Process development of health benefits from corn in pet diets that enhance dog utilization

by

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Abstract

The methods of manufacturing pet diets have evolved over the past 160 years. Starting with a baked biscuit in 1860 to the widely used extrusion technology today. Formulas have also changed with once popular corn now being criticized as inferior and indigestible. Different processing methods can change starch digestion. Less processing can lead to more indigestible starch (resistant starch; RS) within corn diets. This RS may benefit gastrointestinal health. Therefore, the objective of the first study was to determine the effect of process on dietary utilization of corn-based diets, and changes to starch utilization. Experimental diets containing 56% corn as the sole starch source were produced through pelleting, baking, and extrusion and compared to a baked control diet in which the corn was replaced with dextrose. The pelleted diet had the highest ($P < 0.05$) differential scanning calorimetry (DSC) residual gelatinization peak (3.5 J/g) followed by the baked diet (1.4 J/g), and then similar to the extruded diet the control had the lowest result (0.1 J/g). The extruded diet resulted in the highest ($P < 0.05$) level of gelatinized starch (34.2%) by the glucoamylase procedure followed by the baked treatment (14.7%) and pelleted had the lowest ($P < 0.05$) gelatinized starch (8.4%). As a percentage of total starch, the extruded diet had the highest ($P < 0.05$) level of rapidly digestible starch (90.0%), the baked treatment was intermediate ($P < 0.05$, 60.9%) and pelleted the lowest ($P < 0.05$, 34.8%). The pelleted diet had the highest ($P < 0.05$) amount of slowly digestible starch (SDS, 42.6%), with the baked treatment having an intermediate ($P < 0.05$) amount of SDS (27.1%), and the extruded diet had the lowest ($P < 0.05$) amount of SDS (7.1%) Among corn treatments the extruded diet had the lowest ($P < 0.05$) level of RS (1.3%). The baked diet was intermediate ($P < 0.05$, 3.6%) with the highest RS ($P < 0.05$) in the pelleted diet. To evaluate the *in vivo* effects of these process treatments, twelve Beagle dogs were fed the experimental diets for 9 d adaptation

and 5 d of collection in a replicated 4 x 4 Latin square designed study. The data were analyzed using the general linear mixed model with statistical analysis software (GLIMMIX proc; SAS v9.4, Cary NC) and means were considered different at $\propto = 5\%$. The experimental diet was the fixed effect with period and dog as random effects. Dogs were fed to maintain body weight, with food intake similar among corn treatments and lower ($P < 0.05$) for those fed the control diet. Fecal scores (1: soft-liquid to 5: hard-dry) were slightly different across the three corn treatments, but each exceeded 3.5 which was considered ideal. However, dogs fed the control diet (dextrose) had soft-runny stools (average score of 1). Feces from dogs fed the baked treatment had a lower fecal pH (5.37; $P < 0.05$) than the other corn treatments (average 5.71); whereas the pH of feces from the dogs fed the control (dextrose) diet were the highest ($P < 0.05$) at 6.87. The dry matter apparent total tract digestibility (ATTD) was greater ($P < 0.05$) for the dogs fed the extruded corn-based diet and the control diet (average 75.75%) versus the baked and pelleted diets (average 69.49%). In conclusion, the corn diets had different levels of gelatinized starch thereby influencing the *in vivo* digestibility, Resistant starch was higher in treatments of lower cooking intensity including lower fecal pH which may indicate improved colonic fermentation leading to positive impacts on gut health.

To assess the level of RS in commercial products for comparison purposes, 30 baked dog treats were evaluated in a 2 x 3 factorial design. Samples were purchased and separated into main effects of size (small, medium, large), and presence or absence of wheat. Samples were analyzed for their resistant starch concentration. It was determined that the size and presence of wheat did not impact the total starch (average 40.7%), digestible starch (average 39.5%), or resistant starch (average 1.8%) concentration. However, a post-hoc analysis did indicate the few (n=5) grain-free products that were selected had a higher ($P < 0.05$) concentration of resistant starch (average 4.9%).

In conclusion, among treatments like extrusion that had more energy inputs there was a lower concentration of RS while the inverse was observed in processes with fewer energy inputs or lower cooking intensity. This also impacted stool quality and the digestion of some nutrients but significantly increased starch digestion. Treatments with lower levels of RS are common to commercially available foods. So, using different processing methods can change the digestible and indigestible starch ratio, which can have promote several health benefits.

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Chapter 1 - Literature Review

Introduction

Grains are largely included in pet food formulas for their economical, nutritional, and processing characteristics. Cereal grains are nearly 90% carbohydrate which are comprised of starch, oligosaccharides, and fibers (Corsato Alvarenga, Dainton, and Aldrich 2021a). Starches from cereals are digested to glucose in the animal's intestinal tract (K. Englyst and Englyst, 2004) by enzymatic hydrolysis. This is where it is absorbed and transported to the circulation as glucose for metabolic functions and sparing the need for the liver to break down glycogen (Brouns and Dye, 2004). Other minor sources of energy in grains are derived from lipids (fat), or protein. Starches from cereals also play an important role in structure formation in food products during cooking processes like extrusion and baking where the thermal treatment can strengthen the starch granule (Taggart 2004). Corn is one of the most common and abundant grains grown in the United States (*USDA*, 2020) and it is utilized in a wide array of pet diets. These diets can contain whole ground corn, corn flour, or by-products of corn processing from the human food industry, such as distiller's dried grains, or corn gluten meal (Corsato Alvarenga, Dainton, and Aldrich 2021b). Formulations have historically contained varying levels of corn or its by-products; and some studies have reported high digestibility (nearly 100%) of diets formulated to contain between 50 and 70% of corn (Bazolli et al., 2015; Carciofi et al., 2010; Kore et al., 2009; Twomey et al., 2003; Wolter et al., 1998; Walker et al., 1994).

In recent years, misconceptions about corn have surfaced. One such claim is that corn is used as a "filler" (Khuly 2013; Laflamme et al. 2014); meaning it is not an adequate source of nutrients or is a cheap commodity that can be used to substitute for other ingredients. It is true, that when compared to other grains such as wheat, or sorghum, corn is more economical per metric ton. For example as of August 2020 ("Corn Price" n.d.; "US Sorghum Price" 2020; Plecher 2021) the price per metric ton for these grains were $205, $190, and $150, respectively. However just because it is less expensive does not indicate a lack of nutritional value. A poll conducted by Petfood Industry magazine asked consumers a variety of questions regarding the types of food they would feed their pets. Of those surveyed, 43% of pet owners surveyed said that "nutritional value is the most important factor in the foods they feed to their pets" (Pet Food Industry, June 2018). If the consumer has the impression that corn is not a viable nutritional source, then foods containing corn might be avoided. Of consumers who answered the survey, 28% indicated that "natural or organic" foods were preferred. The term "natural" is defined in the AAFCO manual in a way that's different than some may think, as it states, "the food can be subjected to physical or heat processing but not chemical synthetic processes." Under this definition corn is a natural ingredient. The Food and Drug Administration (FDA) reported in 2018 that 92% of planted corn was genetically modified (Food and Drug Administration 2020), and 33% of those surveyed by the Petfood Industry claimed to desire foods that did not contain genetically modified organisms (GMO). The GMOs are produced through genetic modification ("Agricultural Biotechnology Glossar" 2020) to enhance certain traits like larger yield, or tolerance to limited water or pests. If consumers do not want GMOs in their pet's food, corn is automatically eliminated as an option because over 90% of corn has been genetically engineered for a variety of reasons (Dodson n.d.). The reasons can include herbicide tolerance, insect resistance, drought resistance or a combination. Given all these factors, there is much evidence to show that the consumer has a perspective that corn as a negative impact on a pet foods ingredient composition.

Another misconception is that dogs and cats are becoming allergic to grains in their diet. A survey taken in 2020 showed 40% of respondents, answered that when their pet experienced an allergic reaction that involved itchy skin, hair loss, or soft stools (Banton et al. 2021). The itchy skin and hair loss can develop into acute moist dermatitis if the dog constantly licks or scratches the area (Racine 2021). The slang term for this condition is a “hot spot” because of the redness and inflammation that occurs. The same survey also noted that when the pet developed what they believed to be an allergic reaction, the owners were two, to even four times more likely to choose a diet that is labeled to have no grains (not containing corn).

The problem with this logic is that dogs and cats are highly unlikely to have an allergic reaction to grains like, corn, rice, or other grains (Verlinden et al. 2006); rather it is more likely they have a food sensitivity to a protein. The food sensitivity is a simple, gradual reaction unlike the immune response from a true allergy (Burke 2021). Even when diagnosed with a “celiac disease like” condition, which is a sensitivity to gluten proteins found in grains like wheat or barley, it is a small population of dogs (Garden et al. 2000). Further, corn does not contain the same protein that is thought to cause this sensitivity (Verlinden et al. 2006).

The purpose of this research was to determine positive attributes to corn that shifted the narrative from it being a “cheap filler,” allergen, or GMO, to explore its positive attributes such as ease of processing, functionality, and potential to improve the amount and consistency of resistant starch in the final product. Extrusion is the most common method to produce dry dog foods (Sanderson 2021) and it often uses cereals like corn. Previous work would suggest that extrusion is very effective at converting all the starch to be digestible (Alvarenga and Aldrich, 2020; Svihus et al., 2005). Other processes that are less intensive may improve the chances of increasing indigestible RS. For example, baking could be an option. However, no information is available to confirm this. Additional context between cooking intensity and how it affects starch conversion with an emphasis on increasing RS should be explained. The net result of increasing RS could be linked to improvements in gut health if it can be consistently achieved in a processed pet food. This review will explore previous research focused on processes for making pet foods with corn as the primary starch, and how it affects nutrient digestibility, and indirectly if RS levels can be enhanced. This might result in benefits for colonic fermentation in dogs and gut health.

“Extrusion cooking is a process in which food or feed material is forced to flow under elevated temperature, pressure and shear through a die” (Mian N. Riaz 2012). Other methods beyond an unprocessed granular meal that could be used to produce dry pet foods include pelleting or baking. Baking uses thermal energy, while pelleting uses minimal energy from steam to help bind the product. Each of these processes result in some level of starch gelatinization.

Starch gelatinization occurs when moisture (and heat) is applied to the starch granule, causing it to lose crystallinity, to swell and rupture (Cheftel 1986). This swelling unfurls the amylopectin, which makes it more accessible to digestion by mammalian enzymes. The starch fraction which is not digested is a fermentable component called resistant starch (RS) that may provide benefits for colonic microbiota (Jackson et al. 2020; Ribeiro et al. 2019; Peixoto et al. 2018). It is thought to be desirable to include levels of RS and slowly digested starches (SDS) in pet foods. It is our hypothesis that consistent and meaningful levels of RS can be accomplished by utilizing different processing methods.

Corn Composition

When considering the composition of corn, starch is the dominate component that makes up approximately 88.3% of the kernel of the endosperm (Murray et al. 2001). The other components such as the pericarp and germ contribute other nutritional factors such as 5.6% protein, 3.2% fat, 3.0% total dietary fiber (Murray et al. 1999). These values can vary slightly throughout corn harvests. Starch is a carbohydrate consisting of two types of molecules. One is amylose which is primarily a linear α 1-4 linked glucose molecule, and amylopectin which is highly branched due to its α 1-6 glycosidic linkages (Rumney 1991). The ratio of these two components can have an effect on the animal's health (Gajda et al. 2005) as they impact the rate of starch digestion. Typically maize, or corn starch contains a ratio of 23:77 amylose to amylopectin (Xingxun. W. Liu et al., 2009). We can also alter their natural compact structure by applying different methods of cooking.

Starch can be classified by *in vitro* enzymatic digestion into rapidly digested (RDS), slowly digested (SDS) or resistant starch (RS) (Dhital et al., n.d.). By definition RDS is the starch digested within 20 min (*in vitro*) of incubation, while SDS is digested between 20 and 120 min, and RS is the fraction that wasn't digested after 240 min (Mccleary et al. 2012; McCleary 2007; H. N. Englyst, Kingman, and Cummings 1992). The ratios of these different types of starches within corn is approximately (35%), slowly digestible starch (15%), and resistant starch (24%) out of a total starch level of 73% (Bednar et al. 2001; Murray et al. 2001). The proportions of these starch fractions can be altered by food processing conditions, or *in vitro* incubation conditions during analysis.

Starches are broken down into their simpler glucose sugars by acid hydrolysis in the stomach and by enzymes in the small intestine. After digestion they can be absorbed into the bloodstream, which triggers an insulin release by the pancreas. If the majority of starch is RDS, then the blood glucose level can spike quickly following a meal. Long-term this may affect pancreatic insulin resistance (André et al. 2017) and subsequent health risks such as diabetes.

Resistant starch is not digested in the small intestine and can indirectly impact health positively at increased concentrations.

Resistant starch is classified into several categories. These are based on the physical and chemical nature of the starch granule. The RS are classified into 5 groups; 1, 2, 3, 4, and 5 (RS1, RS2, RS3, RS4, RS5, respectively; Raigond et al., 2015). The RS1 is found in raw starchy foods such as pulses or some cereals (Raigond, Ezekiel, and Raigond 2015). Potato starch is an example of RS2. It has a high amylose content and is easily gelatinized. But generally RS2 is resistant to gelatinization at high temperatures (Thompson 2006). Retrograded starches that have been gelatinized then cooled to room temperature are considered RS3. When a starch is chemically modified, for example by cross-linking, it may be described as RS4 (Wang, Kozlowski, and Delgado 2001). The formation of amylose-lipid complexes is RS5. This can be formed from high amylose starches (Raigond, Ezekiel, and Raigond 2015).

After food is digested in the stomach and small intestine, and absorbed prior to the ileum, the remainder passes to the large intestine. This includes carbohydrates like fiber or resistant starch. In the large intestine, fermentation by the host microbiota including saccharolytic bacteria, can take place (Jackson and Jewell, 2019). When bacteria ferment food particles as energy substrates they produce short chain fatty acids (SCFA) such as acetate, propionate, and butyrate. These become energy substrates for the host. Studies have shown that consumption of RS by dogs increases butyrate production in the colon (Jackson et al., 2020; Ribeiro et al., 2019; Peixoto et al., 2018). Of the total SCFA's produced from RS, proportionally 41% was acetate, 21% propionate, and 38% butyrate (Kritchevsky, 1995). Butyrate is the preferred energy substrate of colonocytes (Leonel and Alvarez-Leite, 2012) and has an important role in suppressing colonic carcinoma and regulating local immunological parameters (Lee and Hase, 2014).

There have been numerous studies demonstrating the results of grain-based diet digestibility and fermentation by *in vitro,* and *in-vivo* techniques (Bazolli et al. 2015; Murray et al. 2001; Stroucken et al. 1996; Peixoto et al. 2018). More research with the target species; such as companion animals like the dog or cat, are needed. Further details regarding the effects various types of grains, like corn might have on nutrition and gastrointestinal health.

The Influence of Processing on Corn Characteristics

Dry diets for companion animals are commonly produced by extrusion with a few complete foods, and a wide array of treats produced by baking. The methods differ by time, temperature, moisture, and even pressure inputs. These inputs can influence the characteristics of the final product. One such characteristic is the ratio of amylose and amylopectin. As energy and/or moisture are applied to corn starch, it starts to unfurl the highly branched amylopectin. This allows access by digestive enzymes to degrade the starch to glucose rather than remain in its indigestible crystalline form (X. Liu et al. 2009). Although the act of gelatinization does not require large amounts of energy to occur. Energy (heat) does accelerate the rate of reaction (Bjorck et al. 1994).

Extrusion is the method of production that causes the most gelatinization among various processes (Gibson and Alavi, 2015; İnal et al., 2018). It applies high levels of both mechanical and thermal energy under pressure. Based on previous research, it has been found that changes of processing parameters affect the final concentration of gelatinized starch and thereby influence the amount of resistant starch present (Jackson et al. 2020). It also increases the *in vitro* organic matter digestibility and kibble physical attributes (Pacheco et al. 2018).

As part of the extrusion process there is a preparation step before the extruder barrel proper; wherein the dry ration is “conditioned.” This apparatus called the “preconditioner” is where water, steam, and in some cases fat or meat slurries can be added and mixed before reaching the extruder barrel. While moisture and heat are added at this step, it only partially cooks the ration before entering the extruder barrel (Mian N. Riaz 2012).

Within the extruder barrel the screw transfers mechanical energy in the form of shear when pushing the material against the die. This causes the internal pressure to build and the mix to rub against the screw elements. High levels of mechanical energy are applied to the mixed ration through this shear force, as well as thermal energy that is added from the jacketed barrel segments that surround the barrel, or by direct steam injection. These jackets are temperature regulated using water and steam to add more thermal energy. The net result is a phase transition of the dough from a glassy to a rubbery state. This is where the nearly complete (100%) gelatinization takes place (Moraru and Kokini, 2003). This transition occurs towards the die where pressure and heat are the highest. Once back-pressure is created, the barrel interior reaches pressure which is multiples of atmospheric condition and “melts” the ration prior to exiting from the die. The sudden transition from several atmospheres to ambient causes the water to vaporize to steam. This causes the product to expand (Moraru and Kokini, 2003). The mechanical and thermal energy can be altered during the extrusion process by modifying several variables. Each of which will modify the total energy being applied. To start, the screw speed (RPM) or screw profile (orientation of the screw flights and kneading elements within the barrel) will change the mechanical energy or shear force being applied to the ration. A slower screw speed will result in a lower amount of specific mechanical energy (SME) being applied while the inverse results in a higher SME. A screw profile can be customized to meet the requirements of several product types. Certain screw elements will increase the amount of energy applied by reducing the forward flow of the melt such as a reverse flight element, or a cut flight element. A shear lock acts as a barrier between sections and increases the amount of time the material is receiving energy from the screw. Along with these sources of mechanical energy, there can also be direct steam injection, or changes to barrel temperature which will affect the thermal energy being applied (Baller et al., 2018). When any of these sources of energy is decreased the potential for less starch gelatinization exists and may result in a higher level of RS (Jackson et al. 2020; İnal et al. 2018).

Baking is quite different from extrusion. Unlike, extrusion which relies on both mechanical and thermal energy, baking does not employ mechanical energy input beyond the mixer. The mixing starts at the very beginning where liquid ingredients are mixed into the dry flour ingredients to form a dough; whereas, in extrusion the steam, water and lipids are added in-process. With liquid ingredients able to be added in-process, extrusion is a continuous process. Baking has potential to be continuous instead of a batch method when special equipment such as rotary molder and tunnel oven are used. For baking this mixing to form the dough is very important. Once the dough is formed the combination of moisture when mixing the dry ration with water helps unfurl the amylopectin molecules, then the thermal energy continues to increase the rate of gelatinization. The moisture has an impact on the ability to extract the treat from the die, development of hardness, and overall drying time. Having the correct dough consistency can help with both extracting the treat from the molder or getting stuck in the cutter. One of the main objectives of baking is the removal of moisture (Davidson 2016) followed by structure formation. Thus, minimizing water addition is important. When utilizing a rotary molder there is more consistent size for the final product. Whereas using a shaped cutter the dough will have to be rolled into a sheet or pushed into a mold cavity with the desired shape cut. When sheeting the dough, thickness can play a role in how the piece cooks. A thin sheet can lead to burnt biscuits, and too thick can lead to moisture trapped in the center. Excessive moisture remaining inside the piece can increase water activity potentially leading to mold. Water activity (a_w) is the ratio between vapor pressure of the food itself, when in a completely undisturbed balance with the surrounding air, and the vapor pressure of distilled water under identical conditions (Food and Drug Administration 2014). Monitoring the a_w is critical for mitigating spoilage from fungal growth particularly in cereal grain based baked products (Blackburn 2006). Achieving a a_w less than 0.65 is adequate to retard most mold growth in these products. One approach to the target moisture and lower water activity is the design and utilization of dokker pins in the molder die. These inserts add indentions to the treat which function as a channel for water vapor to escape during baking, and the thereby decrease a_w and reduce the risk of mold or pathogens.

These baking parameters influence the final product. The oven is where the texture and flavor are developed. After the water vapor escapes about halfway through the cooking cycle, color and flavor begin to form and texture develops as a_w decreases (Davidson 2016; Arimi et al. 2010). Although very little occurs during the extrusion or drying process, another way the final product can be influenced by the Maillard reaction is with the heat applied when baking (Davidson 2016). This browning reaction occurs in the dryer, forming acrylamide, which increases the intake and palatability compared to some other products (Di Cerbo et al. 2017). It is a reaction that occurs between amino acids and reducing sugars that lead to a “toasted” flavor. However, too much of this reaction can reduce the bio-availability of some essential amino acids such as lysine (Friedman 1996) and there may be a negative impact on flavor (Boekel et al. 2010). Temperature and moisture play a large role in these chemical reactions. Temperature is also used to assist mitigation of bacteria and decrease pathogen risks when target levels of time and temperature are achieved. For example, when drying an extruded kibble, temperatures can range from 120º C to 150º C (İnal et al. 2018; Fortes et al. 2010; Kawauchi et al. 2011). Reducing the amount of moisture can also accelerate the Maillard reaction as higher moisture can actually inhibit the browning reaction in some model systems (Rooijen et al. 2021). These two factors, heat, and moisture may allow control over this process in the dryer during production.

Pelleting is a method that was common in the early days of pet food and is still used widely in the livestock industry. It differs from extrusion and baking in that it lacks virtually all mechanical energy and imparts minimal thermal energy to the product. The small amount of thermal energy comes from the steam injected in the conditioner to aid compaction of the pellet and more recently to increase the temperature as a kill-step to eliminate pathogens. The pellet conditioner is similar to the pre-conditioner in the extrusion process but lacks the fat or meat slurry addition. In a study by İnal et al., (2018) the authors reported that extruded kibbles had roughly four times more gelatinized starch than a pelleted diet. This resulted in a lower dry matter digestibility in dogs fed the pelleted diet when compared to dogs fed the extruded diet. With the lower cooking intensity when pelleting, it impacts the starch gelatinization, and overall digestibility of nutrients such as organic matter, crude protein, crude fat, or crude fiber (İnal et al. 2018).

When producing any dog food, it is important to assure the particle size of the ingredients making up the ration are ground to a uniform consistency before processing. The particle size can influence the product characteristics and processing conditions. As particle size changes, it can impact how the processing method will alter the starch granules or it may result in damage to the starch granule (Taggart 2004). The particles size can also improve or reduce the digestibility. A larger particle size can lead to reduced dry matter apparent total tract digestibility (DM ATTD) in grains such as corn, or sorghum (Bazolli et al. 2015). The coarser grind reduces the level of gelatinization and increases the RS concentration in the final product (Bazolli et al. 2015; Peixoto et al. 2018).

Starch Digestion

For starch to be effectively utilized it must be digestible. Animal species differ in the methods used to digest starch. Humans, for example start breaking down starch in the mouth with α-amylase enzyme in the saliva (Boehlke, Zierau, and Hannig 2015). Whereas dogs and cats lack the concentration of salivary amylase in the oral cavity compared to humans (Contreras-Aguilar et al. 2017). Further, the particle size of food is reduced by mastication to increase surface area influencing digestion (Ranawana et al. 2010). Once in the stomach, the food is exposed to hydrolysis with hydrochloric acid, and enzymatic pepsin which starts breaking down the starch into maltose and other oligosaccharides (lactose, sucrose or other simple sugars), and the proteins into peptides and amino acids (Guevara et al. 2008), respectively. Passing from the stomach and into the small intestine, where chyme is released (Bednar et al. 2001). The acid chyme passes through a pyloric sphincter to the small intestine (Daristotle et al. 2011). In the duodenum, the sugars are further degraded to glucose and then absorbed by enterocytes through active transport by a sodium-glucose cotransporter (Wachters-Hagedoorn, Priebe, and Vonk 2004). This is followed by a spike in the blood glucose concentration. This leads to a release of insulin from the pancreas to remove the sudden increase of blood sugars in the bloodstream. As the food continues its passage through the digestive system, the material not absorbed in the duodenum or jejunum will move onto the ileum. These residues are typically the indigestible portions of the diet such as fibers or potentially resistant starch which reach the colon (large intestine). Fermentation leading to the formation of SCFA's occurs in the colon. Previous research has reported that there is a large and diverse bacterial population residing in the large intestine of both humans and canines. (Bednar et al. 2001; Ruseler-Van Embden et al. 1992). These bacterial populations are characterized as anaerobic or aerobic with large counts from 2 x 10^9 to 4 x 10^{10} per gram of feces (Simpson et al. 2002).

Health Impacts

After starch digestion and absorption, there is a spike in circulating blood glucose. If the dietary RDS is high and happens repeatedly for an extended time frame, it could lead to diabetes as the dog becomes insensitive to insulin. This could be remedied with a diet change (André et al. 2017). It is possible to slow this time to peak insulin response, and reduce the total amount of insulin the pancreas releases (Ribeiro et al. 2019). Slowly digestible carbohydrates or higher dietary fiber concentration in daily meals can result in a lower glycemic response (Brouns and Dye, 2004). Having a lower level of RS in the diet may also increase the chances of obesity as the excess energy supplied from the RDS that's converted into glucose can form adipose tissue increasing the likelihood of obesity since the dietary glycemic index plays a role in weight regulation (Brand-miller et al. 2013). As many as 53.8% of dogs in the US are considered overweight when compared to body condition scores from the WSAVA Nutritional Assessment Guidelines (Freeman et al. 2011). This number increased to 59.5% in 2018, so there is a trend for increasing obesity in US dogs (*Pet Obesity Survey Results*, 2018). These dogs are most likely consuming an extruded diet as it is the most purchased by consumers. Extruded diets are high in

RDS (Alvarenga and Aldrich, 2020). By increasing the amount of RS and SDS in the diet, the intense glycemic response dampens and there is better control of glucose concentrations (Kimura 2013). Although diet and nutrition are an important factor when addressing issues like obesity and diabetes, the dogs activity level also plays a large part (Sallander et al. 2001).

As discussed above, the RS can result in more SCFA production in the colon which may be beneficial to gut health. One study found that an increased circulation of SCFA's as a result of rats fed a diet with higher levels of fiber, were more resistant to allergic processes in the lungs (Trompette et al. 2014).

When feeding a diet containing resistant starch, it appears to have a larger impact on SCFA production when fed over a longer period, at least when compared to three weeks vs six weeks (Jackson et al. 2020). Some diets higher in RS led to a 36% increase in total SCFA production (Peixoto et al. 2018) and improved colon mucosal functions. A study conducted with swine indicated that a higher RS diet appeared to increase crypt depth and villus height which may improve the absorption of nutrients (Hedemann and Bach Knudsen, 2007; Peixoto et al., 2018).

As discussed above, feeding a diet that contains higher levels of RS can impact the health of different species. There are several studies that raise the possibility that this is also true in canines. One study comparing glucose concentrations in digestible and indigestible dextrins inferred "RS could be valuable for dietetic treatment of diabetes and obesity in dogs" (Kimura 2013). Even at a lower concentration of 0.5%, RS led to an increased gastric emptying rate in dogs fed a grain free diet (Richards et al. 2021). A separate study focusing on gut health found when fed a diet containing 1.46% RS to older dogs, the fecal pH had dropped, as well as more SCFA's were detected, and crypt depth increased. (Peixoto et al. 2018). The range of RS concentrations in these studies seems wide, but the results indicate that at least 1% can impact the health of a canine.

Conclusion

Regardless of trends set by the consumer, corn does have nutritional benefits that the canine digestive system can utilize and should not be considered simply a filler. The question is can a corn-based diet lead to improved gut health? What is important to consider is how the diets are being produced as the cooking intensity can have an impact on the starch fractions as well as nutrient digestibility. Our hypothesis is that decreasing processing intensity will increase dietary RS leading to beneficial changes in the diet utilization and colonic fermentation.

Although there are many studies that have reported benefits to corn inclusion in pet diets, none have effectively determined an optimal level of RS that would be most beneficial to improving the gut health and colonic fermentation. The proposed work will attempt to reduce the gap in our knowledge regarding how the processing methods such as pelleting, baking, and extrusion influence the diets levels of different types of starch and their impact on digestibility.

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Chapter 2 - Process Development of Health Benefits from Corn in Pet diets that Enhance Dog Utilization

Abstract

The methods of manufacturing pet diets have evolved over the past 160 years. Starting with a baked biscuit in 1860 to the widely used extrusion technology today. Formulas have also changed with once popular corn being criticized as inferior and indigestible. Different processing methods can change starch digestion. Less processing can lead to more indigestible starch (resistant starch) within corn diets. These may benefit gastrointestinal health. Therefore, the objective of this study was to determine the effect of process on dietary utilization of high corn diets, and changes to starch utilization which may provide health benefits. Experimental diets containing 56% corn as the sole starch source were produced through pelleting, baking, and extrusion and compared to a baked control diet in which the corn was replaced with dextrose. Among the corn diets, the extruded kibbles had the lowest ($P < 0.05$) differential scanning calorimetry (DSC, 0.469 J/g), pelleted and baked diets were intermediate (average 3.73 J/g) to the control. The extruded diet resulted in the highest ($P < 0.05$) level of gelatinized starch (32.5%) by the glucoamylase procedure and had the highest level of rapidly digestible starch (35.3%) and pelleted had the lowest gelatinized starch (16.8%) followed by the baked treatment (23.4%). Among corn treatments the extruded diet had the lowest level of resistant starch (0.5%) To evaluate the *in vivo* effects from these treatments, twelve beagle dogs were fed the experimental diets for 9 d adaptation and 5 d of collection in a replicated 4 x 4 Latin square designed study. The data were analyzed using the general linear mixed model with statistical analysis software (GLIMMIX proc; SAS v9.4, Cary NC) and means were considered different at

$\propto = 0.05$. The experimental diet was the fixed effect with period and dog as random effects. Dogs were fed to maintain body weight, with food intake similar among corn treatments and lower ($P < 0.05$) for those fed the control diet. Fecal scores (1: soft-liquid to 5: hard-dry) were slightly different across the three corn treatments, but each exceeded 3.5 which was considered ideal. However, dogs fed the control diet (dextrose) had soft-runny stools (average score of 1). Feces from dogs fed the baked treatment had a lower pH (5.37; $P < 0.05$) than the other corn treatments (average 5.71); whereas the pH of feces from the dogs fed the control (dextrose) diet were the highest ($P < 0.05$) at 6.87. This suggests that little to no dextrose reached the large intestine, while the baking process retained more resistant starch and likely increased saccharolytic bacterial fermentation. The dry matter apparent total tract digestibility was greater ($P < 0.05$) for the dogs fed the extruded corn-based diet and the control diet (average 75.75%) versus the baked and pelleted diets (average 69.49%). In conclusion, digestibility was high, and dogs maintained their body weight throughout the study. It was unexpected that dogs fed the control diet in which corn was replaced with dextrose would lead to diarrhea and soft stools. Among the corn containing diets, as the cooking became more intense there was a decline in resistant starch concentration and a corresponding increase in DM digestibility. Dogs fed the baked product had ideal fecal scores and lower fecal pH which suggests that the increased RS may improve colonic fermentation leading to positive impacts on gut health.

Introduction

Grains have lost traction as an acceptable pet food ingredient for reasons ranging from suspected grain allergies to desire for foods with “no fillers” (Banton et al. 2021). This may have been paused or reversed slightly following the July 2018 (Tyler 2021) alert from the Food and Drug Administration suggesting a link between dilated cardiomyopathy and grain-free dog diets (FDA, 2018). Some consumers have developed the perspective that cereal grains in a dog diet are unnecessary (Rasmusen 2010; Becker 2014a) because the dog’s common ancestor the wolf consumed a high protein and high fat diet from prey animals (Bosch, Hagen-Plantinga, and Hendriks 2015). However, the domestic dog (*Canis lupus familiaris*) co-evolved with humans and has adapted to increased grain consumption (Ollivier et al., 2016). As such the dogs digestive system has moved closer to that of an omnivore like humans or pigs (Bosch, Hagen-Plantinga, and Hendriks 2015).

It is a common belief that cereals like corn have poor nutritional value for the dog and are frequently referred to as “filler ingredients” (Khuly 2013; Becker 2014b; Hill’s 2020). That is not necessarily the case, as cereals are rich in starches which are available sources of energy (Sanderson 2021). They also contain dietary fiber, essential amino acids, phospholipids and essential fatty acids that contribute to the nutritional value of the food.

Starches are particularly important in binding and matrix development in processed pet foods. There are various techniques to characterize starch. One focuses on the rate of digestion. This method is described as rapidly digestible starch (RDS) and slowly digestible starch (SDS) which are both digested in the small intestine (K. Englyst and Englyst, 2004). While resistant starch (RS) escapes digestion which can be fermented in the colon. As starch gelatinizes in the presence of water, it becomes water soluble (hydroxyl groups exposed), loses birefringence, and swells (Cheftel 1986). Starch gelatinization provides functionality to the food and makes it more accessible for digestion by mammalian enzymes. Higher process temperatures gelatinize starches are required for RS2 (amylose starches) as compared to RS1 (native to pulses) that only needs to by physically broken down through milling, or even chewing (Peixoto et al., 2018; Ribeiro et al., 2019). Raw starch has the lowest level of digestible starch and highest level of RS.

Common processes applied to animal foods include pelleting, crumbling, steam flaking, expelling, baking and extrusion. These processes apply different levels of moisture, time, temperature and (or) pressure to the raw food matrix during conversion to a finished product. Pelleting applies minimal thermal energy through steam to aid mechanical compaction of the ration to make the pellet. Baking relies exclusively on thermal energy in the form of radiation, conduction and convection. Extrusion relies upon thermal energy from steam and mechanical energy through shear (friction) under pressure to cook the material. Extrusion has been reported to gelatinize the starch and elevate starch digestibility to nearly 100% (Bazolli et al. 2015). This leaves very little RS available in the finished product.

Resistant starch is a substrate that can impact colonic fermentation and increase production of SCFA’s such as butyrate that can have beneficial effects (Jackson et al. 2020). Butyrate is a critical energy source for colonocytes within the colon along with other SCFA’s as RS produces more than other forms of indigestible fibers (Sharma and Yadav, 2008; Vaziri et al., 2014). There have been studies that observed concentrations of RS from 0.5%-1.5% which influenced aspects of health such as obesity, diabetes by changing the concentration of butyrate in the large intestine (Kimura 2013; Richards et al. 2021; Peixoto et al. 2018). One study found that regardless of whether it was low or high temp processing, RS found in grains decreased after processing (Murray et al. 2001).

If one wanted to increase the amount of RS, a lower energy/gelatinization process would need to be identified and optimized. Pelleting and baking may be processes which could address this gelatinization and resulting RS values. Finding a moderate level of energy input can lead to an optimal concentration of RS remaining in the product. It was our hypothesis that processing at decreasing cooking intensities would lead to increasing levels of starch cook and thereby concentrations of resistant starch. The objective of our research was to determine if the change in concentration of resistant starch resulted in different levels of digestibility and impacted stool consistency.

Materials and Methods:

Fat Application Test:

For the corn-based formulas a preliminary experiment was designed to determine the optimal level of fat that can be externally coated without wicking onto the packaging. Small bags similar to commercial packaging were constructed out of multi-layered poly lined paper bags. The miniature bags were fabricated to be 16 cm x 10 cm. The pellets were sourced from a prior research study and consisted of a corn/soybean meal-based poultry feed. The baked biscuits were from a previous experiment and were predominantly red sorghum as the base starch source. The extruded kibble was a millet/barely based dog food formula produced on a pilot scale extruder for educational purposes. While the ingredient composition for these products differed in compositions to the corn-based products, they were able to help evaluate the fat holding capacity of the particular food form. All three products were coated with increasing levels of fat (2%, 4%, 6% and 8% on a weight/weight ratio). Every level of fat application was evaluated in triplicate. In a stepwise manner, the empty bags were weighed, then the food and fat were weighed (each in separate containers). To account for the fat that might adhere to the mixing bowl, it was filled with fat then emptied to leave a thin residual coating. The fat was then drizzled onto the food while being mixed in a folding action. Total mix time was one minute once all fat was applied. After mixing, the coated product was added to the empty bag and weighed before being stapled closed. Bags were then placed in a storage container at room temperature (22° C) for two weeks. After storage the food was removed from the bag and the empty bag was weighed. The change in bag weight was recorded and the difference in the amount of fat that wicked into the bag was calculated. Results are reported as the proportion of fat lost from the pellet/biscuit/kibble relative to the starting weight by the following equations. The accumulation of fat on the interior of the bag is based on the assumption that no fines from the products remained in the bag.

Equation 2.1: Determination of fat accumulation onto packaging

$$\%\, fat\ lost = \frac{final\ bag\ weight - initial\ bag\ weight}{fat\ applied\ to\ product}$$

Results (Figure 2.1) from this evaluation along with processing information was used to determine the proportion of fat to be applied internally and externally for each given process (Table 2.1).

Experimental dietary treatments

The diets for the animal evaluation were formulated (Concept5; Creative Formulation Concepts Staples, MN) to contain similar levels of crude protein (CP), crude fat (CF), ash, and crude fiber and were also formulated to be nutritionally complete for dogs at maintenance (AAFCO, 2018). Minor ingredient differences between the control and corn treatments were compensated by adding corn protein concentrate and slightly adjusting the chicken meal to help achieve the target nutrient content among the treatments (Table 2.1). The dry ration ingredients for the corn-based treatments was pre-mixed by a commercial pet food ingredient supplier (Lortchers, Animal

Nutrition Inc. Bern KS). The ingredients for the control diet were sourced as individual components from the same supplier. Chicken fat (IDF, Springfield MO) and flavor digest (AFB International, St. Charles MO) were sourced prior to the study conduct.

Control diet production

The dry ingredients were weighed and added to the mixer bowl. Based on the preliminary fat application experiment it was determined that 9% fat would be added internally and the remaining 1% externally as a topical coating to help adhere to the dry palatant. To accomplish this, the internal fat was mixed with the dry ingredients in a paddle mixer (Hobart A200, Troy Ohio) for a total batch size of 4.4 kg. The fat was added while mixing. Once all the fat was added, the batch was mixed for two minutes, then water was added, and the dough was mixed for an additional two minutes. The dough was placed on a smooth plastic work surface and rolled into sheets to a thickness of approximately 1 cm using rolling pins. Thickness was controlled using 1 cm thick metal guides. The sheet was cut with a rotary knife into 2 cm x 2 cm squares, and these were placed on metal cooking sheets. The sheets were placed in a convection oven and squares were cooked for 20 minutes at 150°C. Once removed the squares were placed onto cooling racks. Once cooled, the liquid fat (1%) was coated onto the squares while tumbling in a drum style mixer for a total of 60 seconds. The same procedure was then followed for the dry palatant. After coating, the final product was placed in Kraft paper poly-lined bags for two weeks until the feeding study.

Pelleted diet production

Before pelleting it was determined that the dry mix could contain up to 4% liquid fat before adversely affecting the final product. Thus, 4% liquid fat was added to the dry ingredients in a double ribbon mixer (Hayes and Stolz 23 kg HR2SSS-0106 Burleson, Texas). Once all the fat was added, the batch was mixed for five minutes. The pelleted diet was manufactured with a laboratory scale pellet mill (CPM California Pellet Mill, model CL-5 Crawfordsville, IN) equipped with a steam injection conditioner. The downspout conditioned mash temperature was 80º C. The die openings were 4.7 mm diameter with a knife cut-off at 12.7 mm. Once the temperature was achieved, pellets were diverted to a lab scale cooler for 15 minutes and cooled with ambient air until the pellets were equilibrated to ambient conditions. During production pellet temperature was recorded by collecting pellets at the discharge and immediately placing them into an insulated container with a thermometer inserted. Processing data were recorded and included flow rate (kg/min), motor load (%) and bulk density (g/L) in triplicate. The final product was coated in a manner similar to that described for the control diet with 3.3% fat surface applied along with the dry flavor. After coating the final product was stored in bags for two weeks until the animal feeding study was conducted.

Baked diet production

For production of the baked dietary treatment the internal fat was included at 6.3% into the dry ration which was weighed and blended into the dry mix with a paddle mixer (Hobart A200, Troy Ohio) for two minutes. A total of 1.2 kg of water was added then mixed for two more minutes. The dough was placed onto the work surface where it was rolled to a thickness of 1cm. Using a rotary knife, the dough was cut into 2 cm x 2 cm squares and placed on a cookie sheet. This was baked in a convection oven (Sunfire SDG-1 Cleveland, Ohio) for 25 minutes at 180º C. The biscuits were removed and placed on racks to cool. After the biscuits were cooled the same procedure for coating as described for the control diet was followed with a fat level applied to the exterior at 1% of the formula. After the final product was removed from the tumbler, they were stored in bags until for two weeks the animal feeding study.

Extruded diet production

The extruded diet was produced on a pilot scale extruder (Model: X-20/E325 Wenger Mfg/Extrutech Inc., Sabetha KS) equipped with a differential diameter preconditioner with steam and water injection set to nine and ten kg/hr, respectively. The screw profile was set to a standard pet food production setup (Figure 2.2). The kibble had specific product parameters to reach before drying. The discharge temperature out of the preconditioner was 85º C was required. Kibble size was measured with a micrometer. Once the desired size of 10±0.2 mm was reached, a density (g/L) measurement was taken of kibble coming off the extruder. Once all target parameters were achieved the wet mass flow rate was measured by collecting all product coming off the extruder for 60 seconds. Then kibbles were pneumatically conveyed to a double pass gas-fired pilot-scale dryer/ cooler system (Wenger 4800 Series Manufacturing, Sabetha, KS) and dried at 121º C for 5 minutes on the top belt, 8 minutes on the bottom belt, then cooled in a single pass cooler for 5 minutes with ambient air. The extruded kibbles were coated using the same tumbling process as described previously with all the formulated chicken fat (7.3%) topically applied followed by the 1% dry palatant. The final product was then packaged into bags and stored for two weeks until the animal feeding study.

Equation 2.2: Specific Mechanical Energy (SME)

$$SME\ (kJ\ /kg^{-1}) = \frac{\left(\frac{\tau - \tau_0}{100}\right) * \frac{N}{N_r} * P_r}{m}$$

where, τ is operational torque (%); τ_0 is the no load torque (24%); N is the extruder screw speed; 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 throughput.

Feeding and Stool Consistency

Experimental diets were fed to 12 Beagle dogs (4 spayed females, 8 castrated males) of similar age (6.5 +- 0.23yr), and body weight (BW) (11.6 +- 1.8kg) in a replicated 4 x 4 Latin Square design. Dogs were randomly assigned to experimental treatments and period according to the Balanced Latin Square Designed Excel spreadsheet-based program (Kim and Stein, 2009). The dogs were housed individually in pens (1.83 m x 1.20 m) with acrylic-coated mesh floor to allow for separation of urine and feces at the Large Animal Research Center (Kansas State University, Manhattan KS). The dogs were maintained in temperature controlled (23^{o} C) rooms with 16 hr light and 8 hr dark. Each period consisted of two weeks with an adaptation period during the first nine days, followed by a five-day fecal collection period. Every dog was weighed on d 1 and d 8, in order to adjust daily intake to correct for BW change. Each dog was fed twice daily and fresh water was provided at all times. Initial food amounts for each period were calculated from the equation based on their weight for inactive lab kennel dogs (NRC 2006) as follows:

Equation 2.3: Metabolizable Energy Requirements (MER)

$$MER = 95 * BW^{.75}$$

After determining the MER for the dog based of their BW, the caloric density of the diets was determined for the mass of food to feed to the dogs.\

Equation 2.4: Mass of diets fed

$$food(g)/day = \frac{MER}{(food\ kcal/g)}$$

The animal feeding protocol was approved by the Kansas State University institutional animal care and use committee under protocol #4097.5.

Sample Collection

After the nine days of acclimation, all feces were evaluated at least three times daily over the five d collection period. They were subjectively scored on a 5 point scale for consistency; wherein 1 = liquid stool; 2 = soft consistency, unformed stool; 3 = very moist stool that retains shape; 4 = well-formed stool that does not leave residue when picked up; 5 = very hard, dry pellets that crumble when pressed. A fecal score of 3.5 was considered ideal. After scoring, feces were collected in individual Whirl-pak bags, weighed, and stored frozen at -16° C pending further analysis. During each 5-d collection period, one fresh fecal sample from each dog was collected within 15 min of excretion and measured for pH by inserting a calibrated glass-electrode pH probe (FC240B, Hanna Instruments, Smithfield, RI) directly into the sample. The pH was analyzed in triplicate. Six 2-g aliquots of the fresh sample were transferred into plastic microcentrifuge tubes and stored at -80° C for later analysis of SCFA, and ammonia. After each collection period, feces were thawed at room temperature, pooled by dog, and dried in a forced air oven at 55° C for up to 48 h until dry to the touch. Diets and dried fecal samples were ground using a fixed blade laboratory hammermill (Retsch, ZM200, Haan, Germany) fitted with a 0.5 mm screen. Ground samples were stored in glass jars until nutrient analysis.

Processed Diet Nutrient Analysis

Processed diets from three replicates were ground, analyzed for dry matter, moisture, organic matter, and ash according to methods of the Association of Official Analytical Chemists (AOAC, 2019; methods 934.01 and 942.05). Crude protein content of the samples was determined by the Dumas combustion method (AOAC 990.03) using a nitrogen analyzer (FP928, LECO Corporation, Saint Joseph, MI). Crude fat was determined by acid hydrolysis (AOAC 954.02). Gross energy was determined by bomb calorimetry (Parr 6200 Calorimeter, Parr Instrument Company, Moline, IL). Titanium content in the samples was determined according to the colorimetric method described by Myers et al. (2004). Total dietary fiber (TDF) was determined (AOAC 2019; method 985.29) using the TDF assay kit (Megazyme TDFR-200a, Bray Ireland). Ground diets were tested for total starch, rapidly digestible starch, slowly digestible starch, and resistant starch using the digestible and resistant starch assay kit (K-DSTRS Megazyme Bray, Ireland). Total dietary starch and gelatinized starch were measured in a commercial laboratory (Wenger Manufacturing, Sabetha, KS) according to Mason et al. (1982). The energy absorption properties were determined by differential scanning calorimetry (DSC) (Q200, TA Instruments Waters- LLC, New Castle, DE). Each diet was put into solution of two parts water to one part dry matter. 25 to 40 milligrams of the solution was placed into a stainless steel high volume pan and closed with a lid that had an O-ring insertion. The DSC run parameters were to equilibrate at 10˚ C and ramp up to 140˚ C at 10˚ C /min for each test pan. Each product was run in duplicate. Integration of the endothermic curves provided by the DSC was completed using Universal Analysis 2000 software (version 4.7A, TA Instruments Waters-LLC, Newcastle, DE). The degree of cook was calculated as described below:

Equation 2.7: Degree of cook

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

The non-structural carbohydrate (NSC) value was determined as the sum of moisture, fat, TDF, protein, and ash subtracted from 100 which results in a crude starch value similar to the traditional NFE values under the Wende system. All nutrient analysis were performed in triplicate unless otherwise specified.

Fecal Nutrient Analysis

Dried feces were analyzed in triplicate for dry matter, organic matter, and ash according to methods of the Association of Official Analytical Chemists (AOAC, 2019; methods 934.01 and 942.05). Crude protein content of the samples was determined by the Dumas combustion method (AOAC 990.03) using a nitrogen analyzer (FP928, LECO Corporation, Saint Joseph, MI). Crude fat was determined by acid hydrolysis (AOAC 954.02). Gross energy was determined by bomb calorimetry (Parr 6200 Calorimeter, Parr Instrument Company, Moline, IL). Titanium content in the samples was determined according to the colorimetric method described by Myers et al. (2004). Total dietary fiber (TDF) was determined (AOAC 2019; method 985.29) using the TDF assay kit (Megazyme TDFR-200a, Bray Ireland). Two methods were utilized to estimate apparent total tract nutrient digestibility. The total fecal collection (TFC) method is widely used in animal nutrition research and requires the collection of all fecal material excreted by the animal. However, due to instances of occasional coprophagia by the dogs and loss of sample residue during daily pen sanitation, this method may lead to an overestimation of apparent total tract nutrient digestibility compared to the use of an indigestible dietary marker

(Alvarenga et al., 2019). Thus digestibility, was also determined using the marker method. Apparent total tract digestibility (ATTD) of dry matter, organic matter, crude protein, crude fat, ash, and gross energy was calculated by both the TFC (NRC, 2006) and marker methods (AAFCO, 2020b) by the following equations (2.5 and 2.6), respectively.

Equation 2.5: TFC Method

$$Nutient\ Digestibility\ \% = \frac{nutrient\ consumed\ \left(g*d^{-1}\right) - nutrient\ excreted\ \left(g*d^{-1}\right)}{nutrient\ consumed\ \left(g*d^{-1}\right)} * 100$$

Equation 2.6: Marker Method

$$Nutrient\ Digestibility\ \% = 1 - \frac{\%\ Nutrient\ in\ Feces * \%\ TiO_2\ in\ Food}{\%\ Nutrient\ in\ Food * \%\ TiO_2\ in\ Feces} * 100$$

Statistics

Data were analyzed as a replicated 4 x 4 Latin square design with 4 dietary treatments randomly assigned to 3 dogs during each of the 4 periods. Dogs were randomized to treatment and square by the procedure of Kim and Stein (Kim and H. H.Stein, 2009). Apparent total tract digestibility by total fecal collection and fecal marker titanium dioxide (TiO_2) methods, as well as intake and fecal parameters (fecal pH, wet and dry fecal outputs) were analyzed using the generalized linear mixed model (GLIMMIX) procedure with statistical software (SAS version 9.4, SAS Institute, Inc., Cary, NC), with diet as the fixed effect and period, square and dog nested within square as random effects. Least square means were assessed using the Tukey's post hoc test for multiple comparisons. Fecal scores were analyzed as ordinal data using a multinomial distribution in the GLIMMIX procedure with diet as the fixed effect and dog and period as random effects. Frequency of fecal scores within each diet were determined with the frequency (FREQ) procedure (SAS version 9.4, SAS Institute, Inc., Cary, NC). Results were considered significant at ($P < 0.05$).

Results

Processing data

Statistical analysis was not possible for all variables as some treatments did not have a sufficient sample replicates. The pelleted diet (82.8º C, Table 2.2) had the lowest ($P < 0.05$) temperature, extrusion was intermediate ($P < 0.05$, 100.2° C), and higher ($P < 0.05$) for the control diet (148.9º C), and baked treatment (176.6º C). Residence time was 20 min for the control and 25 min for the baked treatments. The pelleting process then had the lowest residence time (30 seconds). The extrusion residence time was not measured during production. The density of the final products was highest ($P < 0.05$) for the pelleted diet (606.2 g/L), then the baked treatment (498.0 g/L), followed by the control diet (424 g/L), and lowest ($P < 0.05$) density for extrusion (310.6 g/L). Pelleting had 0.6 kg/min flow rate and extrusion an estimated rate of 1.7 kg/min.

Diet composition

The moisture composition of experimental diets (Table 2.3) was higher ($P < 0.05$) in the control diet (13.9%) as compared to the corn containing diet (average8.1%), and lowest ($P < 0.05$) in the extruded treatment (5.2%). However, the water activity was low enough (average 0.4, Table 2.3) to not be a spoilage concern. The pelleted and baked diets had similar moistures and were below the maximum 10% (average 9.5%) threshold. On a dry matter basis, the protein (25.8%) was similar between the control, pelleted, and extruded treatments, but was slightly higher ($P < 0.05$) for the baked treatment (29.9%). The TDF was similar among the corn treatments, and the pelleted treatment (9.6%) was similar to the control treatment ($P < 0.05$, 9.6%). The fat (average 12.4%) and ash (average 7.2%) levels were similar for all treatments. The NSC estimation resulted in a lower ($P < 0.05$) concentration in the baked diet (35.8%), with the remaining diets similar to each other (average 40.5%). Across the diets, the TS measured with the Megazyme assay, was similar between the pelleted and extruded diets (average 40.0%). Although formulated to be similar, the baked treatment had less ($P < 0.05$) TS at (36.3%) than the corn-based treatments but it was similar to the control treatment (average 33.7%). Total dietary starch (TDS) measured with the amyloglucosidase assay from Wenger method followed a similar relationship. Wherein, the pelleted and extruded diet had similar TDS values (average 42.4%), while the control and baked were lower ($P < 0.05$) (average 33.9%). The percentage of gelatinized starch increased as the cooking intensity associated with each treatment increased. Among the corn-based treatments extruded kibble (85.5%) had the highest ($P < 0.05$) level of starch gelatinization as a % of total starch followed by the baked treatment (40.5%), and the pellets were the lowest (21.0%). Percentage of cook measured by the glucoamylase method followed the same order, apart from the control (95.4%) which had the highest ($P < 0.05$) level of cook. The kibble treatment (86.1%) was highest ($P < 0.05$), for the corn-based treatments, intermediate ($P < 0.05$) for baked (40.3%), and lowest ($P < 0.05$) for pelleted (19.9%). The RDS levels increased as cooking intensity increased, whereas SDS concentration declined with more intensive cooking processes. Similarly, RS yields were lower when cooking intensity increased with the lowest ($P < 0.05$) concentration in the extruded food (1.3%). The enthalpy of the diets was highest ($P < 0.05$) for the pelleted diet (3.5 J/g) and with baking (3.4 J/g) intermediate ($P < 0.05$) and the extruded and control treatments (average 0.6 J/g) were the lowest ($P < 0.05$).

Intake and fecal characteristics

All dogs were healthy throughout the study with a mean BW of 11.6 kg (range 8.6 to 14.1 kg) at day 0 and 12.3 kg (range 8.8 to 14.9 kg) at day 56. Food intake did not differ among dogs fed the corn treatments but was lower ($P < 0.05$) for the control treatment. Dogs fed the control food had diarrhea episodes (fecal score 1; Figure 2.3) during collection, thus had the lowest fecal scores (1, Figure 2.3), which made sample collection difficult. Stool scores for dogs fed the pelleted and baked diet were similar with most feces being consistent and firm (range of 3 to 4). Whereas dogs fed the extruded diet had slightly firmer feces than the other treatment groups (range 3 to 4.5; average 3.84; Table 2.4). The wet fecal output of dogs fed the pelleted and baked diets was greater (Table 2.4; $P < 0.05$) than dogs fed the control diet (average 107.9 vs 70.8 g/day), and wet fecal output of dogs fed the extruded treatment was similar to the extremes (91.1 g/day; Table 2.6). After drying, there was more fecal dry mass (Table 2.4) when dogs were fed the pelleted, baked and extruded foods compared to the control diet (average 38.3 g/day vs $P < 0.05$). The fecal output differed slightly among the corn diets ($P < 0.05$). The dogs fed the pelleted and baked diets had similar fecal output (range of 105-111.1 g/d). The feces from dogs fed extruded kibble were intermediate to the other three treatments (91.1 g/d), and slightly lower than the pelleted, baked, but were higher than the control diet (70.8 g/d). The fecal pH for dogs fed the control diet (6.87) was the highest among treatments ($P < 0.01$), followed by the kibble and pellets that had similar values (average 5.7) and the baked treatment which had the lowest fecal pH measured (5.37; Table 2.4) among the treatments.

Apparent Total Tract Digestibility

The apparent total tract digestibility (ATTD) of nutrients were determined by the total fecal collection (TFC) (Table 2.5) method, and by indigestible marker titanium dioxide (TiO_2) (Table 2.6). For the estimate of TFC digestibility, dogs fed the control diet had the highest ($P < 0.05$) digestibility across all measured parameters (Table 2.5). For dogs fed the corn containing diets, no differences were observed in ATTD among processed treatments for DM, OM, CF, and TDF. However, the crude protein (CP) ATTD was the highest ($P < 0.05$) for those dogs fed the extruded diet relative to the baked and pelleted diet. The GE digestibility among dogs fed the corn diets was highest ($P < 0.05$) for those fed the extruded (87.8%), dogs fed the pelleted diet were lowest (84.9%), and dogs fed the baked diet (85.5%) were intermediate. The ash digestibility for dogs fed the corn diets was highest ($P < 0.05$) for those fed the baked diet (45.5%) and extruded, followed by the pelleted diet (24.8%). The NSC digestibility was highest ($P < 0.05$) for dogs fed the extruded diet (98.8%). The dogs fed the control and pelleted had similar digestibility to each other (average 96.4%), and the baked diet. Dogs fed the baked diet did have the lowest ($P < 0.05$) digestibility (94.9%).

Dry matter digestibility estimated by TiO_2 marker for dogs fed the control diet was different ($P < 0.05$) from all other diets and was between the pelleted and baked diets (76.9%) The dogs fed the extruded kibble had the highest ($P < 0.05$) ATTD compared to those fed the baked, and pelleted diets. (84.9, 79.7, and 72.4% respectively; Table 2.6). The same trend was observed for the ATTD of OM. The digestibility was highest ($P < 0.05$) in the extruded diet (88.1%), then the baked diet (82.6%), with the pelleted diet (77.5%) having the lowest ($P < 0.05$) digestibility. The CP digestibility was highest ($P < 0.05$) when dogs were fed the extruded kibble (88.9%), and lower when they were fed the baked treatment (83.2%), pelleted or control diet (average 75.1%). Crude fat digestibility was not different for dogs fed the control, kibble, or baked diets (average 94.5%) but was lower ($P < 0.05$) for dogs fed the pelleted diet (92.1%). Similarly, TDF ATTD was the greatest ($P < 0.05$) when dogs were fed the control and extruded diets (39.7 and 46.3%) and intermediate when they were fed the baked (24.2%), relative to the pelleted food (5.2%). Gross energy ATTD was highest when dogs were fed the extruded kibble (89.6%), intermediate for the baked and control diets (average 84.1%) then lowest for dogs fed the pelleted treatment (80.1%). Ash digestibility was lowest ($P < 0.05$) for the pelleted diet (4.4%), with ATTD for dogs fed the baked diet intermediate digestibility (38.3%) to the dogs fed the extruded (47.6%). Dogs fed the control diet (41.9%) resulted in a digestibility between the baked and extruded diet. The NSC digestibility was lowest ($P < 0.05$) for dogs fed the control diet (92.4%). Dogs fed the baked diet (94.7%) were similar to the control and pelleted diets (95.9%). The dogs fed the pelleted diet were close to the ones fed the extruded diet which had the highest ($P < 0.05$) NSC digestibility (99%).

Discussion

One of the goals of the study was to determine the impact of different processing methods on the corn-based pet food/treat with an emphasis on the effect processing intensity has on starch digestion as it relates to retention of RS. The resistant starch (RS) fraction by definition escapes digestion in the small intestine and reaches the colon where it is available for fermentation by the native bacteria. The result of which would be short chain fatty acid production that are beneficial to gut health (Flint et al. 2012). This study processed a corn-based diet under different cooking intensities, using practical methods currently utilized in the pet food industry. Corn was selected even though its use has become less popular relative to the gain in market share of grain-free diets that exclude corn and other cereals. Corn is a good source of energy, and remains the highest volume ingredient used in pet food (IFEEDER, 2020) and there is published data about how it behaves with processing to achieve a desired level of resistant starch (Ribeiro et al. 2019; Peixoto et al. 2018).

After producing the diets there was concern regarding the shelf-life of the control diet due to the high moisture content in the final product. To verify the food was not susceptible to spoilage by mold growth, the water activity was measured in all the diets. Although the moisture in the final product for the control treatment was high enough for mold growth to occur (14%), the humectant characteristic of dextrose reduced the free water and the a_w (Table 2.3) to (0.4). For dry pet foods have a long shelf life at a_w<0.85 (Santillana Farakos, Frank, and Schaffner 2013) but we targeted a level of a_w<0.65 and this was achieved across all diets.

The total starch for the baked treatment was lower than the pelleted and kibble treatments, even though they were produced from the same pre-mixed ration. No previous research was found in published literature that reported a similar outcome. To verify this difference, the samples were re-analyzed and similar results were observed. The measurement for TS and TDS were analyzed using wet chemistry. Whereas the NSC value is a calculation derived from adding all non-starch components and subtracting from 100. Interestingly, this yielded a similar observation where the NSC calculation was lower ($P < 0.05$) in the baked treatment than the other corn-based treatments. The reason for this difference is not currently obvious given each corn-based treatment was produced from the same base ration of ingredients. Furthermore, the control diet analysis for total starch yielded similar results to the corn treatments. This may be due to dextrose being an α-glucose molecule, which is the compound that is measured in the starch assay after enzymatic hydrolysis of the starch. Thus, this procedure is measuring glucose and not starch per se, and so dextrose will appear as if it were starch. This factor is also why the dextrose control diet had the highest gelatinized starch (98.7%) and RDS (98.1%) values as the assay ultimately measures starch by conversion to glucose. As anticipated the extruded kibble resulted in the highest concentration of gelatinized starch (85.7%) among the corn-based treatments which was similar to other studies that extruded a corn-based diet compared to other processing methods (Bazolli et al. 2015; İnal et al. 2018; van der Sman et al. 2018). Extrusion imparts mechanical and thermal energy, which accelerates the rate of starch conversion and gelatinization reducing SDS and RS (Zhang, Ao, and Hamaker 2006). This was similar to a study by Inal et al. (2018), in which extrusion resulted in at least four times more gelatinization than in a pelleted diet. The same study led to lower digestibility of the pelleted diet when compared to the extruded diet. This lower digestibility is likely due to the nearly raw nature of the starch in the diet using this processing method. The pelleting process includes some added moisture and heat from steam to aid forming the pellets. Therefore, small amounts of gelatinization is inevitable as moisture is added. During baking, the residence time (Table 2.2) was nearly six times longer, as well as a higher temperature compared to pelleting and may explain the intermediate gelatinization and digestibility measured. Digestibility is directly affected by cooking intensity, such that applying incremental levels of specific mechanical energy among various treatments will increase starch utilization by the animal (Alvarenga and Aldrich, 2020; İnal et al., 2018).

The enthalpy measured using the DSC to determine the starch gelatinization further supports this data (Table 2.3). The more energy (J/g) the sample can absorb corresponded to the potential for more energy applied which increased the level of starch cook and gelatinization (Pungor 1995). These results confirm the experimental premise regarding the added cooking intensity for the treatments in the current experiment. Wherein, the pelleted and baked diets absorbed at least two times more J/g than the extruded or control diet. This differential shows how much of an influence energy input can have on aspects of starch digestibility and conversely the level of RS. Employing this analysis for starch gelatinization had a similar outcome in another study that determined that extrusion left the final product almost completely gelatinized (Gibson andand Alavi, 2015).

The total starch (TS) was analyzed three ways. One was the amyloglucosidase digestion method according to Mason et. Al. (1982) performed by Wenger Mfg to determine gelatinized starch and compute starch cook. A second is the Megazyme kit digestion assay in which TS, RDS, SDS, and RS were determined. The third method was the estimation made by a calculation of NSC by difference. Interestingly, each of these methods had similar results across all treatments with the exception of the results for the baked treatment.

The conversion of starch observed as “percent of starch cook” in the convection oven for the baked treatment was 24.8% (Table 2.3). The pelleted diet had the lowest percent of cooked starch likely since it had only thermal energy applied in the form of steam rather than any mechanical energy. This steam application is primarily utilized as a kill-step and to aid in the compaction and adhesion of the ration to form the pellet. With the more cook that takes place there is an increase in RDS, with an inverse relationship with SDS (Zhang, Ao, and Hamaker 2006). It is important to note, that the measurement of gelatinized starch and starch cook are important. Though they were measured using different assays, both resulted in similar values across all treatments. Following this same relationship for RS, as it declined with increased cooking intensity. The pelleted food had the lowest cooked or gelatinized starch, which resulted in the highest concentration of RS, while extrusion had the opposite effect to produce lower RS.

It is possible that the undigested starch led to an increase in fermentation, as the amount of RDS increased and RS decreased.

The dogs remained healthy throughout the study with small body weight increase during the 56 days of the experiment. Although the amount of food fed per meal was adjusted weekly to maintain BW, the slight gain in weight may be attributed to inaccurate estimates of food energy due in part to the process differences and how they affected nutrient utilization. When producing the diets with different processes the expectation is that the intensity of cooking applied may have an impact on more than just the starch fractions. This also affected nutrient digestibility. The protein and fat digestibility, as well as ash mineral absorption could vary between extremes because of pelleting and extrusion (Stroucken et al. 1996; İnal et al. 2018) like those observed in this study.

When considering the total fecal collection (TFC) digestibility (Table 2.5) there was a distinctive difference between those values and the marker method (Table 2.6) as it relates to the control diet. For this treatment there was a nearly 10% higher nutrient ATTD for the TFC method compared to the marker method. This was primarily due to the difficulty in collecting all feces since dogs on this diet developed diarrhea. This inability to collect all of feces led to an underestimation of fecal excretion and thereby overestimation of digestibility. This further reinforced the potential unreliability of the TFC method and points to the value in having confirmation using two analysis methods. The TiO_2 marker would, in this case be considered the more reliable method (Alvarenga et al., 2019).

As expected, the ATTD (TiO_2 marker, Table 2.6) of DM, OM, CP, CFat, TDF, and GE increased as cooking intensity increased (pelleting < baking < extrusion). A previous study reported similar findings in which extrusion led to higher nutrient digestibilities when compared to a low cooking intensity process like pelleting (İnal et al. 2018). A different processing method is not the only option to change the cooking intensity but there is also the potential within a process like the extrusion itself to manipulate the energy input in terms of thermal energy or shear force on the product (Jackson et al. 2020; Pacheco et al. 2018) and to affect nutrient digestibility and(or) starch digestibility estimates (Dust et al. 2004; Murray et al. 2001). However, that is not always the case. In a study with some similar variables to the current study, the nutrient digestibility for DM, crude fat, or nitrogen/crude protein were not influenced (Stroucken et al. 1996). However, this may have been a function of the use of a twin-screw extruder in their work; whereas, in the current experiment a single screw was utilized to produce this experimental treatment.

In the current study, stool consistency was negatively affected for dogs fed the control diet. These stools also had a higher pH and were unexpected. The feces for dogs fed the treatments with higher concentrations of SDS and RS (Ribeiro et al. 2019) had a lower pH. The lower fecal pH may have been associated with elevated lactic acid fermentation due to the increased RS concentration in the diet. Although this was not measured. Overall digestibility increased as cooking intensity increased. Although some studies (Boehlke, Zierau, and Hannig 2015; Ribeiro et al. 2019; Dust et al. 2004) indicated that RS affected the micro-biome with changes in SCFA concentration. This change may improve the overall health of the host. Future work should focus on the colonic fermentation effects from these treatments.

The results of higher concentrations of SDS and RS is the potential for positive change to overall health of pets through several avenues. This starts with influencing factors like lowering blood glucose levels to reduce the risk of diabetes (Maki et al. 2012) by protecting the insulin receptor and the insensitivity to insulin. This could be due to repeated spikes in glucose from foods high in RDS. Canine obesity may be linked to these changes in insulin sensitivity (LaFlamme, 2011). In turn obesity has been linked to heart disease and cancer (Weeth 2016; Thengchaisri et al. 2014). Thus, altering the cooking intensity and reducing RDS has the potential to reduce the risk of diabetes, obesity, heart disease and cancer in dogs and thereby benefit health maintenance avoid chronic diseases.

In future studies, an alternative non-starch ingredient to replace dextrose should be considered. Although it had no slowly digestible or resistant starch present as seen in the glucoamylase tests conducted at Wenger measuring glucose, it led to problems during the feeding portion of the study. An alternative to glucose might be a pre-gelatinized starch.

The application of fat in this study was slightly inconsistent across treatments. However, they were similar to how commercial products are manufactured so the results are representative to commercial products. All treatments had a portion of the formulated amount of liquid fat added internally to the product apart from the extruded treatment in which all liquid fat was applied as a coating.

Suggested next steps for this research topic would be to measure the SCFA concentrations in the fresh feces to determine if diets influenced the gut microbiome population with changes of resistant starch. Conducting a dose study by changing the concentration of starch ingredients and utilizing a single process to explore the impact specified levels of RS has on canine gut health. Changing the cooking intensity of a single process to target a desired RS concentration by reducing the mechanical energy input. Either through changing the machine settings, or lubricants in the formulation to reduce shear. Another opportunity would be to develop a potential corn-based baked food or treat that contains higher resistant starch concentration for improved health.

Conclusion

The results of this study determined that starch concentration of RDS, SDS, and RS of a corn-based diet was affected by the processing method employed during production. As the cooking intensity increased from pelleting to baking to extrusion, the RDS increased, SDS and RS decreased. The digestibility for the experimental diets increased for each diet, as the cooking intensity increased.

The change in cooking intensity not only increased concentration in RS but also appears to have affected the fecal characteristics such as fecal score and pH. A lower pH observed for the baked and pelleted diet would suggest higher organic matter fermentation was taking place in the colon. This could potentially result in the improved gut health through increased levels of SCFA's such as butyrate, acetate, and propionate that should be explored in future work.

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Figure and Tables

Table 2.1: Ingredient composition (% basis) of experimental treatments for process development of differences in resistant starch

	Dextrose	Corn		
Ingredients	Baked	Pelleted	Baked	Extruded
Corn	-	56.2	56.2	56.2
Dextrose	45.40	-	-	-
Chicken By-Product Meal	14.00	13.80	13.80	13.80
Spray Dried Plasma	10.00	10.00	10.00	10.00
Corn Protein Concentrate	7.00	-	-	-
Fish Meal	5.00	5.00	5.00	5.00
Cellulose	4.00	4.00	4.00	4.00
Dicalcium Phosphate	1.75	1.00	1.00	1.00
Potassium Chloride	0.65	0.40	0.40	0.40
Titanium Dioxide	0.40	0.40	0.40	0.40
Calcium Carbonate	0.25	0.35	0.35	0.35
Choline Chloride (60% dry)	0.20	0.20	0.20	0.20
Potassium Sorbate	0.10	0.10	0.10	0.10
Trace Minerals Premix[1]	0.10	0.10	0.10	0.10
Vitamin Premix[2]	0.15	0.15	0.15	0.15
Chicken Fat, Internal	9.00	4.00	6.30	0.00
Chicken Fat, External	1.00	3.30	1.00	7.30
Flavor	1.00	1.00	1.00	1.00

[1] Vitamin Premix: Vitamin E Supplement, Niacin Supplement, Thiamine Mononitrate, d-Calcium Pantothenate, Vitamin A Supplement, Sunflower Oil, Pyridoxine Hydrochloride, Riboflavin Supplement, Vitamin D3 Supplement, Biotin, Vitamin B12 Supplement, Folic Acid.

[2] Trace Mineral Premix: Zinc Proteinate, Calcium Carbonate, Zinc Sulfate, Iron Proteinate, Ferrous Sulfate, Copper Proteinate, Copper Sulfate, Manganese Proteinate, Sunflower Oil, Sodium Selenite, Manganous Oxide, Calcium Iodate, Ethylenediamine Dihydroiodide.

Table 2.2: Processing parameter averages from production of experimental corn and control diets

	Control	Pelleted	Baked	Extruded	SEM	*P*-value
Process Temp (°C)	148.9^{a}	82.8^{b}	176.6^{c}	100.2^{d}	0.6	<.0001
Residence Time (min)	20.0	0.5	25.0	*NM	**N/A	**N/A
Density (g/L)	424.0^{a}	606.2^{b}	498.0^{c}	310.6^{d}	6.9	<.0001
Final Product Flow Rate (kg/min)	*NM	0.6	*NM	1.7	**N/A	**N/A
Operating torque %	*NM	57.0	*NM	44.0	**N/A	**N/A
Discharge Temperature	*NM	180.0	*NM	203.0	**N/A	**N/A
Feed Rate (kg/hr)	*NM	1.4	*NM	80.8	**N/A	**N/A

*NM = Not Measured

**N/A = Not Available

abc in each row means with different superscripts differ $P<0.05$

Table 2.3: Nutrient composition of a dextrose control diet and corn based pet foods produced by pelleting, baking, and extrusion (DM basis)

Item	Control	Pellet	Baked	Extruded	SEM	*P*-value
Moisture, %	13.9^{a}	9.6^{b}	9.4^{b}	5.2^{c}	1.3	<.0001
Water Activity	0.4	0.5	0.5	0.2	N/A	N/A
Dry Matter, %	89.7^{a}	91.1^{b}	90.7^{b}	94.8^{c}	0.1	0.1376
Protein, %	26.6^{a}	24.8^{a}	29.9^{b}	26.1^{a}	0.5	0.0003
Fat, %	12.7	12.2	12.4	12.1	0.3	0.1421
Total Dietary Fiber, %	9.6^{a}	10.8^{ab}	11.1^{b}	11.5^{b}	0.3	0.0163
Ash, %	7.5	6.9	7.6	6.9	0.4	0.656
Gross energy kcal/g	4449.3^{a}	4409.9^{b}	4445.8^{b}	4612.9^{b}	273.7	<.0001
NSC, %*	39.1^{a}	41.3^{a}	35.8^{b}	41.1^{a}	1.2	0.0021
TS^{+++} %	31.0^{a}	40.0^{b}	36.3^{c}	40.0^{b}	0.8	<.0001
TDS, %$^{+}$	31.2^{a}	42.4^{b}	36.6^{c}	42.4^{d}	4.8	<.0001
Gelatinized starch, %	29.8^{a}	8.4^{b}	14.7^{c}	34.2^{d}	0.6	<.0001
Gelatinized starch % of TS	96.1^{a}	21.0^{b}	40.5^{c}	85.5^{d}	.02	<.0001
Starch Cook %	95.4^{a}	19.9^{b}	40.3^{c}	86.1^{d}	4.9	<.0001
RDS, %**	30.2^{a}	13.9^{b}	22.1^{c}	35.8^{d}	0.4	<.0001
SDS, %***	0.9^{a}	17.0^{b}	9.8^{c}	2.8^{a}	0.7	<.0001
RS, %$^{++}$	0.1^{a}	2.1^{b}	1.3^{c}	0.5^{d}	0.1	<.0001
RDS, % of TS**	97.5^{a}	34.8^{b}	60.9^{c}	90.0^{d}	4.2	<.0001
SDS, % of TS***	3.1^{a}	42.6^{b}	27.1^{c}	7.1^{a}	6.4	<.0001
RS, % of TS^{++}	0.3^{a}	5.2^{b}	3.6^{c}	1.3^{d}	0.1	<.0001
Enthalpy J/g (DSC)	0.1^{a}	3.5^{b}	1.4^{c}	0.1^{a}	0.1	<.0001

*Non-structural carbohydrates diets (NSC) **rapidly digestible starch (RDS), ***slowly digestible starch (SDS), $^{+}$total dietary starch (TDS), $^{++}$resistant starch (RS), $^{+++}$total starch (TS)

*****NSC determined by adding the macro-nutrient (Moisture, CP, Cfat, TDF, Ash) percentages and subtracting them from 100
NSC = 100 - (Moisture + Protein + Fat + Fiber + Ash)

^{abc} Means in a row with unlike superscripts differ $P<0.05$

Table 2.4: Intake and fecal characteristics measured during the feeding study of a dextrose diet and corn based experimental diets

	Dextrose	Corn				
Item	Control	Pellet	Baked	Kibble	SEM	*P*-value
Food Intake, g DM/d	158.0^{b}	195.0^{a}	194.0^{a}	204.0^{a}	10.40	0.0015
Wet fecal Output, g/d	70.8^{b}	111.1^{a}	104.7^{a}	91.1^{ab}	10.88	0.0005
Dry Fecal output g/d	18.7^{b}	40.8^{a}	38.0^{a}	36.1^{a}	3.23	<.0001
Fecal DM %	26.4^{b}	36.7^{a}	36.3^{a}	39.6^{a}	1.02	<.0001
Fecal Score	1.0^{c}	3.8^{b}	3.6^{b}	3.8^{a}	0.05	<.0001
Fecal pH	6.9^{a}	5.5^{bc}	5.4^{c}	5.8^{b}	0.10	<.0001

Abbreviations: DM: Dry matter

^{abc} Means in a row with unlike superscripts differ P<0.05

Table 2.5: Apparent total tract digestibility (ATTD) of experimental diets by dogs using total fecal collection (TFC) method of estimates

	Dextrose	Corn				
ATTD TFC, %	Control	Pelleted	Baked	Extruded	SEM	*P*-value
Dry Matter	88.6^{a}	79.2^{b}	80.6^{b}	82.3^{b}	1.38	<.0001
Organic Matter	90.4^{a}	83.0^{b}	83.4^{b}	86.0^{b}	1.21	<.0001
Crude Protein	87.2^{a}	82.4^{c}	84.0^{bc}	87.0^{ab}	1.41	0.0004
Crude Fat	96.6^{a}	93.5^{b}	93.5^{b}	94.3^{b}	0.53	<.0001
TDF*	70.3^{a}	28.5^{b}	27.8^{b}	37.0^{b}	4.10	<.0001
Gross Energy	91.8^{a}	84.9^{c}	85.5^{bc}	87.8^{b}	1.01	<.0001
Ash	66.9^{a}	24.8^{c}	45.5^{b}	39.6^{b}	4.38	<.0001
NSC**	95.9^{ab}	96.9^{ab}	94.9^{b}	98.8^{a}	0.88	<.0001

*Total dietary fiber (TDF) **Non-structural carbohydrates

^{abc} Means in a row with unlike superscripts differ $P < 0.05$

Table 2.6: Apparent total tract digestibility (ATTD) of experimental diets by dogs determined by indigestible marker method using titanium dioxide

	Dextrose	Corn				
ATTD TiO_2	Control	Pelleted	Baked	Extruded	SEM	*P*-value
Dry Matter	76.9^{c}	72.4^{d}	79.7^{b}	84.9^{a}	0.65	<.0001
Organic Matter	80.5^{c}	77.5^{d}	82.6^{b}	88.1^{a}	0.62	<.0001
Crude Protein	73.5^{c}	76.6^{c}	83.2^{b}	88.9^{a}	1.60	<.0001
Crude Fat	94.0^{a}	92.1^{b}	94.0^{a}	95.5^{a}	0.47	<.0001
TDF*	39.7^{a}	5.2^{c}	24.2^{b}	46.3^{a}	2.27	<.0001
Gross Energy	83.4^{b}	80.1^{c}	84.7^{b}	89.6^{a}	0.52	<.0001
Ash	41.9^{ab}	4.4^{c}	38.3^{b}	47.6^{a}	1.92	<.0001
NSC**	92.4^{c}	95.9^{ab}	94.7^{bc}	99.0^{a}	0.86	<.0001

*Total dietary fiber (TDF) **Non-structural carbohydrates

^{abc} Means in a row with unlike superscripts differ $P < 0.05$

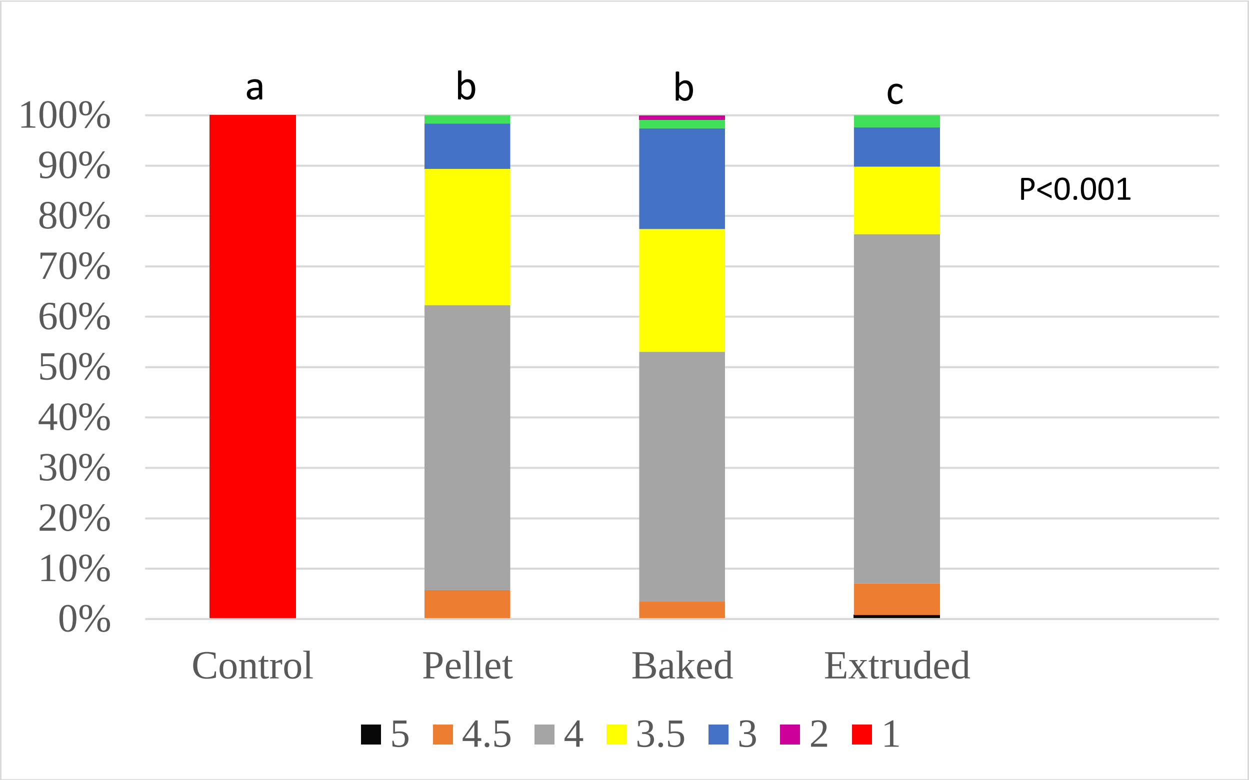

Figure 2.1: Distribution of fecal scores from dogs fed a control diet formulated of dextrose and experimental diets with corn, and 3 corn diets of which the processes of production were of different cooking intensities in which they were pelleted, baked or extruded in comparison to the control which replaced corn with dextrose. Scores were 1-5 on .5 intervals and represent 1 as soft and without form, 3 as soft with form and 5 as hard and dry and the ideal score being 3.5. Each color represents the percentage of collections that were collected in relation to the correlating color.

^{abc} Means fecal score among treatments with unlike letters differ $P < 0.05$

Figure 2.2: Images of the dextrose and corn based final products

A: Control Treatment (Dextrose)

B: Pelleted Treatment (Corn)

C: Baked Treatment (Corn)

D: Extruded Treatment (Corn)

Chapter 3 - Starch Characterization of Baked Dog Treats

Abstract

Baking is a common process to produce pet foods and treats. Especially treats. These commercial products could serve as a sample set to determine the range of starch gelatinization and RS due to baking. Our hypothesis was baked treats would contain a wide range of resistant starch levels. The specific objective was to evaluate the nutrient composition, total starch, digestible starch, and resistant starch from a sample size of 30 total treats. The study was conducted in a 2 x 3 factorial arrangement of treatments, with the main effects of size (small, medium, large), and presence or absence of wheat. For the starch fractions, the size of the treat did not affect the total starch (P=.91), digestible starch (P=.89), or the resistant starch (P=.23). The same result for the main effect of wheat wherein; there was no effect on the total starch (P=.56), digestible starch (P=.51), or resistant starch (P=.91). Since total starch was not different, comparing the starch fractions as a percentage of total starch also did not result in differences for digestible or resistant starch. However, there was a wide range (1.4% to 3.5%) in RS on a % of TS for samples that contained wheat vs those without (1.1% to 2.1%). This was also observed in the different sizes of treat indicating that there was wide variation among the samples collected. A post-hoc analysis between whether the treat was grain-free or not, did determine that the grain-free treats had higher concentrations of resistant starch ($P < 0.05$) and might point to a new direction of inquiry.

Introduction

Dog treats are intended for supplemental or intermittent feeding so are not required to be nutritionally complete or balanced (AAFCO 2012). Although treats are not necessary in a dogs' diet, a majority of owners have stated they use treats as a reward. They can be beneficial as aids to dental health, or as a means to increase bonding between pet and owner, or simply for pampering (Morelli et al. 2020). Though there may be more, very little published research is available regarding the nutrition of baked foods or treats for dogs. Typically the nutrition of a baked treat is not considered since they are not meant as a meal, but for supplementation. If a consumer were to follow the guidelines to the letter, and reward their pet with a treat, they would likely exceed the estimated caloric needs for their pet. There are many dog treat options available in the pet food market which are produced in a wide array of processes, forms and ingredients. They include baked biscuits and cookies, meaty chunks, bones, rawhide chews, and injection molded dental treats to name a few. Many of the products manufactured are rotary molded baked treats similar to a "Milk-Bone™" product. Most of these are produced with wheat as the primary grain ("Pet Food Production and Ingredient Analysis" 2020).

The baking process depends exclusively on thermal energy through convection, radiation and conduction. The process starts when liquid ingredients are mixed with dry ingredients to form a dough. This dough production is generally a batch process prior to molding or forming the biscuit. Though beyond the mixing, baking has potential to be a nearly continuous process (like extrusion) if a rotary molder and tunnel ovens are used. Mixing to form the dough is very important as the moisture can impact treat extraction from the die, hardness, drying, and, flavor. Achieving the correct consistency can help with extraction of the treat from the molder or help with uniform flow of the sheeting or cutting operation. Sufficient water to hydrate the dough is vital for starting the gluten generation or other binding activation. However, excess moisture can be a challenge for production when that moisture has to be removed. This can take time to get down to the level of water activity that will not cause mold growth. One of the main functions of baking is the removal of moisture (Davidson 2016) followed by structure formation and starch gelatinization. For gelatinization to occur all that's required is added water and the granules start to swell unfurling the structure into more digestible fractions of starch decreasing the amount of resistant starch (X. Liu et al. 2009).

There are many starch flour sources used in the baking industry that can be used such as wheat, corn, potatoes, or rice. Various ingredients for structure building and binding are needed in the baking process. As an example, wheat or potato starches have their own unique binding characteristics. When creating the dough the starch granules begin to absorb a limited amount of water, start to swell, and gelatinization begins (Davidson 2016). The gelatinization that occurs results in higher levels of rapidly digestible starch and lower levels of RS that might pass into the colon.

The assumption was that there would be a lower level of starch gelatinization or cooking from baking than extrusion and quite possibly more raw starch resistant to digestion. However, one study noted that while a larger particle size reduced gelatinization, the overall starch digestibility was not affected (Bazolli et al. 2015). Resistant starches bypass intestinal digestion and become a fermentable substrate in the colon. Thereby acting as a prebiotic that may benefit colonic health through bacterial fermentation and production of short chain fatty acids like butyrate (Corsato Alvarenga et al., 2021; Jackson et al., 2020; Ribeiro et al., 2019; Peixoto et al., 2018). A higher concentration can lead to increased fermentation of SCFA's that can improve canine gut health (Murray et al. 2001; Ribeiro et al. 2019; Raigond, Ezekiel, and Raigond 2015). This could be an added benefit to baked foods/treats which has been overlooked in the past.

Most baked treats are produced with wheat flour as a primary ingredient. This has been exploited to aid development of texture and durability. Some new products are being explored which eliminate wheat, such as grain-free, or even those that use an alternate sources of cereals like sorghum or oats (Almeida and Aldrich, 2021). Tubers and legumes which comprise most of the starch in grain-free products might be higher in RS type 2 (Corsato Alvarenga et al., 2021) due to their starch granule structure. Unlike cereals, tubers and legumes do not contain pores and channels on their starch granules that serve as attachment sites for saccharolytic enzymes to digest the starch (Bhattarai et al., 2018, 2017). Barley and oats as an alternate source of starch have been shown to lower pre-cecal digestibility in the dog when compared to corn or rice (Walker et al., 1994) which contribute more RS. Previously, a study to quantify the starch components of extruded dog and cat foods which were either grain-based or grain-free foods. In this reported work, extruded foods had low levels of RS among all foods evaluated (Corsato Alvarenga and Aldrich, 2020). There were minimal differences in overall concentrations of RS in extruded kibbles, with many samples below the limit of detection. There was also a high variability associated with the RS assay.

Presuming that RS is beneficial, evaluation of another food type processed in a different manner might provide some insight on where the opportunity to increase RS exists. Our hypotheses was that baked treats would contain a meaningful concentration of resistant starch (RS). The objective was to determine the connection products and whether factors such as size or ingredient composition had an effect. Since a key step in baking is moisture removal by heat, the penetration of heat may be affected by the size and dimension of the treat, it may take more heat or residence time with larger products as the moisture has more mass to travel through and(or) the mass may potentially reduce heat penetration. The longer time before moisture removal takes place, the longer the starch has the moisture to gelatinize. All that is needed for gelatinization to occur is moisture and heat simply accelerates the rate (Davidson 2016).

Materials and Methods

Power analysis

To determine the number of samples required to address our hypothesis, a power analysis was conducted using the POWER procedure from statistical software (SAS v 9.4, SAS Institute, Inc., Cary, NC). Data from a previous study in which the RS least square means were 1.062 and 0.828% for grain-free and grain-based diets, with a pooled standard deviation of 0.18% was used (Corsato Alvarenga and Aldrich, 2020). Assuming a greater variation, the power calculation using a 2-sided t-test with an α= 0.05 and power of 90% indicated that we would need 14 samples per treatment group to identify a difference if one truly existed. To assure that there were plenty of samples it was determined that 30 products would provide a difference if one truly existed.

Sample selection and purchasing

The sampling frame was selected according to a market research website (Statista, 2021). All adult dog baked treats from the top four US pet food companies by sales of treats were included: Smuckers, Nestle Purina Petcare, Mars Petcare and Blue Buffalo. A list was created by selecting only dry baked treats with company name, treat brand, product name, the ingredient composition (with and without wheat), flavor designation, and caloric content per kg and per treat. There was a total of 85 baked treats identified among all treats sold by each company (Table 3.1). Treat mass in grams was calculated by multiplying treat calories by 1000 and dividing by the caloric content per kg reported on the package. From this the treats were divided into three sizes according to their estimated mass: small, medium and large. The small size ranged from 0.95 to 4.21 g with an average (± SD) of 2.45g ± 0.973 and median of 2.35 g. The medium size ranged from 4.33 to 9 g, had an average (±SD) of 7.40 g ± 1.522 and median of 7.82 g. The large size ranged between 9.03 and 39.68 g, averaged (±SD) 17.47 g ± 8.90 and had a median of 13.64 g. The sampling frame was divided into main effects for selection criteria: small, medium, large (3), and with or without wheat (2; Table 3.1).

Samples were assigned a random number using the RAND function (Microsoft Excel) then sorted in descending order and the first 5 treats for each treatment combination were randomly selected resulting in 30 total products (Table 3.2). These products were purchased from online retailers.

Sample processing

Upon receipt of the baked treats, a subsample (100 g of each product) ground into crumbles through a disc-type laboratory mill (3303 Perten, Springville, IL, U.S.A.). These crumbles were further ground to 0.5 mm sieve using a laboratory fixed blade impact mill (Retsch, type ZM200, Haan, Germany). Each sample was stored in 4 oz. plastic bags (Whirl-Pak®, Madison, WI, U.S.A.) at room temperature until analyses were conducted.

Nutrient analyses

Total starch (TS), digestible starch (DS), RS and total dietary fiber (TDF) were determined using enzymatic kits (K-RSTAR for total starch and RS and K-TDFR-200A for

TDFs; Megazyme Inc., Ireland). All samples were analyzed for dry matter, organic matter, and ash according to the methods of Association of Official Analytical Chemists (AOAC, 2019; methods 934.01 and 942.05). Crude protein content of the samples was determined by the Dumas combustion method (AOAC 990.03) using a nitrogen analyzer (FP928, LECO Corporation, Saint Joseph, MI). Gross energy was determined by bomb calorimetry (Parr 6200 Calorimeter, Parr Instrument Company, Moline, IL). Fat by acid hydrolysis (AOAC Official Method 954.02) and crude fiber analysis (AOAC Official Method 978.10) were conducted at a commercial laboratory (Missouri Analytical lab Inc. Columbia, MO 65211). A mathematical estimate of starch, as non-structural carbohydrates (NSC) was also calculated using the following equation:

Equation 3.1:

$$NSC = 100\% - [Protein\% + Ash\% + Moisture\% + TDF\% + Fat\%]$$

Resistant starch was reported as a proportion of total starch. This allows for comparison of diets with different formulas.

Statistical Analysis

All variable concentrations for treats with wheat vs without wheat, and large vs medium vs small treats were analyzed as a 2 x 3 factorial arrangement of treatments using statistical software (SAS v. 9.4 Carry, NE). Main effects of treat composition (with and without wheat), treat size (small, medium, and large), as well as their interaction were included in the model. Product characteristic of grain-free or grain-based was added as a random factor, and a natural log transformation was used on RS as a percent of total starch to help meet studentized residuals and model assumptions. Significance was considered at a $P < 0.05$, and marginal significance at a $0.05 < P < 0.10$. Then a post-hoc analysis of grain-based vs grain-free treats was also evaluated with the products that fit these categories to help explain some of the results.

Results

The sampling frame included 85 products of which more were without wheat (Table 3.1). Only 30 products were selected that fit the experimental criterium. Of the 30 products, the first five ingredients identified on the product labels are listed (Table 3.2). In this, 24/30 products had a starch source as the first ingredients and 16/30 and had a starch source as the second ingredient.

There were no significant interactions between main effects. Therefore, the main effect means will be described. The main effect means for treats with wheat were similar (Table 3.3) in DM, OM, TS, DS, RS, NSC, TDF, GE, CP, Cfat, and ash compared to treats without wheat. The main effect means for size (small, medium, and large) did not differ for (Table 3.3) DM, OM, TS, DS, RS, NSC, TDF, GE, CP, Cfat, and ash either. There was a large range in RS as a percentage of TS between the samples with wheat (1.4% to 3.5%), and without wheat (1.1 to2.1%) which suggests large variation in the analysis, and also that there were some samples that had higher than expected concentrations of RS. In two samples that were grain free, (samples 27 and 30) the RS was very high relative to others (9.1% and 14.5% respectively).

Discussion

Based on the results from our previous study where different processing methods have been used to determine how RS concentrations changed with cooking intensity, the goal was to determine if commercially baked products had similar levels of RS to what was observed previously. Having a starch source as one of the first five ingredients reinforces how critical this ingredient class is to the structure formation in baked treats. Although the formulation can vary significantly based on the desired product characteristics, baked goods generally have 71 to79% carbohydrates (Taggart 2004). Grains such as wheat and barley are common grains used to produce biscuits (Davidson 2016). Not because of consumer popularity, but in large part due to their functionality. Once water is added to a flour like wheat, it reacts with the gliadin and glutenin to make the protein gluten which becomes extensible and elastic (Davidson 2016). This elasticity develops, and the starch begins to gelatinize as water is absorbed and the starch granule starts to swell , disrupting that crystalline structure making the starch more digestible (Lai and Kokini, 1991; Sharma and Yadav, 2008). For treats that do not contain gluten like potato starches, binding agents must be added to aid gelling and thickening. An option is the use of an animal protein such as spray dried egg or animal plasma that enhance the dough (Nogueira and Steel, 2018).

There wasn't an observable difference between the wheat vs. no wheat treats. This comparison was explored because wheat as a grain possesses either the same or higher concentrations of RS compared to other cereals like corn, barley, rice, or sorghum based on the percentage of total starch (Murray et al. 1999; 2001; Dupuis, Liu, and Yada 2014). These RS concentrations in grains may be influenced through processing; for example, baking differs from extrusion. Extrusion converts the starch to be nearly completely digestible; whereas baking might offer a way to retain a meaningful amount of RS in the final product.

Among the treatments the low RS was unexpected. Rather it was anticipated that a higher percentage of resistant starch (as a % of the total starch) would be much greater than in a baked food was reported previously for extruded pet foods such as that reported by Alvarenga et al. (2018). There was some expectation that the larger biscuits might have more RS since there's more material for the heat to penetrate. However, for gelatinization to occur only moisture is required, heat simply accelerates the reaction. If more time was required to remove the moisture, then the extra time with the moisture could have led to increased gelatinized starch and a reduction in RS. This may further increase the digestible starch levels as well. Also this could have been influenced by the duration of time the dough spent during baking as high heat can further disrupt the starch granules structure and increase gelatinization (Y. Liu et al. 2019). A previous study observed that differential scanning calorimetry (DSC) that the minimum water levels required for the gelatinization of starch was only 21-29% (Unasekaran 2006). Rotary molded products can be around 25% total moisture (Davidson 2016).

There were two samples that had much higher resistant starch levels, (9.8%, 15.5% RS of TS respectively). Both products incorporated legume/lentil ingredients in the top five of the ingredients listed. This could increase the overall RS concentration since lentils are high in RS, at about 40% (Lintas and Cappelloni, 1992). Legumes typically have higher resistant starch than conventional cereal grains (Bednar et al. 2001). There are conflicting studies that indicate as a percent of total starch, potatoes have lower RS compared to other grains such as wheat, sorghum, corn, or rice (Murray et al. 1999). Another study found that potato starch has higher levels of RS than corn and wheat (Murray et al. 2001; Bednar et al. 2001).

To explore this further, samples were also analyzed grain-free (n=5) vs. grain-based (n=25) treats although the sample size for grain-free was small. When products were extruded, variation in the RS concentration was lower regardless of whether the diet was grain-free or grain-based (Alvarenga and Aldrich, 2020). This may have been due to the high cooking intensity that in extrusion as most of the starch fractions were converted to rapidly digestible starch or slowly digestible starch (Murray et al. 1999). The overall average RS for the 30 treats that products was 0.705% and for 20 commercial extruded samples was 0.945% of the TS (Alvarenga and Aldrich, 2020).

Future research should evaluate the starch fractions over different baking times, moisture, and temperature. For these commercially produced products, processing times and temperatures were not disclosed. Additionally, it would be beneficial to know how the mix and hydration time influence gelatinization in these baked products. Clearly a few of the grain-free products had high RS which may provide an avenue to exploit this benefit.

Conclusion

The commercial baked treats were not different for the analyzed nutrients or for digestible or resistant starch whether they contained wheat or not. The same was observed across the different product sizes. When comparing whether the products were grain-based or grain-free, there were some high values for RS which should be explored further. Commercial baked pet treats were remarkably similar in proximate composition and starch digestibility (*in vitro*) assays regardless of brand and composition. The range of RS, as a percentage of TS (1.48-2.21) was higher when compared to the extruded samples previously discussed in this thesis as other studies exploring RS in extruded diets.

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Tables and Figures:

Table 3.1: Sampling frame of baked treats within small, medium and large, as well as with and without wheat categories.

	Small	Medium	Large	**Total**
With Wheat	7	10	11	28
Without Wheat	22	19	16	57
Total	29	29	27	85

Table 3.2: List of first five ingredients on purchased treat product labels

Sample #	Ingredients
1	Ground Whole Wheat, Wheat Flour, Meat And Bone Meal, Beef Fat, Poultry By-Product Meal
2	Wheat Flour, Chicken By-Product Meal, Dried Beet Pulp, Natural Chicken Flavor, Dicalcium Phosphate
3	Rice Bran, Whole Grain Wheat, Wheat Flour, Chicken, Potato Protein, Potato Starch
4	Wheat Flour, Beef Tallow, Wheat Gluten, Mono And Dicalcium Phosphate, Bacon Flavor
5	Ground Whole Wheat, Wheat Flour, Meat and Bone Meal, Poultry By-product Meal, Beef Fat
6	Oatmeal, Oat Flour, Barley, Rye, Chicken Meal
7	Whole Brown Rice, Oatmeal, Chicken Meal, Cane Molasses, Dried Apple,
8	Whole Brown Rice, Oatmeal, Chicken Meal, Cane Molasses, Dried Apple
9	Ground Whole Wheat, Wheat Flour, Meat and Bone Meal, Poultry By-product Meal
10	Wheat Flour, Wheat Bran, Meat and Bone Meal, Milk, Wheat Germ
11	Ground wheat, Grain Sorghum, Animal fat, Meat by-products, Meat and bone meal
12	Whole Wheat, Wheat Flour, Rolled Oats, Turkey, Bacon
13	Chicken, Sugar, Soy Grits, Dried Potato, Rolled Oats
14	Oat Flour, Oatmeal, Chicken Fat, Chicken Meal, Beef, Oat Fiber
15	Dried Potatoes, Sweet Potatoes, Potato Protein, Cane Molasses, Venison
16	Oat Flour, Oatmeal, Chicken Fat, Chicken Meal, Chicken
17	Rice Bran, Whole Grain Wheat, Wheat Flour, Chicken, Potato Protein
18	Wheat Flour, Meat and Bone Meal, Wheat Bran, Milk, Beef Fat
19	Wheat Flour, Chicken By-Product Meal, Dried Beet Pulp, Natural Chicken Flavor, Brewers Dried Yeast
20	Wheat Flour, Ground Whole Wheat, Meat and Bone Meal, Poultry By-Product Meal, Beef Fat
21	Wheat flour, wheat bran, beef meal and beef bone meal, beef fat, wheat germ
22	Oatmeal, Ground Barley, Ground Whole Oats, Pumpkin, Cinnamon

23	Oatmeal, Barley, Oat Flour, Beef, Flaxseed
24	Chicken, Potatoes, Peas, Glycerin, Carrots
25	Potatoes, Sweet Potato, Potato Protein, Bison, Canola Oil
26	Beef, Fish Meal, Potato, Flaxseed, Sunflower Oil
27	Dried Potatoes, Potato Starch, Dried Peas, Turkey Meal, Beef Tallow
28	Duck, Chicken Meal, Potatoes, Chicken Fat, Flaxseed
29	Turkey, Chicken Meal, Potatoes, Chicken Fat, Flaxseed
30	Salmon, Chicken Meal, Potato, Chicken Fat, Flaxseed

Table 3.3: Commercial baked treats proximate analysis and with total starch and resistant starch sorted by containing with wheat or no wheat (DM basis).

Sample	**Wheat ?**	**Size (S, M or L)**	Total starch, %	RS, % of whole kibble	RS, % of TS on DMB	Digestible starch, %	DM	OM	GE	Protein DMB
1	Yes	M	51.00	0.36	0.72	50.63	0.92	0.94	4548.65	0.21
2	Yes	S	48.44	0.34	0.70	48.10	0.94	0.94	4771.43	0.26
3	Yes	S	44.68	0.57	1.28	44.11	0.94	0.92	4350.38	0.19
4	Yes	S	52.42	0.38	0.72	52.05	0.95	0.96	4941.29	0.19
5	Yes	M	57.94	0.34	0.59	57.60	0.93	0.95	4559.40	0.21
6	No	S	51.50	0.36	0.70	51.14	0.95	0.95	4662.61	0.20
7	No	S	58.45	0.17	0.29	58.29	0.93	0.95	4628.27	0.19
8	No	S	53.27	0.21	0.38	53.07	0.93	0.96	4682.40	0.20
9	Yes	S	56.99	0.39	0.69	56.59	0.92	0.95	4572.37	0.20
10	Yes	L	53.17	0.45	0.84	52.72	0.93	0.94	4465.85	0.19
11	Yes	M	60.13	0.74	1.22	59.40	0.93	0.96	4553.10	0.13
12	Yes	S	43.71	0.51	1.16	43.21	0.91	0.96	4749.12	0.19
13	Yes	M	13.13	0.34	2.59	12.79	0.80	0.93	4912.12	0.25
14	No	M	42.09	0.19	0.44	41.90	0.93	0.95	4783.21	0.17
15	No	M	47.95	0.89	1.86	47.06	0.91	0.96	4767.54	0.22
16	No	M	43.31	0.20	0.47	43.10	0.94	0.96	4899.02	0.17
17	Yes	L	52.00	0.52	0.99	51.48	0.94	0.92	4348.43	0.20
18	Yes	L	59.35	0.46	0.77	58.89	0.92	0.95	4463.19	0.19
19	Yes	L	44.59	0.26	0.59	44.33	0.94	0.93	4469.00	0.29
20	Yes	L	51.64	0.40	0.78	51.24	0.93	0.94	4565.55	0.22
21	Yes	M	51.18	0.39	0.76	50.79	0.92	0.94	4439.56	0.20
22	No	L	51.92	0.46	0.89	51.46	0.91	0.96	4453.59	0.14
23	No	L	53.07	0.30	0.56	52.77	0.94	0.96	4645.65	0.13
24	No	S	32.25	1.01	3.12	31.25	0.80	0.97	5365.68	0.16

25	No	S	41.48	0.98	2.36	40.50	0.93	0.95	4722.74	0.24
26	No	M	24.95	0.34	1.35	24.61	0.95	0.90	5122.33	0.35
27	No	M	44.08	4.03	9.14	40.05	0.93	0.95	4638.32	0.21
28	No	L	23.22	0.48	2.07	22.74	0.96	0.91	5287.63	0.36
29	No	L	22.61	0.38	1.69	22.23	0.94	0.91	5166.63	0.39
30	No	L	31.52	4.59	14.57	26.93	0.94	0.92	5175.74	0.37

Abbreviations: S= small, M= Medium, L= Large, RS= Resistant starch, TS= Total starch, DS= Digestible starch, DM= Dry matter, OM= Organic matter, GE= Gross energy

Table 3.4: Starch fractions (% DM) least square means [95% CI] of baked treats with or without wheat at small, medium and large sizes.

Nutrient, DM %	With wheat	Without wheat	*P*	Small biscuit	Medium biscuit
*DM	91.5 [88.8, 94.3]	92.6 [90.7, 94.5]	0.5187	91.6 [89.0, 94.2]	91.3 [88.7, 93.9]
**OM	85.1 [82.6, 87.6]	87.3 [85.5, 89.1]	0.1423	86.4 [84.0, 88.8]	85.6 [83.2, 88.0]
TDF[3]	1.90 [1.14, 2.65]	2.46 [1.93, 3.00]	0.2071	1.97 [1.25, 2.69]	2.57 [1.84, 3.29]
Gross energy, kcal	3994 [3770, 4219]	4183 [4025, 4340]	0.1598	4068 [3853, 4283]	4037 [3822, 4252]
Crude Protein	22.4 [18.5, 26.3]	20.5 [17.8, 23.3]	0.4115	20.1 [16.3, 23.8]	20.5 [16.7, 24.3]
Crude Fat, acid hydrolysis	7.84 [6.31, 9.37]	8.85 [7.78, 9.92]	0.2635	8.32 [6.85, 9.79]	8.39 [6.92, 9.85]
Ash	6.97 [5.77, 8.17]	5.65 [4.81, 6.49]	0.0686	5.62 [4.47, 6.77]	6.21 [5.06, 7.36]
NSC[2]	52.4 [47.5, 57.4]	55.1 [51.6, 58.6]	0.3581	55.6 [50.9, 60.3]	53.6 [48.9, 58.4]
Total starch	40.4 [32.9, 47.9]	40.9 [35.7, 46.1]	0.9069	43.0 [35.8, 50.1]	38.2 [31.1, 45.4]
Digestible starch	39.2 [32.0, 46.5]	39.8 [34.7, 44.9]	0.8895	42.0 [35.1, 49.0]	37.0 [30.0, 43.9]
Resistant starch	0.826 [0.50, 1.35]	0.581 [0.41, 0.82]	0.2287	0.645 [0.40, 1.04]	0.728 [0.45, 1.17]
Digestible starch, % of TS[1]	96.9 [94.8, 98.9]	97.0 [95.6, 98.5]	0.8798	97.5 [95.5, 99.5]	96.7 [94.7, 98.7]
Resistant starch, % of TS[1]	2.21 [1.40, 3.50]	1.48 [1.08, 2.05]	0.1470	1.56 [1.00, 2.41]	2.07 [1.34, 3.22]

*Dry matter, **Organic matter, 1: Total Starch, 2: Non-structural carbohydrates, 3: Total dietary fiber

Samples ran in duplicate

Appendix

Evaluation of applied fat on pelleted, extruded, and baked animal foods

Liquid fat is typically applied to the exterior of kibble to meet the guarantee of crude fat as well as essential amino acid. When on the exterior of the product, there is the possibility that some will be wicked onto the interior of the packaging. Different final products will have varying levels of surface area, or porosity which allows the fat to be absorbed or adhere to the surface of the product. A pelleted, extruded, and baked foods were used in this evaluation to determine how much liquid fat at varying levels, remained on the package by difference in weight. Using small experimental Kraft multi-layered poly lined bags with dimensions of approximately 17.7 cm x 10.1 cm, all products were coated in 2%, 4%, 6%, and 8% fat in a weight/weight ratio with a combined weight of 100 grams.

Each bag was sealed and stored horizontally for two weeks at room temperature. After which, each bag was opened, and the product removed. The difference in weight between the empty bag weight and the final bag weight is how the determination of the amount of fat remaining on the bag was completed. As the amount of fat applied to the outside increased, the amount of fat remaining inside the packaging also increased apart from the baked biscuits from 2% to 4%. Due to the diets formulated in the study previously conducted in this paper requiring 7% fat, the 8% fat application level was focused on. The pellets, baked biscuits, and extruded kibble lost the 1.2%, 0.7%, and 1.4% of fat applied respectively, at the 8% fat application.

Based on the results of this preliminary study, fat was added both internally and externally with the exception of the extruded kibble for the aforementioned feeding study.

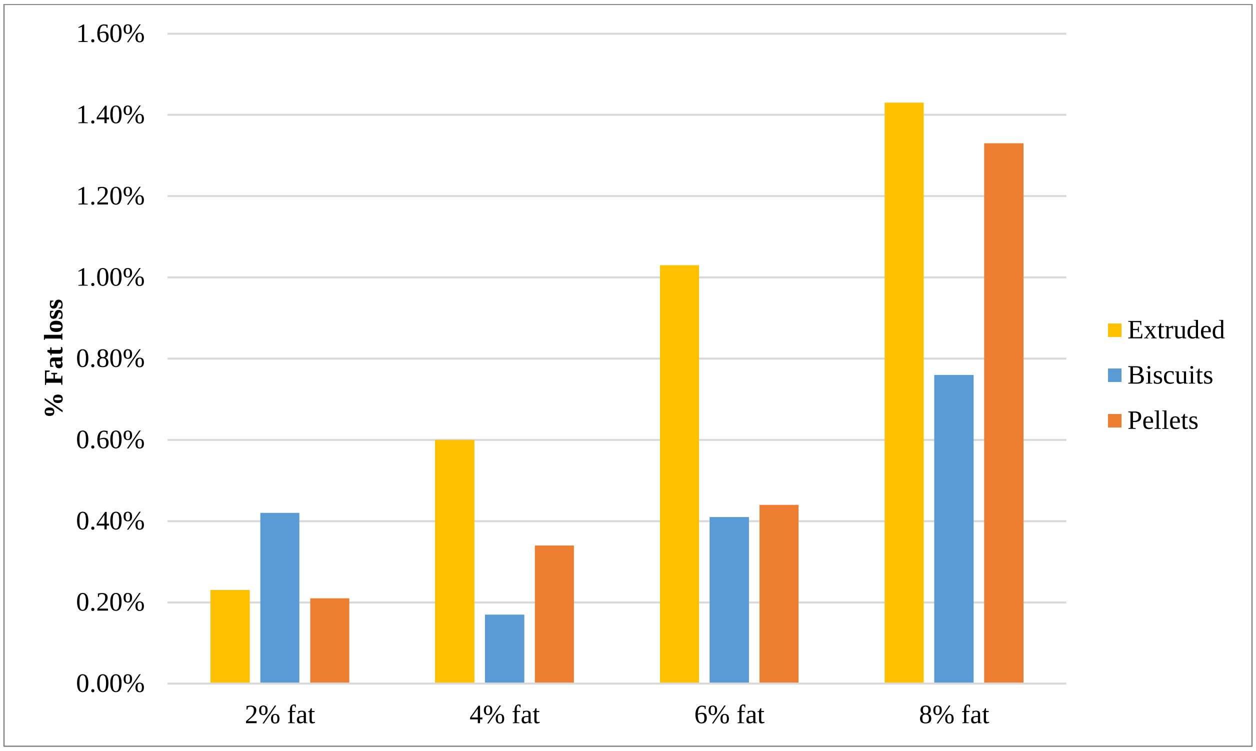

Figure A.1: The mean proportion of fat wicked from the pellets, biscuits, and kibble absorbed onto the experimental bags in the preliminary study, vertical lines indicate the standard deviation