber is better utilized by bacteria in the colon and fermented to various end-products (lactate, acetate, propionate, butyrate, valerate, H_2, CO_2, and methane) (Donadelli and Aldrich, 2020).

The dietary inclusion of dried apple pomace did not affect first-choice results. However, a higher IR of the diet containing dried apple pomace was found. Although the dehydrated apple has a sugar content of 106.8 g kg^{-1} of DM and this can influence the palatability of the food, cats are strictly carnivorous

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animals and do not have sugar taste receptors (Félix et al., 2010). This way, a possible explanation for the preference for the diet with dried apple pomace in the present study is the Maillard reaction, which occurs between reducing sugars and amino acids of the diet as a result of the high temperatures applied during the extrusion process. The sugar levels present in apple pomace may have benefited this reaction. According to Zaghini and Biagi (2005), the formation of Maillard reaction products during extrusion improves the palatability of cat foods.

5. Conclusions

Adding dried apple pomace (60 and 90 g kg^{-1}) to cat diets reduces the digestibility of crude protein, dietary metabolizable energy content, and fecal dry matter content. However, these parameters are not changed with the inclusion level of 30 g kg^{-1} of dried apple pomace. Besides, at the levels evaluated, it does not affect fecal score or fecal sialic acid concentration. Diets containing dried apple pomace are palatable and can be used for reducing the energy content of cat food.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: C.B.M. Brito, D.C. Lima, C.M.M. Souza, R.S. Vasconcellos, S.G. Oliveira and A.P. Félix. Data curation: C.M.M. Souza and A.P. Félix. Formal analysis: C.B.M. Brito, C.M.M. Souza and A.P. Félix. Funding acquisition: A.P. Félix. Investigation: C.B.M. Brito, D.C. Lima, C.M.M. Souza, R.S. Vasconcellos, S.G. Oliveira and A.P. Félix. Methodology: C.B.M. Brito, D.C. Lima, C.M.M. Souza, R.S. Vasconcellos, S.G. Oliveira and A.P. Félix. Project administration: C.B.M. Brito, D.C. Lima, R.S. Vasconcellos, S.G. Oliveira and A.P. Félix. Resources: C.B.M. Brito, D.C. Lima, C.M.M. Souza, R.S. Vasconcellos, S.G. Oliveira and A.P. Félix. Software: A.P. Félix. Supervision: R.S. Vasconcellos, S.G. Oliveira and A.P. Félix. Validation: A.P. Félix. Visualization: C.B.M. Brito, C.M.M. Souza and A.P. Félix. Writing-original draft: C.B.M. Brito, C.M.M. Souza, and A.P. Félix. Writing-review & editing: C.B.M. Brito, C.M.M. Souza and A.P. Félix.

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