Effect of ancient grains and grain-free carbohydrate sources on extrusion parameters and nutrient utilization by dogs

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ABSTRACT: The aim of this study was to evaluate the impact of ancient grain and grain-free carbohydrate sources on extrusion process, nutrient utilization, and palatability by dogs. Two maintenance dog diets were formulated with same proportions of carbohydrates: 1) ancient grain diet (**AG**) with spelt, millet, and sorghum; and 2) grain-free diet (**GF**) which had potato, peas, and tapioca starch. Experimental diets were extruded over 5 replicates in a completely randomized experimental design. Digestibility was carried out with 12 dogs in a switch-back experimental design. The GF diet required 22.6 and 25.9% more ($P < 0.05$) specific mechanical energy and in-barrel moisture input, respectively, than AG to produce kibbles out of the extruder with similar bulk density ($P > 0.05$). After drying, GF kibbles were less dense and more expanded, but harder than AG kibbles ($P < 0.05$). Dogs preferred GF over AG in the palatability assessment of uncoated kibbles. Apparent nutrient digestibility of dry matter, organic matter, gross energy, crude protein, and crude fat were not affected by treatment ($P > 0.05$). However, total dietary fiber (TDF) digestibility was 31.9% greater for dogs fed GF ($P < 0.05$). Moreover, wet fecal output was higher, and fecal dry matter was lower for dogs under GF ($P < 0.05$). The results demonstrated that GF and AG diets behaved differently during extrusion, but were similarly utilized by dogs, with exception of TDF. Thus, fiber content of grain-free diets should be monitored to maximize fecal quality.

Key words: digestibility, dog food, grains, legumes

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INTRODUCTION

Dogs and cats are some of the most popular pets owned in the United States, and are found in more than 60 and 47 million households in country, respectively (APPA, 2018). This directly impacts the pet supply industry which was worth $69.51 billion in 2017 (APPA, 2018). Humanization of pets has shifted the pet food industry toward a diet perceived as healthy for pet owners. Within these trends, the "grain-free" and the "ancient grain" claims have become popular as many pet owners consider traditional cereal grains to be unhealthy for their companion animals (Laflamme, 2014).

Ancient grains are typically considered those that have been cultivated for centuries with little genetic modification, such as sorghum, millet, quinoa, chia, and spelt. Some of these grains have perceived health benefits (Tang and Tsao, 2017), which might open them for consideration as alternatives to the grain-free diets in the market. These grain-free diets are commonly formulated with tubers and legumes such as potato, peas, and tapioca starch as replacements for conventional grains. Although tubers, legumes, and grains are all carbohydrate sources, each class has a unique nutritional composition which impacts their processing (Riaz, 2007), and nutrient utilization (Fortes et al., 2010). The effects of pea, lentil, sorghum, and traditional grains on dog food digestibility have been assessed by previous authors (Carciofi et al., 2008). However, these ingredients

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were evaluated as sole carbohydrate sources, and were included at high levels compared to practical diets. While this is valuable information, most commercial dog diets are formulated with a combination of carbohydrate sources.

Evaluation of these ingredients in a commercial dog food scenario may provide important information to maximize processing and ensure proper nutrition for dogs. To our knowledge, there is no published study comparing an ancient grain and a grain-free dog food that were formulated with a combination of different carbohydrate sources. Thus, the objective of our study was to evaluate a grain-free diet compared to ancient grain diet on processing conditions and nutrient utilization by dogs. We hypothesized that experimental diets will require different processing parameters to produce a product with similar bulk density, but nutrient utilization by dogs will not be affected by treatment.

MATERIALS AND METHODS

Diet Formulation and Production

Two dog maintenance diets were formulated containing the same proportion (50% of total) of carbohydrate sources: an ancient grain diet (**AG**) with spelt, millet, and sorghum, and a grain-free diet (**GF**) which had potato, peas, and tapioca starch (Table 1). Ancient grain sources were included at the same level (16.78%) while inclusion of tapioca starch was limited to 7.32% to prevent clumping during extrusion. Peas were added at the expense of tapioca starch to achieve similar proportion of carbohydrate sources as in AG. This extrusion trial was designed as a completely randomized design with diets produced over 5 replicates. Experimental diets were produced in a pilot scale single-screw extruder (X-20, Wenger Manufacturing, Sabetha, KS) with the following extruder profile: zone 1—single flight small and steam lock; zone 2—single flight and small steam lock; zone 3—single flight and medium steam lock; zone 4—large steam lock and uncut cone screw. Two circular dies (4.0 mm diameter) were used to produce standard size kibble for dogs. Similar bulk density out of the extruder (~415 g/L) was set as the targeted parameter during production, and adjustments of processing conditions were allowed to keep product bulk density as specified above. After processing stabilization, experimental diets were produced in cycling order over 5 replicates each. Processing parameters and material out of the extruder were collected for each replicate. Extruder mass flow rate was measured by collecting material out of the extruder in a bucket for 1 min. Product bulk density was measured using a 1-liter cup. Specific mechanical energy (**SME**) was calculated according to the equation below:

$$\mathrm{SME}\left(\frac{\mathrm{kJ}}{\mathrm{kg}}\right) = \frac{\frac{\tau-\tau_o}{100} * \left(\frac{N}{N_r}\right) * P_r}{m} \quad (1)$$

where τ is the % torque, or motor load, τ_o is the no-load torque (34%), N is the screw speed in RPM, N_r is the rated screw speed (508 RPM), P_r is the rated motor power (37.3 kW), and m is the total mass flow in kg/s. In-barrel moisture (**IBM**) was calculate as described below:

Table 1. Ingredient composition of experimental rations processed by extrusion

Item	Treatments[1] AG	GF
Ingredient composition, % as-fed		
Hydrolyzed pork protein	41.99	41.99
Potato, white	–	16.78
Peas, green	–	26.23
Tapioca starch	–	7.32
Spelt	16.78	–
Millet	16.78	–
Sorghum	16.78	–
Salt	0.48	0.48
Potassium chloride	0.30	0.30
Choline chlorine, 60% dry	0.24	0.24
Vitamin premix	0.24	0.24
Dicalcium phosphate	0.24	0.24
Calcium carbonate	0.24	0.24
Trace mineral premix	0.17	0.17
Fish oil, Menhaden	0.12	0.12
Taurine	0.12	0.12
Natural antioxidant	0.12	0.12
Chicken fat	4.00	4.00
Dry digest	1.00	1.00
Titanium dioxide	0.40	0.40
Chemical composition, % DM basis		
Moisture	5.41	5.49
Crude protein	37.00	38.00
Crude fat	15.80	12.50
Total dietary fiber	6.91	10.07
Insoluble fiber	5.25	3.85
Soluble fiber	1.66	6.22
Starch	38.04	35.32
Gelatinized starch	32.96	34.18
Degree of cook	86.65	96.77
Ash	4.24	4.33
Calculated metabolizable energy, kcal/g DM basis[2]	3.84	3.63

[1]AG = ancient grain; GF = grain-free.

[2]Metabolizable energy = 8.5 kcal ME/g fat + 3.5 kcal ME/g CP + 3.5 kcal ME/g nitrogen-free extract.

$$\text{IBM}\ (\%) = \frac{mf*Xf + mps + mpw + mes + mew}{mf + mps + mpw + mes + mew} \quad (2)$$

where *mf* is the dry feed rate, *Xf* is moisture content of the feed material, *mps* is the steam injection rate in the preconditioner (kg/h), *mpw* is water injection rate in the preconditioner (kg/h), *mes* is water injection rate in the extruder, *mes* is the steam injection rate in the extruder (kg/h), and *mew* is the rate of water injected in the extruder.

The kibbles were dried in a double pass oven drier (Series 4800, Wenger Manufacturing, Sabetha, KS) at 104 °C for 8 min each pass targeting a moisture content below 10%. Bulk density of dried kibbles was measured in each replicate. Dried product was coated with chicken fat (4%), dry palatant (1%), and titanium dioxide (0.4%), which was added as an external marker to estimate apparent total tract digestibility.

Kibble Characteristics

Kibble samples out of the drier were collected in each replicate to evaluate final product macrostructure characteristics. Twenty kibbles of each replicate were randomly selected, and their diameter, length, and weight were assessed to calculate piece density (**ρ**), sectional expansion ratio (**SEI**), and specific length (**l_{sp}**) as following:

$$\rho = \frac{4m_e}{\pi * l_e * d_e^2} \quad (3)$$

$$\text{SEI} = \frac{d_e^2}{d_d^2} \quad (4)$$

$$l_{sp}(\text{m/kg}) = \frac{l_e}{m_e} \quad (5)$$

A TA-XT2 Texture Analyzer (Texture Technologies Corporation, Hamilton, MA) was used to determine kibble hardness and toughness. A total of 15 kibbles per treatment were randomly selected for evaluation. A compression test was performed using a 25-mm cylindrical probe at a pretest speed of 2 mm/s, test speed of 1 mm/s, a posttest speed of 10 mm/s, and strain level of 90%. The first peak fracture force was taken as a measure of hardness. Toughness was defined as the total energy required to break the sample at the specified strain level, and it was calculated as the total area under the fracture curve.

Palatability Assessment

Experimental diets with no external addition of chicken fat (4%), dry palatant (1%), and titanium dioxide (0.4%) were used to assess palatability to evaluate the intrinsic effects of ingredients on palatability without dilution by topical coating with fat and flavor. The 2-bowl method (Griffin, 2003) was performed with a trained dog panel consisting of 20 Beagle dogs at a commercial kennel (Summit Ridge Farms, Susquehanna, PA). The kennel facility is registered with the USDA No. 23-R-0126 under the Animal Welfare Act. For 2 consecutive days, approximately 400 g of each experimental diet were presented simultaneously to the dogs in separate bowls once a day for 30 min. Bowl position was switched in the next day to prevent side bias. The amount of food offered in each bowl exceeded the dog's daily energy requirement to allow leftovers. First choice (**FC**; first product eaten by the animal) was recorded by technicians, and intake ratio (**IR**) was calculated according to the formula below:

$$\text{IR} = \frac{consumption\ of\ diet\ A}{(consumption\ of\, diet\ A + consumption\ of\ diet\ B)} \quad (6)$$

Digestibility Assessment

The experimental protocol was reviewed and approved by the Institutional Animal Care and use Committee at Kansas State University under protocol #3883. The digestibility trial took place at the Large Animal Research Center at Kansas State University where 12 castrated Beagle dogs (8 males, 4 females) of similar age, and initial body weight (12.56 kg ± 1.34, mean ± SD) were used. Dogs were individually housed in cages (1.83 m × 1.20 m) equipped with an acrylic-mesh floor and a pan underneath to allow separation of feces and urine. All cages were located in a temperature (22–23 °C) light-controlled (16 h light:8 h dark cycle) building. Food was provided twice daily (0800 and 1630 h) to maintain body weight. Food leftover was weighed at each meal, and food consumption was recorded. Daily metabolizable energy was calculated as an average for inactive dogs (ME, kcal/d = 95 × $BW^{0.75}$) according to the National Research Council (NRC, 2006). Body weight and body condition score (**BCS**) were measured biweekly, and food amount was adjusted accordingly. The BCS was determined using a 1 to 9 points scale, where a score 1 represented an extremely cachectic animal, and a score 9 an extremely obese dog. A score 4 or 5 was considered ideal.

The study was conducted as a switch-back design consisting of 2 periods of 9 d of acclimation to the diet followed by 5 d of fecal collection. Dogs were randomly assigned to experimental diets. Each dog received both diets at the end of the second period, and served as its own control. After the 9 d of acclimation, feces were collected and scored on a 5-point scale increment wherein: 1 = watery; liquid that can be poured; 2 = soft, unformed stool; assumes shape of container; 3 = softer stool; retains shape; 4 = hard, formed stool; 5 = very hard, dry pellets. A 3.5 score was considered ideal. Fecal samples were stored in individual plastic bags, and frozen at −15 °C until further analysis.

Digestibility Calculation

Feces were placed in an aluminum pan, and dried in an electric oven (Cat 52755-20, Matheson Scientific, Morris Plains, NJ) at 55 °C until constant weight was achieved (24 to 48 h). Following drying, feces were ground through a 1-mm screen in a fixed blade laboratory mill (Retsch, type ZM200, Haan, Germany). Concentration of titanium was determined in fecal and food samples according to Myers et al. (2004). Absorbance values were read at 410 nm using a microplate reader (Synergy H1, Biotek, Winooski, VT) Apparent total tract nutrient digestibility (ATTD) was calculated using the following equation:

$$\text{Nutrient digestibility} = \frac{[1-(\%Ti\ in\ food*\%nutrient\ in\ feces)]*100}{(\%Ti\ in\ feces*\%\ nutrient\ in\ food)} \quad (7)$$

Nutrient Analysis

Dry matter (DM; AOAC 930.15), organic matter (OM; AOAC 942.05), crude protein (AOAC 990.03), and fat by acid hydrolysis (AOAC 954.02) were analyzed in fecal and food samples in a commercial laboratory (Midwest Laboratories, Omaha, NE). Total dietary fiber (TDF; AOAC 985.29) was analyzed using a commercial kit (TDF-100A; Sigma-Aldrich; Saint Louis, MO). Gross energy was determined with a bomb calorimeter (Parr Instrument Company, Moline, IL). Fecal starch content was determined using a total starch Megazyme kit (K-TSTA-100A, Megazyme International Ireland Limited, Ireland). Total dietary starch and gelatinized starch were measured in a commercial laboratory (Wenger Manufacturing, Sabetha, KS) according to Mason et al. (1982). Degree of cook was calculated as described below:

$$\text{Degree of cook}\ (\%) = \frac{(gelatinized\ starch\ \%)*100}{(total\ starch\ \%)} \quad (8)$$

Statistical Analysis

A total of 5 and 12 replicates were achieved for the processing and the digestibility trial, respectively. Extrusion conditions, kibble macrostructure, and digestibility data were analyzed using the GLIMMIX procedure in SAS (SAS Inst. Inc., Cary, NC). For the digestibility experiment, diet was used as fixed effect, and animal nested within sequence was used as random effect. Means were separated using Fisher's LSD, and a probability of $P < 0.05$ was accepted as significant. In the palatability trial, first choice and intake ratio were analyzed using chi-square test and 2-way ANOVA, respectively.

RESULTS

Diet Formulation and Production

Diets contained similar concentrations of most nutrients (Table 1). A lower CF content and a greater TDF content were reported for GF compared to AG diet (CF, 12.5 and 15.8%; TDF, 10.7 and 6.9%, respectively). This same diet also resulted in a greater starch content (38.04 vs. 35.32%), and a lower degree of cook (86.65 vs. 96.77%) compared to GF. Similar bulk density out of the extruder was achieved through adjustment of the following parameters: preconditioner feed rate and steam were increased ($P < 0.05$), and extruder screw speed was decreased ($P < 0.05$) for AG compared to GF diet, respectively (Table 2). The IBM and SME input were 25.9 and 22.6% greater ($P < 0.05$) for GF compared to AG, respectively. In addition, AG diet was processed at a faster knife speed (2,381 vs. 1,904 RPM) and had a lower mass flow rate compared to GF ($P < 0.05$). Cone head pressure at the extruder barrel was greater ($P < 0.05$) for AG than GF. Water injection into the preconditioner and into the extruder did not differ between diets.

Kibble Characteristics

The bulk density of AG was greater ($P < 0.05$) than GF diet after drying (389 vs. 367 g/L; Table 2). Accordingly, AG kibbles were heavier ($P < 0.05$),

Table 2. Processing parameters and kibble traits of dog diets formulated with different carbohydrate sources

Item	Treatment[1] AG	GF	SEM	P-value
Raw material				
Feed rate, kg/h	166	82	2.24	<0.0001
Preconditioner				
Temperature, °C	91.6	71.8	2.56	<0.0001
Steam injection, kg/h	14.46	5.5	0.61	<0.0001
Water injection, kg/h	5.06	4.98	0.04	0.242
Extruder				
Water injection, kg/h	12.68	12.04	0.36	0.189
Extruder screw speed, RPM	442	637	0.84	<0.0001
Knife speed, RPM	2,382	1,905	73.84	0.002
Motor load	49	41	0.55	<0.0001
Cone head pressure, PSI	460	188	16.59	<0.0001
Mass flow, kg/h	0.046	0.024	0.002	0.002
Bulk density, g/L	418	427	6.04	0.346
Other data				
Specific mechanical energy, kJ/kg	115	141	7.37	0.038
In-barrel moisture %	30.3	38.2	0.54	<0.0001
Kibble traits				
Bulk density, g/L	389	367	3.84	0.004
Weight, g	0.16	0.1	0.01	0.004
Piece density, g/cm^3	0.6	0.54	0.01	0.009
Specific length, cm/g	4.41	4.24	0.08	0.174
Sectional expansion index	3.03	3.5	0.073	0.005
Hardness, kg	3.12	6.36	0.22	<0.0001
Toughness, kg * mm	2,427	1,778	220	0.070

[1]AG = ancient grain; GF = grain-free.

and exhibited greater piece density ($P < 0.05$) and lower sectional expansion index ($P < 0.05$) compared to GF kibbles. Interestingly, hardness was greater for GF compared to AG kibbles (6.36 vs. 3.12; $P < 0.05$). Specific length and toughness did not differ between treatments.

Palatability and Digestibility Assessment

Food intake was similar between diets (Table 3). Dogs fed GF had a greater wet fecal output compared to those fed AG (69.57 vs. 59.60 g/d), and 15% decrease in fecal dry matter ($P < 0.05$). No differences were observed among treatments for defecations per day, and fecal score. The IR results indicated a significant preference of dogs for GF over AG diet (IR of 0.84), which was also approached first by dogs (37 vs. 3 times). No differences were observed for DM, OM, CP, CF, and gross energy digestibility between AG and GF (Table 3). Total dietary fiber digestibility was 32% greater for dogs fed the GF when compared to those fed AG. Starch digestibility was statistically lower for dogs fed GF ($P < 0.05$); however, the numerical difference observed between treatments (99.4 vs. 99.6%) would not likely have an impact in a practical situation.

DISCUSSION

The first aim of this study was to evaluate the effects of ancient and grain-free carbohydrate sources on extrusion parameters, and kibble characteristics. It was not our intention to evaluate single ingredients, but rather the overall effect of dog diets formulated with different carbohydrate sources to simulate the performance of commercial diets. Thus, only 2 diets were tested in this study. It was not our intention to formulate isonutritional diets, but rather to evaluate how different of carbohydrate sources would affect nutritional composition, and then processing and animal responses. The results reported herein demonstrated that a similar bulk density out of the extruder could be achieved for AG and GF with minor processing adjustments. Processing difficulties have been reported during extrusion of tuber starches, mainly potato starch (Della Valle et al., 1995). Thus, to gain better control during the process, material

Table 3. Feed intake, fecal characteristics, and apparent total tract digestibility of dogs fed ancient grains and grain-free diets

	Treatment[1]			
Item	AG	GF	SEM	*P*-value
ATTD[2], %				
Dry matter	85.8	85.8	0.53	0.939
Organic matter	87.7	87.0	0.48	0.259
Energy	87.5	87.3	0.48	0.660
Crude protein	88.1	87.2	0.48	0.155
Crude fat	93.1	93.6	0.21	0.133
Starch	99.6	99.4	0.06	<0.0001
Total dietary fiber	39.3	51.8	2.33	0.026
Feed intake and fecal characteristics				
Feed intake, g/d	151.0	149.0	4.17	0.664
Wet fecal output, g/d	59.6	69.57	2.66	0.007
Fecal DM, %	33.9	28.8	0.45	<0.0001
Defecations per day	1.44	2.56	0.47	0.074
Fecal score	3.15	3.33	0.07	0.099

[1]AG = ancient grain; GF = grain-free.

[2]Apparent total tract digestibility.

feed rate into the preconditioner was 50.6% lower for GF compared to AG. No challenges were faced during extrusion of experimental diets, which demonstrates that material feed rate can be used as a tool to closely monitor the process. Nevertheless, this may not be translated to a commercial scale where production needs to be at its maximum efficiency. Future studies may consider keeping a constant feed rate to describe the challenges that might be experienced during extrusion of dog diets.

Steam injection into the preconditioner was decreased during extrusion of GF. Steam is the main source of thermal energy in the process due to vapor condensation on particle surfaces (Riaz, 2007), and impacts preconditioner temperature the most. As tubers and legumes gelatinize at lower temperatures when compared to cereal grains (Mishra and Rai, 2006), steam was decreased to prevent complete gelatinization of these starches in the preconditioner. Moreover, tubers are known for their high swelling power compared to cereal grains (Swinkels, 1985). Greater swelling power indicates that more water is being bound by starch molecules, resulting in greater resistance to shear force and greater final viscosity (Wang et al., 2011). Further, potato starch has a high swelling power due to the high content of phosphate groups bound to amylopectin. The repulsive force between phosphate groups weakens the bonding within the starch crystalline domain, thus increasing hydration of starch granules (Galliard and Bowler, 1987). In order to decrease viscosity and increase material flow within the extruder barrel, screw speed was increased for GF. Under high shear condition, the viscosity of starch pastes decrease as the molecules are progressively oriented in the direction of flow, and the hydrogen bonds between amylose-amylopectin-water are ruptured (Cornell, 2004). The greater screw speed resulted in a more fluid mash inside the extruder for GF, and may explain the lower cone head pressure and motor load observed for this treatment. In an attempt to achieve similar kibble length, the knife speed was set at a lower RPM for GF due to the lower feed rate established for this treatment.

The SME and IBM are critical parameters for extrusion process, and are influenced by processing variables. The SME can be defined as the amount of frictional/mechanical energy input per unit feed input or mass, and it is transferred to the dough due to the friction action between the material against the extruder screw and barrel. As a result, greater screw speed leads to greater SME (Riaz, 2007). Due to lower feed rate and greater screw speed, the SME input was greater for GF compared to AG. Domingues et al. (2019) also reported an increase in SME with inclusion of potato starch in dog diets. The greater energy requirement during extrusion of potato starch and other tubers is a result of their high melt viscosity and early melting in the extruder compared to cereal grains.

On the other hand, IBM represents the moisture as a percentage of the total mass inside the system. Water acts as a plasticizer during extrusion (Guy, 2001) decreasing material viscosity, and friction between material and extruder. Consequently, an increase in moisture content within the system leads to a decrease in SME (Riaz, 2007). For example, Pacheco et al. (2018) reported an inverse relationship between IBM and SME. However, this was not observed in our study. The lower feed rate and greater screw speed in GF compared to AG had a greatest impact on SME rather than IBM.

Water and steam addition into the system, as well as feed rate have an impact on IBM. Although steam input into the preconditioner was lower for GF, the IBM was greater for this treatment due to its lower feed rate. The difference in raw material composition of experimental diets used in our study required changes in processing conditions. As mentioned above, tubers required more water during processing as a result of their high swelling power, which also explains the greater IBM during extrusion of GF diet. Our findings are in agreement with Senouci and Smith (1986), who also reported a greater water addition during potato starch extrusion.

Processing conditions as well as raw material may impact kibble macrostructure, and consequently diet palatability. Although bulk density out of the extruder was similar for both diets, it was lower for GF after drying. The greater IBM observed in GF may have resulted in a product out of the extruder with greater moisture content. This excess of water was probably removed during drying, resulting in a decreased bulk density. During extrusion, the material is under a high-pressure and high-temperature environment (Riaz, 2007). Upon exiting the die, the melted mash is exposed to ambient pressure and temperature, causing it to expand and solidify. The extruded material can expand both longitudinally and radially. These variables are assessed by l_{sp} and SEI calculations, respectively. To evaluate overall expansion of an extrudate, one should calculate piece density, as it considers both radial and longitudinal expansion. In our study, l_{sp} was similar among treatments, but a greater SEI was observed for GF kibbles. Thus, the lower bulk and piece density reported in GF kibbles are mainly due to their greater SEI. Also, bulk density has an inverse relationship with SME (Riaz, 2007). Consequently, the greater SME input in GF resulted in lower bulk and piece density of the final product compared to the AG.

Furthermore, SME can also impact cell structure, and consequently product texture. Hardness is a mechanical property commonly used in pet food to access product texture, and it is characterized by the material resistance to deformation. The greater input of SME during extrusion of GF led to harder kibbles. A smaller and more uniform cell structure is observed in extrudates as SME increases. Smaller cell walls reinforce each other, thus requiring more force to break the kibble (Dunsford et al., 2002). It was previously reported that increasing levels of potato starch in a dog diet required greater SME and resulted in harder kibbles with greater number of cells (Domingues et al., 2019). Toughness is another way to assess product texture in which the total force to completely disintegrate the kibble is evaluated. In the current study, toughness was similar between treatments, although with the large numerical differences. Large variation in toughness was reported in previous studies (Alvarenga et al., 2018; Alvarenga and Aldrich, 2019), and was attributed to the nonuniform air cells in the extrudate. Specific thermal energy (**STE**) can also influence kibble characteristics. Unfortunately, it was not possible to calculate this parameter in the current study. Not only processing conditions, but also raw material can impact final product characteristics. Potato and tapioca dried starch films have a greater internal and tensile strength compared to maize and wheat starch (Swinkels, 1985). However, because processing conditions were not kept constant between treatments in this study, it is difficult to evaluate the single effect of raw materials on final product traits.

The second aim of this study was to evaluate the effect of grain-free and ancient grain carbohydrate sources on palatability and nutrient utilization by dogs. Uncoated diets were used to assess palatability. Although commercial diets typically undergo a coating step, where fat and other ingredients may be applied topically, our intention was to evaluate the intrinsic effects of ingredients on palatability alone. In our study, dogs exhibited a preference toward the GF diet. While sorghum, which was included in AG, has been associated with bitter and astringent notes (Kobue-Lekalake et al., 2007), Donfrancesco and Koppel (2017) reported that these characteristics can be reduced in the final product after extrusion. However, in a prior study a higher concentration of volatile compounds was observed—mostly hexanal in grain-based diets when compared to grain-fee pet food products (Koppel et al., 2013). This may have influenced the preference toward the GF in the current study. Thus, some component present in GF may be more attractive and palatable for the dogs. In another study, diets with greater inclusion of potato starch were also preferred by dogs (Domingues et al., 2019). Tubers release ribonucleotides after cooking as RNA is degraded. Ribonucleotides are precursors for umami compounds, and act as flavor enhancers (Jansky, 2010). Thereby, tubers might have an important impact on palatability. However, further studies should investigate the sensory characteristics of these ingredients, and their correlation with dog food preference. It is noteworthy that processing conditions also play a role in palatability, and they were not kept constant between experimental diets. The greater SME input for GF might have enhanced the flavor compounds in the diet due to greater degree of cooking. In accordance, Trivedi and Benning (2003) reported that dogs preferred diets processed with higher SME input. However, Dunsford et al. (2002) showed that dogs had a greater preference toward a more thermally cooked diet while no preferences were observed by Pacheco et al. (2018) when different levels of SME and STE were evaluated. In our study, the experimental diets were formulated with different raw materials, and were extruded under different conditions. Thus, a combination of factors may be playing a role on palatability beyond the SME:STE ratio.

Although dogs showed a greater preference toward GF in the palatability trial, no signs of refusal were observed for the AG diet during the digestibility study. However, diets were coated with fat and palatant before digestibility assessment, and this may improve overall diet acceptability or mask any off flavors. In our study, the ATTD of most nutrients was not affected by different starch sources. Carciofi et al. (2008) reported a lower ATTD of DM, OM, and CP for dogs fed a diet containing peas compared to those fed cereal grain-based diets. This was not observed in the present study. However, GF contained tapioca starch which was reported by the same author to be more digestible than diets containing corn, sorghum, lentil, and peas. High digestibility for tapioca starch in dogs was also reported by Kamalu (1991). If the grain-free ingredient sources were included at the same proportion in our study, a digestibility improvement may have been observed as a result of an increase in tapioca starch and decreased peas. Furthermore, dried potato was reported to be highly digestible by dogs (Kendall and Holme, 1982). Despite these previous reports, the overall GF digestibility was similar for all nutrient measured with the exception of TDF. It must be noted though that only 1 level of grain-free carbohydrate source was tested in this study. Evaluation of increasing levels of grain-free carbohydrate sources may provide a better understanding of the impact of these ingredients on nutrient utilization.

The lower content of gelatinized starch in AG resulted in a lower degree of cook in this diet compared to GF. Starch gelatinization has been found to increase with increased SME during extrusion (Ilo et al., 1996) which corroborates with our results. Starch digestibility is highly dependent upon full gelatinization; thus, we were expecting a lower starch digestibility for dogs fed AG. Although our results indicate a statistical difference in starch digestibility between dietary treatments, this difference is minimal under practical conditions. The lower degree of starch gelatinization observed in AG was sufficient to enable full digestion of starch. Our results are in agreement with Pacheco et al. (2018) who did not observe differences in digestibility of a dog food even though gelatinized starch differed among dietary treatments. There is no published information regarding the amount of gelatinized starch needed to allow proper digestibility in dogs. Although the AG required lower energy input through SME and steam addition, it did not impair nutrient digestion; resulting into similar digestibility of most nutrients between diets. These results suggest that mild processing of dog foods may be sufficient to allow proper nutrient digestion.

The greater digestibility of TDF for GF may indicate a higher large intestinal fermentation of the fibers present in this diet compared to those in AG. This result is in accordance with other studies that also found a high digestibility of TDF for legume-based diets compared to grain-based ones (Carciofi et al., 2008). Legumes such as peas contain greater concentration of soluble fibers compared to cereal grains, which results in a greater TDF value for those ingredients (Bednar et al., 2001; de-Oliveira et al., 2012). Most soluble fibers are indigestible by the dog due to the lack of specific enzymes, but are readily fermented in the lower intestinal tract (Jezierny et al., 2010). Consequently, TDF digestibility increases as these fibers are converted to fermentative end products by colonic bacteria.

Moreover, fermentation of soluble fibers by colonic bacteria produces gases and short chain fatty acids (**SCFA**). Although SCFA can improve gut health and reduce inflammation (Sivaprakasam et al., 2016), their overproduction can attract water and sodium to the lumen due to their osmotic power and result in increasing fecal moisture content (Binder, 2010). Unfortunately, fecal pH and SCFA were not determined in the current study. These results could have provided a more concrete understanding with regards to lower bowl fermentation. Dogs fed GF diet exhibited greater wet fecal output, and lower fecal DM probably due to fermentation of soluble fibers in the lower gut. Carciofi et al. (2008) also reported a lower fecal DM in dogs fed diets containing peas and lentil. Similar results were observed by Fahey et al. (1990) who reported a linear increase in wet fecal weight as percentage of dietary beet pulp increased. Intriguingly, fecal score and number of defecations per day were not statistically different. High variation within treatments may explain the lack of significance; but, it is worth noting that dogs fed GF diet had on average 1 more defecation per day compared to those fed AG.

CONCLUSION

The 2 classes of carbohydrate sources behaved differently during extrusion; prompting changes to the processing parameters to produce diets with similar bulk densities. The GF diet had a greater energy and moisture requirement during extrusion compared to AG. This resulted in more expended and harder kibbles for GF over AG. Dogs exhibited similar digestibility of most nutrient, besides for TDF, which had a greater disappearance for those dogs fed GF. Moreover, a greater fecal output, which had greater moisture content, was observed for dogs fed the GF diet. Grain-free and ancient grain carbohydrate sources were well utilized by dogs, but close attention should be given to the fiber content of GF diets to maximize fecal quality.

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