Next-generation distillers dried grain as a potential dietary ingredient in dog and cat diets

by

Spencer C. Smith

B.S., Kansas State University, 2016

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

College of Agriculture

KANSAS STATE UNIVERSITY

Manhattan, Kansas

2018

Approved by:

Major Professor

Dr. C. G. Aldrich

Abstract

Novel ingredients have been a source of innovation and growth in the pet food market. Further, with rising trends in the humanization of pet food, there has been increased competition between the human food systems and pet food industry for high quality ingredients. Next-generation distillers dried grains (NG-DDG) are a sustainable alternative protein source that show a strong potential for use in companion animal diets. The objectives of this work were to determine the effect of NG-DDG on the extrusion of dry kibbles, the utilization of diets by dogs, the palatability of diets by dogs and cats, and to evaluate the amino acid profile and protein quality through a chick growth assay. Corn gluten meal (CGM) and soybean meal (SBM) were used as standards for comparison. Diets were extruded over 3 days in a complete block design. During extrusion, the NG-DDG kibbles had less radial expansion ($P<0.05$) compared to the CGM and SBM kibbles (2.62 vs. average 3.10 mm^2/mm^2, respectively). The NG-DDG kibble also required a smaller ($P<0.05$) mass restriction-valve opening to increase die back-pressure. No other differences in extrusion parameters or kibble texture were observed. Twelve beagle dogs were arranged in a 3x3 replicated Latin Square and were each fed the 3 experimental diets to evaluate digestibility by use of titanium dioxide. Diet produced with CGM was more digestible ($P<0.05$) in terms of dry matter, organic matter, crude protein, crude fat, and gross energy. Additionally, dogs fed NG-DDG diets had larger ($P<0.05$) fecal mass than both CGM and SBM (55.65 vs 35.91 and 43.25 g/d, respectively), and a higher ($P<0.05$) fecal score than dogs fed the CGM diet (3.63 vs. 3.27). Diets were fed to both dogs and cats to assess palatability via a two-bowl test. Dogs had a preference ($P<0.05$) for CGM over SBM and NG-DDG, but cats showed a preference ($P<0.05$) for SBM and NG-DDG over CGM. To assess protein quality, one-day old chicks (CobbxCobb; n=120) were fed semi-purified diets containing test ingredients at a 10%

crude protein inclusion level, as well as spray dried granulated egg (SDG) and a nitrogen-free basal diet (NEG) to serve as positive and negative controls, respectively. Chicks were arranged in a randomized block design with 6 chicks per pen, 1 pen per battery, and 4 pens per treatment. The protein efficiency ratio (PER) of each treatment was calculated as weight gain (g) per protein intake (g). All experimental treatments had a lower ($P<0.05$) PER value than the positive control. The PER for NG-DDG and CGM did not differ from each other and had the lowest value of all treatments ($P<0.05$; average 1.17). In summary, next-generation distillers dried grains can be used to make a similar kibble to CGM and SBM, are similar to SBM in terms of digestibility, and would be an acceptable source of protein in companion animal diets when paired with a supplemental protein source.

Table of Contents

List of Figures

List of Tables

CHAPTER 1 – LITERATURE REVIEW

Pet Food

About two-thirds of U.S. households own pets, making the pet industry worth $66.75 billion in 2016. Pet ownership is dominated by dogs and cats, with an estimated 60.2 million households owning dogs and 47.1 million households owning cats (APPA, 2016). The pet food industry in the U.S. commands a market worth more than $30 billion with growth projected to be more than $37 billion by 2020 (Packaged Facts, 2016). While there are constantly new varieties of pet foods being introduced to the market (i.e. freeze dried, raw, etc.), dry foods have consistently dominated the market year after year for both dogs and cats (PetfoodIndustry.com, 2013).

The majority of these dry foods are produced through extrusion. During this high-temperature, short-time process the ingredient mixture is steam conditioned, compressed, and forced through a die at the end of the barrel. It allows for expansion, dehydration and shaping of kibbles. This high-pressure process leads to a large input of both thermal and mechanical energy and requires a well-balanced mix of ingredients, and an understanding of how ingredients will perform under the processing parameters.

Humanization has been the driving factor behind market trends in the pet food industry. Today's "pet parents" want their pets to eat as well as them and are seeking options in ingredient composition that reflect their own dietary choices. According to PetfoodIndustry.com (2015), 55% of pet owners are concerned about the amount of "fillers," such as grains and meat by-products, in their pet's diets. These consumer preferences are driving companies to search for novel ingredients to replace these customary ingredients. Novel proteins such as bison or kangaroo have been incorporated into pet foods with some success (Anderson et al., 1961; Wall, 2018). However, there is limited research exploring the use of many of these ingredients. Further, the sustainability of these novel protein sources is questionable. Meat-based diets require more energy, water, and land to produce, and as a result have higher environmental consequences than their plant-based counterparts (Okin, 2017). With ongoing trends towards humanizing pet foods, today's pet owners are seeking foods with increased meat quantity and quality and are more likely to purchase foods with premium cuts of meat (Okin, 2017). These ingredients compete with the human food system, and as a result many nutritionally adequate co-products go to waste (Swanson et. al, 2013). Subsequently, with increase demand for these products to be used by the human food industry, availability for animal proteins to the pet food industry are decreasing (Fiacco et al., 2018).

Both canines and felines have a dietary requirement for amino acids. However, the protein source (plant vs. animal) does not matter as long as the dietary amino acid profile is sufficient to fulfill the animal's requirements. Additionally, pet food companies tend to formulate their diets based on total protein inclusion at a level in excess of the amino acid requirements for dogs or cats (Fiacco et al., 2018). Amino acids are required by dogs and cats for two reasons. First, dietary protein provides the amino acids the animals are not able to synthesize on their own, also known as essential amino acids (EAA). These amino acids become the building blocks used to synthesize other proteins needed by the body. Second, protein provides an additional source of amino acids that the body can synthesize on its own, known as non-essential amino acids (NEAA). These amino acids are needed for normal physiological functions such as maintenance or growth. The body is also able to break these NEAA down to their structural components, which can then be repurposed to use as a source of energy, for gluconeogenesis, or to synthesize compounds such as hormones, heme, purines, pyrimidines, and more (NRC, 2006). In addition to the complete amino acid content of an ingredient, their bioavailability must also be considered. Protein bioavailability is directly related to the digestibility of a protein and the ability of an animal to utilize its amino acids. Processing can induce modifications in the proteins that are positive or negative. For example, cooking will denature the protein, which is desirable as it will increase digestibility. However, overheating can damage the amino acids present in an ingredient through transformations such as aggregation and Maillard reactions, making them unusable by the animal (Hodge, 1953; van Rooijen et al., 2013; Salazar-Villanea et al., 2016). Proteins from animal sources are particularly susceptible to these degradations due to the higher degree of processing necessary to destroy pathogens and create safe ingredients (van Rooijen et al., 2013). The pet industry generally utilizes rendered meat meals in pet foods. Rendering utilizes high temperatures over many hours of processing. Research completed by Wang and Parsons (1998) compared the bioavailability of amino acids in meat and bone meal cooked at different temperatures and found that the amino acid digestibility of the ingredients processed at high-temperatures was less ($P<0.05$) than those processed at low temperatures when fed to cecotomized roosters. Plant sourced proteins, while generally lower in initial amino acid concentrations, tend to have less damage to amino acids as a result of processing (Yen et al., 1988; van Rooijen et al., 2013). Additionally, plant proteins tend to have less variability in their nutritional composition when compared to animal-based proteins (Bednar et al., 1991).

Despite trends towards new ingredients and higher animal-based protein inclusions, 81% of dog foods and 85% of cat foods still utilize traditional ingredients like corn, wheat, and soy (Packaged Facts, 2016). The pet food industry has been practicing sustainability by utilizing coproducts from plant derived protein sources, such as soybean meal, or corn gluten meal, for decades (Alonzo, 2017). Ingredients derived from further natural processing like fermentation have been also popular and controversial (e.g. brewers dried yeast vs distillers dried grains and solubles). These ingredients have been researched in the past for their application into pet foods with good results (Allen et al., 1981; Swanson and Fahey, 2006; Silva et al., 2016). New alternatives may be available that should be evaluated such as Distillers Dried Grains from new processes, or Next-Generation Distillers Dried Grains (NG-DDG).

Distillers Dried Grains

Distillers Dried Grains with Solubles (DDGS) are a co-product of corn ethanol production, which is a rapidly growing renewable energy (Batal and Dale, 2006; de Godoy et al., 2014). Over 150 billion metric tons of corn were used for ethanol production in the month of October 2017, with almost 450,000 metric tons of DDGS produced as a co-product (Renewable Fuels Association, 2017). Ethanol is produced by two methods: dry grinding (DG) and wet milling. Dry Grind corn plants tend to be smaller than wet milling, and overall require less capital; however, the coproducts produced from the DG method, mainly DDGS, tend to be less valuable than those of wet milling (Singh et al., 2002). Because of this, the capital made from DDGS sales are crucial to economic success of the DG industry (Belyea et al. 2004). This production process is constantly evolving in order to yield more ethanol and higher quality products (Rho et al., 2017). A modified DG was developed in the 1990s that combines some steps from wet milling with traditional DG processing. Singh et al. (2002) reported that this process had a significant effect on the nutrient profiles of DDGS by increasing crude protein (CP) levels and decreasing acid detergent fiber (ADF), which is highly indigestible by the animal. Advancements in the technology used in the DG process can improve the nutritional qualities of DDGS and increase their value.

In the traditional DG process, DDGS are produced by blending and drying the non-fermentable residues left after the fermentation of corn starch: wet distillers grains and condensed distillers solubles. After fermentation, the stillage is centrifuged to remove insoluble solids, also known as wet distillers grains, and liquids. Water is then evaporated from liquid portion, resulting in the condensed distillers solubles. The two products are dried in a rotatory drum dryer to produce DDGS (Kingsley et al. 2009). Additionally, the wet distillers grains may be dried without the condensed distillers solubles, resulting in distillers dried grains (DDG). New technology has been developed in the ethanol industry to improve these co-products that may make them more suitable to use in companion animal food. Next generation-distillers dried grains (NG-DDG) are produced using post-fermentation separation technologies to separate protein and yeast from fiber prior to drying. This process utilizes a series of screens and centrifuges to separate approximately twenty percent of total DDGS volume as the new high protein ingredient, while the remaining eighty percent is marketed as DDGS. These NG-DDG contain twice as much protein as traditional DDGs and may be a viable option for inclusion as a novel ingredient in pet foods.

Traditionally, DDGS boast an affordable cost and a high CP, fat, and fiber contents (Lodge et al., 1997). When compared to their plant of origin, corn, DDGS have higher concentrations of nutrients, likely due to the removal of starch during fermentation (Widyaratne and Zijlstra, 2006). While nutrient composition can vary based on growing conditions for the corn, the ingredient typically has a protein content between 27 and 35% (Belyea et al., 2004). This is supported by research done by Widyaratne and Zijlstra (2006), in which the nutritive values for corn distillers dried grains were identified to determine their value as a feedstuff for finishing pigs. Their analysis found a CP value of 30%, along with a crude fat (CFat) value of

12.8%, an ADF value of 14.6%, and neutral detergent fiber (NDF) value of 31.2%. Lodge et al. (2006) found comparable results when analyzing DDGS used in cattle feed. They reported CP of 29.2%, 11.4% lipid content, and 51.2% NDF. *In vivo* studies have also been conducted to explore the protein quality of DDGS. A chick protein efficiency ratio (PER) assay done by de Godoy et al. (2014) found that chicks fed a diet containing 10% CP from DDGS resulted in a PER value of 2.63, which was equivalent to that of corn germ meal, and higher than corn gluten meal, a coproduct commonly utilized in animal feeds, and two other corn protein products. This is likely due to a high quality amino acid profile. Lysine is known to be the first limiting amino acid in corn (0.24g/100g) but can be found in much higher concentrations in DDGS (0.77g/100g), with a digestible lysine:CP ratio of 2.54 (Belyea et al., 2004; de Godoy et al., 2014). This higher concentration of essential amino acids is largely due to the high presence of yeast in DDGS.

The protein content in DDGS is derived from two sources: corn and yeast. While values have been found to vary between samples, some studies suggest that yeast accounts for nearly 4% of the dry weight in DDGs and can make up 50% or more of the CP in the ingredient (Stein and Shurson, 2009; Belyea et al., 2004). Amino acid concentrations are considerably higher in yeast protein when compared to corn protein. As a result, DDGS are a more balanced and complete source of amino acids in comparison to corn based protein meals, such as corn gluten meal (Belyea et al., 2004). In addition to increased nutritional values, yeast also serves as a functional ingredient. Components of yeast have been found to bind to mycotoxins to reduce concentrations in feeds, reduce abilities of harmful bacteria to colonize the bodies of companion animals, increase gut health, and increase immunity (Swanson and Fahey, 2006). Furthermore, certain qualities of yeast may serve as a palatability enhancer in companion animal foods. Yeast has been added to human foods for years as a source of savory and meaty flavors (Diehl, 2006). Varieties of yeast have also been added to companion animal foods as a palatant. A palatability test examining the addition of brewer's yeast and corn wet milling yeast found that both cats and dogs consumed the foods with brewer's yeast almost twice that of their consumption of the food with corn wet milling yeast (Swanson and Fahey, 2006). It has been found that cats have a taste preference for amino acids and nucleotides, both of which have a high concentration in yeast (White and Boudreau, 1975; Swanson and Fahey, 2006; Belyea et al., 2004). As cats have been known to be harder to appease than dogs, the acceptance of yeast may prove to be helpful in creating a more palatable food for cats (White and Boudreau, 1975; Li et al., 2006). These attributes, in combination with the functional benefits of yeast, are one of the reasons DDGS are popular in many livestock feeds and may further support the inclusion of DDGS in pet foods.

DDGS in Companion Animal Foods

DDGS have been utilized as an animal feed ingredient for decades (Batal and Dale, 2006). While historically its use began in ruminant feeds, it has since been used successfully by the poultry, swine, and aquaculture industries as well (Parsons et al., 1983; Stein and Shurson, 2009; Overland et al., 2013). The Renewable Fuels Association (2017) has reported that 74% of DDGS consumption is attributed to cattle, while swine and poultry accounts for 16 and 9%, respectively. The remaining 1% of use is made up of aquaculture, sheep, and horses. While there is an expansive selection of research examining the effect use of DDG and other commonly used coproducts in livestock feed on apparent total tract digestibility (ATTD), average daily gain (ADG), and feed-to-gain ratio (F:G), there remains a deficit in the research of exploring their use in companion animal feeds (Silva et al., 2016). Early research in the use of DDGS in dog foods dates back to the 1950s (McCay et al., 1957; Wanner et al., 1958). However, further research since this time has remained limited, warranting a need for further exploration. As a result, it is necessary to review the research regarding the use of DDGS fields of swine nutrition and poultry nutrition, which have proven to serve as a similar in vivo model, as well as the use of other comparable plant-based proteins, such as corn gluten meal and soybean meal, in companion animal foods in order to truly explore the potential of DDG in companion animal foods.

Allen et al. (1981) examined the inclusion of DDGS in dog foods through a series of 4 trials. Trials 1 and 2 observed the effect of graded levels of DDGS (0, 4, 6, and 8%, and 0, 8.9, and 15.7%, respectively) on the ATTD of DM and starch, as well as the effect on the dry matter content and wet weight in the feces. Results from Trial 1 found no differences between treatments for either dry matter or starch digestibility, with an average ATTD of 82.85 and 97.63%, respectively. There was also no difference in fecal DM or fecal weight. The second trial again reported no differences between treatments for starch digestibility (average 98.2%). The high levels of starch digestibility are supported by the work done by Batajoo and Shaver (1998) in which the nutrient degradability of many grains and grain co-products, including DDGS, were measured in cattle. Batajoo and Shaver found that 85% of the starch in DDGS was ruminally available, higher than all other ingredients apart from wheat middlings, which were equivalent. This percentage was calculated by multiplying the sum of the rapidly and slowly digestible starch portions by the rate of degradation and dividing by the sum of the rate of degradation and the rate of passage. High values indicate that the starch in the ingredient is readily accessible by the enzymes needed for digestion, allowing the animal to fully utilize the starch. In contrast to the unchanging ATTD of starch in trials 1 and 2, ATTD of DM decreased between 8.9 and 15.7% inclusion of DDGS in the diet (83.6 vs. 79.9%). Additionally, fecal DM and weight increased with higher inclusion of DDGS. Similar levels for DM digestibility were reported by

Lei et al. (2017) in a study comparing the use of DDGS and solvent-extracted canola meal to soybean meal (currently a standard ingredient in swine diets). Lei reported a DM ATTD of 78.04% for the DDGS in a diet containing 52.17% DDGS. The comparable levels of DM digestibility between the diets including DDGS at 15% and 52.17% may suggest that ATTD does not significantly decrease past 15% inclusion. In a third study Allen et al. (1981) examined the ATTD of DM, energy, and CP for dogs fed diets containing 0, 13.1 and 26.1% DDGS. The DM digestibility decreased in diets containing between 13.1 and 26.1% DDGS (74 vs. 69.6%). Digestibility of energy also decreased between the 13.1 and 26.1% diets, dropping from 78.2 to 74%. Conversely, ATTD of CP did not differ between diets, averaging 74.4% digestion. Similar results were found by Rho et al. (2017), who evaluated the digestibility of a conventional DDGS product in comparison to two high-protein DDGS when fed to growing pigs. At a 65% inclusion of the conventional DDGS, the authors reported a DM ATTD of 75.9%, a CP ATTD of 71.1%, and an energy ATTD of 71.4%. It should be noted that the studies done by Lei et al. (2017) and Rho et al. (2017) calculated digestibility by use of chromic oxide or titanium dioxide, whereas Allen et al. (1981) utilized a total fecal collection (TFC) method. Chromic oxide and titanium dioxide are indigestible markers that are added to feed in known concentrations which can then be used to calculate digestibility. This method is more reliable than TFC, as a TFC method requires that every quantity of feces is collected (Brandyberry et al., 1991). This can be difficult as a clean fecal collection is highly dependent on the consistency of the feces, which may be altered due to a new diet. Dogs have also been known to engage in coprophagy, which can also interfere with TFC data (Nijsse et al., 2014).

As seen in the results by Allen et al. (1981), ATTD tended to decrease with increasing levels of DDGS. It has been suggested by many authors that this is likely due to non-starch polysaccharides (NSP) which are present in most cereal grain co-products and may impede the absorption of nutrients in the intestine and alter the intestinal microbiome (Yamka et al., 2003; Bobeck et al., 2014; Yang et al., 2014; Silva et al., 2016). During the production of DDGS, the removal of ethyl alcohol and carbon dioxide from the corn causes the remaining nutrients in the grain to become more concentrated. This results in the high level of protein and amino acids for which the ingredient is traditionally known. However, this concentration is also applied to the negative nutritional attributes of the grain, such as NSP (Swiatkiewicz et al., 2016). Widyaratne and Zijlstra (2006) found that on a dry matter basis, NSPs made up 19.24% of DDGS from corn. The authors further broke this data down into soluble and insoluble NSP, reporting 1.39 and 17.85%, respectively. These oligosaccharides decrease nutrient digestion by increasing bacterial activity, or fermentation, in the intestines, increasing the rate of passage and, consequently, decreasing absorption time. Additionally, the fiber can absorb some of the proteins and obstruct digestive enzymes, preventing the breakdown and absorption of these nutrients (Yamka et al., 2003). To combat these NSPs, some researchers have explored the effect of adding digestive enzymes to the diets, such as xylanase. Exogenous feed enzymes like xylanase can potentially degrade NSP or other indigestible nutrients, like starch-protein matrices, to aid in digestibility (Twomey et al., 2003). For example, Bobeck et al. (2014) found that the addition of xylanase to hen diets comprised of 10% DDGS increased egg weight, number of eggs laid daily, and increased apparent metabolizable energy. Additionally, the hens fed diets with added xylanase had a higher carcass fat content.

Silva et al. (2016) examined the use of DDGS in dog food with and without the addition of xylanase. Eight treatments were fed to eight beagle dogs in an 8x8 Latin Square. Diets were formulated to contain 0, 60, 120, or 180 g/kg of DDGS, with or without added xylanase.

Digestibility was calculated using TFC, and feces were scored and measured for DM, ammonia content, and faecal pH. Digestibility declined in a linear fashion with increasing levels of DDGS for DM, OM, ether extract after acid hydrolysis (EEAH), gross energy (GE) and metabolizable energy (ME) for both diets (with and without xylanase). Crude protein ATTD was only reduced for animals fed the diets containing no xylanase. There was no effect on fecal score or ammonia content, but a decrease in faecal pH was observed with increasing DDGS levels, indicating a possible prebiotic effect in the large intestines. The ATTD results of Silva et al. (2016) concur with those found by Allen et al. (1981) in that increased levels of DDGS resulted in decreased digestibility of nutrients. A lack of effect for enzyme addition to pet food was also observed by Twomey et al. (2003) and Sa et al. (2013) who examined the use of carbohydrase mixtures (which included xylanase) when added to pet foods utilizing plant proteins known for their high NSP contents. Both authors found that the added enzymes had no effect on digestibility of starch, fat, protein, gross energy, or dry matter of the experimental diets when fed to 30 mixed-breed dogs in each study. Additionally, Twomey et al. (2003) found that the addition of the enzymes caused an increase in fecal score, causing stool to be more loose and runny. A review by Swiatkiewicz et al. (2016) summarized the research of over 20 studies examining the use of enzymes to supplements pig diets with DDGS and concluded that the addition of carbohydrases had no positive effect on either digestibility or performance in nursery or grower-finishing pigs. Swiatkiewicz also summarized similar research with poultry and found that the efficacy of added enzymes was inconclusive based on highly varied results. For these reasons, enzymes do not merit evaluation in the exploration of NG-DDG use in dog and cat foods.

While the digestibility of the pet food is clearly important, the nutritive values are moot if the animal will not consume the food. Only a few studies have examined the palatability of pet foods containing DDGS. Silva et al. (2016) examined the palatability of pet foods containing 60g/kg and 180g/kg DDGS when compared to a diet containing no DDGS and found that the dogs had a preference for the diet containing 180g/kg DDGS when compared to the diet without. Whereas, Widyarante and Zijlstra (2006) noticed a reduction in voluntary feed intake for pigs fed DDGS diets. These authors expressed concern regarding the palatability of the ingredient. Similar findings have been reported in other swine feeding studies (Whitney and Shurson, 2004; Yang et al., 2004). There remains a need for further research to examine the palatability of this ingredient for companion animals. Additionally, the ingredient may have potential for improved palatability in cat diets due to its high levels of yeast. As DDGS are a product of fermentation, much of their protein content can be attributed to yeast. This has been observed as highly palatable to cats, perhaps due to the presence of copious nucleotides (White and Boudreau, 1975; Belyea et al., 2004; Swanson and Fahey, 2006). Flavor may also be affected, positively or negatively, by the processing of the ingredient or diets (Whitney and Shurson, 2004; van Rooijen et al., 2013).

DDGS in Extrusion

While a wide variety of processes are used to produce pet food, such as freeze drying, canning, or baking, extruded dry foods remain the most popular choice for pet owners (PetfoodIndustry.com, 2013). Extrusion cooking is not a single-unit operation (Camire et al., 2009). For pet food production, it combines the operations of a feeder, pre-conditioner, extruder barrel, die and knife assembly, followed by the drying and coating of the material (Rokey et al., 2010). In the preconditioner, the raw material is mixed, heated and hydrated through the rotation of the paddles and the injection of steam and water, respectively. The steam and water begin the cooking process of the ingredients by providing thermal energy. The water penetrates the dry ingredients while the steam adds heat (Kvamme and Phillips, 2003). This begins the process of starch gelatinization, which is essential to extrusion. The starch granules begin to swell, retaining the water. The extrudate mixture then moves into the extruder barrel where the screw conveys the material through to the end of the barrel. As the screw pushes the extrudate through the barrel, the material becomes viscous and dough-like (Tran et al., 2008). Different screw configurations may be used to create different effects in the products. Adding restriction within the barrel causes increased friction, increasing the amount of mechanical energy applied, and further cooking the ingredients (Rokey, 2003). At the end of the barrel, the die creates restriction to flow, generating pressure, shear, and heat before the product is pushed through a shaped hole. Alterations at any step in this process can result in changes in the final product. Additionally, ingredient selection plays a large role in the final product, with the ability to change the texture, expansion, uniformity, and nutritional quality of the final product (Rokey et al., 2010).

Starch plays a large role in the final product created in extrusion. The gelatinization that occurs during the extrusion process causes the expansion of the material. As it exits the extruder through the die, the drastic change in pressure causes the water trapped in the gelatinized starch molecules to vaporize, expanding the piece, leaving a light, puffy product. The amount of starch gelatinization that occurs during extrusion is directly related to the expansion of the final kibble (Kannadhason et al., 2010). During DDGS production, the majority of the starch is converted to ethanol (Stein and Shurson, 2009). Reported starch values for DDGS have been quite variable, with values ranging between 5.1-14.5% (Batajoo and Shaver, 1997; Belyea et al., 2004; Kim et al., 2008). However, these values are still low when compared to its plant of origin, corn, which averages 71.4% starch (Belyea et al., 2004). This can be problematic for creating an ideal kibble through extrusion. Starch can be broken down into two main polymers: amylose and amylopectin. Each component serves a function in extrusion. Amylose has stronger binding capabilities and can improve uniformity and cohesion in final products, whereas amylopectin provides more of the expansion that takes place during extrusion and is responsible for the light, porous structure of kibble (Rokey et al., 2010; Kannadhason et al., 2010). There is no published work identifying the ratio of amylose:amylopectin in DDGS, however, corn is known for having a higher amylopectin content, making it a highly expansive ingredient. However, information about the starch content of DDGS can be inferred from studies examining its extrusion properties.

Kannadhason et al. (2010) examined the use of DDGS in aquaculture diets produced with a twin-screw extruder. The objective of the study was to examine the use of graded levels of DDGS, screw speeds, and multiple die dimensions on their effect to final products and processing parameters. It was found that no matter the die size or screw speed, the addition of DDGS decreased the expansion ratio of the feed when compared to the control diet, which contained no DDGS. However, the offsetting matrix had no impact on expansion. Chevanan et al. (2004) also found a decrease in expansion with increasing DDGS in extruded aquatic feeds. However, they detected a difference between expansion between the inclusion levels. The ability to detect this difference is likely due to the larger difference between DDGS levels (20, 40, 60%) in the experiment by Chevanan et al. (2004) compared to the subtler differences used in the experiment by Kannadhason et al. (2010; 17.5, 20, 22.5, 25, and 27.5%). A general increase in bulk density was also observed by Kannadhason et al (2010) with increasing levels of DDGS, however the increase was not linear. Increased inclusions of DDGS also resulted in a higher pellet durability index, which implies the pellets would store and travel well, and resist breakage. This is a positive finding, as traditional DDGS are high in fiber, which can decrease the binding capabilities and strength of products in an extruder (Chevanan et al., 2004; Monti, et al. 2016). Similar results have been found in other extrusion studies with DDGS included at levels between 20-60% (Chevanan et al., 2004; Chevanan et al., 2008).

While Kannadhason et al. (2010) and Chevanan et al. (2004) examined the effects of graded levels of DDGS on the extrudate, Chevanan et al. (2007) examined the effects of altering various parameters on the extruder on the extrudate while keeping the level of DDGS constant. The barrel temperature, moisture content, and nozzle diameter were all altered and effects on bulk density, pellet durability, and water absorption index (WAI) were recorded. The effect on other extruder parameters, such as mass flow rate (MFR), specific mechanical energy (SME), apparent viscosity, absolute pressure, and temperature of the dough in the barrel, and at the die were also recorded. They observed that the WAI increased, indicating a higher level of gelatinization. As a starch granule is increasingly damaged by gelatinization, more water is able to bind to the molecule, therefore WAI is a good measure of the degree of gelatinization (Ding et al., 2007). The study also found that increasing the barrel temperature decreased the bulk density and the pellet durability index of the extrudate, resulting in a more fragile pellet. The increasing temperature also decreased the specific mechanical energy, viscosity, and absolute pressure in the die. These changes can all result in a less expanded kibble (Plattner, 2007). While the expansion of kibble was not directly measured, kibble expansion may be inversely related to density. It would be expected that the extrudate from this experiment would increase in density as the temperature increased based on the effects on the other processing parameters; however, the opposite effect was observed. As moisture content increased, there was a slight decrease in bulk density when changed from 15 to 20%. This is supported by the increase in WAI that was also observed, indicating more gelatinization, and therefore more expansion in the kibble. The change in moisture also caused an increase in pellet durability. It appears that 25% in-barrel moisture was appropriate for this material to create more durability in the final product. To a certain level, water can act as a binding agent in extrusion. However, if too much is added, the additional moisture can result in a plasticized dough, making it slippery, and preventing mechanical energy from being applied (Ding et al., 2006). This is apparent in the results of the affect of added moisture on extrusion processing parameters. As moisture levels increased from 15 to 25%, MFR decreased, SME decreased, viscosity decreased, pressure decreased, and dough temperature in the barrel decreased. Once again, it is surprising that these changes did not result in a denser, less expanded kibble. The last effect examined was the results of changing the diameter of the die. As the diameter increased, bulk density increased, and pellet durability and WAI decreased. This is expected, as a larger opening at the die would create less restriction, and therefore less added mechanical energy from pressure and friction (Rokey et al., 2010). This results in less gelatinization and less expansion, making a denser and more fragile kibble. This is supported by the effects on the processing parameters. The MFR increased as die diameter increased, which results in less retention time in the extruder. The SME, viscosity, pressure, and dough temperature in the barrel and at the die all decreased.

Understanding the effects of extrusion on an ingredient, and vice versa, the effects of an ingredient on extrusion, is imperative to creating a uniform product. The likelihood of an animal to consume a product is not only based on the taste of the kibble, but also the shape and texture. Each of the aforementioned parameters can have an effect on the appearance and texture of the final product, both of which can influence the acceptability of the kibble to an animal (Hullar et al., 1998). For example, cats have been found to prefer a more mechanically cooked kibble, while dogs seem to prefer a product cooked with more thermal energy (Tran et al., 2008). The ratio of thermal to mechanical energy can has been found to alter the porosity, graininess, and fibrous texture of kibbles (Koppel et al., 2014). A study by Koppel et al. (2015) examined the influence of fiber amount and fiber particle size on the palatability of dry dog foods and examined correlations between palatability and sensory attributes. The results of the test found that the dogs preferred the kibbles that had higher fracturability and crispness. This indicates that texture can influence palatability, and that ingredient inclusion and processing parameters may affect this. Knowledge of the extrusion process and how an ingredient will react under the processing conditions enables producers to achieve the ideal texture for their product and increases the chance of its success.

Conclusion

The pet food industry is rapidly growing and, like human food systems, has an increasing need to seek more sustainable options for their foods. Next generation-distillers dried grains are a high-protein, readily available ingredient that may be an appropriate solution for this need. There is currently no work examining the use of these high-protein DDGS in pet foods, and there is very limited work available examining the use of traditional DDGS in this matrix. There remains a need to examine the potential of this ingredient from both a production and nutrition basis. Therefore, the objectives of this work was to examine the effects of NG-DDG on the extrusion and texture of dog and cat foods, to examine the effect of NG-DDGS on digestibility, fecal scores, fecal output, and fecal weight in dogs, and palatability in dogs and cats, and the protein quality of NG-DDGS through a chick growth assay.

CHAPTER 2 – EFFECTS OF NEXT-GENERATION DISTILLERS DRIED GRAINS ON PROCESSING PARAMETERS OF EXTRUDED DOG AND CAT FOODS

Abstract

The majority of pet foods utilize traditional ingredients like corn, wheat, and soy. These ingredients and others such as distillers dried grains (DDG) have been evaluated in the past with good results. Next generation-DDG (NG-DDG) are a nutrient dense improvement on DDG but have not been evaluated in pet food. Therefore, it was the objective of this project to determine the effect on processing parameters and kibble textures resulting from incorporating NG-DDG into extruded dog and cat foods. Experimental diets with treatment protein sources [corn gluten meal (CGM), soybean meal (SBM) and NG-DDG] were produced in triplicate using a single screw extruder. Processing parameters and kibble samples were collected every 20 minutes. Kibbles were evaluated for physical dimension and texture. There was no difference in kibble diameter, longitudinal expansion, or piece density between treatments. The NG-DDG kibbles had less ($P<0.05$) radial expansion than SBM and CGM kibbles. There was no difference among treatments for kibble texture. The NG-DDG kibble required a smaller ($P<0.05$) mass restriction-valve opening, to increase die back-pressure. There was no difference in specific mechanical energy among treatments. These results suggest that inclusion of NG-DDG can be readily managed relative to conventional plant proteins in extruded pet food applications.

Introduction

The pet food market is rapidly growing and currently contributes nearly 50% to total sales in the pet industry, more than any other factor (Packaged Facts, 2016). Much of this growth is driven by the development of new products, both in the inclusion of novel ingredients and in new forms of foods and treats. While products like baked, freeze-dried, and raw foods have risen in popularity, extruded kibble has continuously dominated the volume of purchases (PetfoodIndustry.com, 2013).

Extrusion is a high-temperature, short-time production process during which the ingredient mixture is steam conditioned, mechanically compressed by a screw, and forced through a die at the end of the barrel. It allows for expansion, dehydration and shaping of kibbles (Rokey et al., 2010). This high-pressure process leads to a large input of both thermal and mechanical energy and requires a well-balanced mix of ingredients, and an understanding of how ingredients will perform under the processing parameters. Humanization has been a driving factor in product development and, as a result, new ingredients are regularly being introduced into the market with little to no knowledge of how they will react under traditional processing methods. Today's pet owners are scrutinizing ingredient lists in pet foods more than ever before and are seeking pet food with ingredients reflecting their own diet (PetfoodIndustry.com, 2015). This has led to an avoidance of more traditional ingredients, such as meat by-products that are not utilized by the human food systems, and a strong movement towards grain-free diets. However, while there has been momentum behind this grain-free movement, over 80% of dog and cat foods still include traditional ingredients such as corn and soybean co-products (Packaged Facts, 2016). Protein concentrates from corn and soy have been used successfully in pet foods for decades (Alonzo, 2017). Improvements in processing technology and co-products from distilling grains from ethanol production have created new variations in these base proteins (Rho et al., 2017). Therefore, new alternatives such as distillers dried grains from new processes (Next Generation-Distillers Dried Grains; NG-DDGS) should be considered.

Traditional distillers dried grains with solubles (DDGS) have been utilized by the livestock industry for decades due to their high-levels of protein, fat, and fiber (Lodge et al., 1997; Batal and Dale, 2006). As a co-product of ethanol production, the ingredient is readily available and boasts affordable costs (de Godoy et al., 2014). Additionally, the ingredient is highly sustainable. The pet food industry currently practices sustainability by utilizing coproducts from plant derived protein sources, such as soybean meal, or corn gluten meal (Alonzo, 2017). While these common coproducts have been utilized by the industry for decades, it would be relevant to look for similar alternatives, such as NG-DDG. Next generation-distillers dried grains are produced using post-fermentation separation technologies to separate protein and yeast from fiber prior to drying. This process utilizes a series of screens and centrifuges to separate approximately twenty percent of total DDGS volume as the new high protein ingredient, while the remaining eighty percent is marketed as DDGS. These NG-DDG contain twice as much protein as traditional DDGs and may be a viable option for inclusion as a novel ingredient in pet foods (Belyea et al., 2004; Table 2.1). However, while promising, the ingredient has yet to make its way into the companion animal market, likely due to the limited knowledge of its effects on extrusion processing, nutrient utilization, and animal acceptance. While a few studies have examined the digestibility of the traditional ingredient in dog diets with some success (Allen et al., 1981; Swanson and Fahey, 2006; Silva et al., 2016), there remains a need for further exploration of its potential. Additionally, there is a distinct gap in research regarding the use of DDGS in extruded dog and cat foods. Although some research has been published regarding its use in extruded aquaculture feeds (Chevanan et al., 2004; Chevanan et al., 2007; Kannadhason et al., 2010), the specifications for those feeds are different than pet foods (i.e. sinkability, water durability, size, shape, texture, etc.), leaving limited information to interpret for their potential in companion animal diets. Before producers begin to consider the possible nutritional benefits to the animal, they should first consider the feasibility of creating a product that will be appealing to both the consumer and the animal in color, size, shape, and texture. Therefore, it was the objective of this study to determine the effect of the addition of NG-DDG on processing parameters and kibble texture in extruded dog and cat foods.

Materials and Methods

Diet Formulation

Diets with three different plant protein sources [corn gluten meal (CGM; Fairview Mills, Seneca, KS, U.S.A.), soybean meal (SBM; Fairview Mills, Seneca, KS, U.S.A.), and next generation-distillers dried grain (NG-DDG; Flinthills Resources, Wichita, KS, U.S.A.)] were formulated to be isonutritional and to contain equal amounts of protein from each test variable ingredients, with remaining mass made up of corn starch (Table 2.2). Diets were formulated to meet nutritional requirements of both dogs and cats (NRC, 2006). Chromium sesquioxide (0.25%) and titanium dioxide (0.40%) were added to serve as external markers to estimate fecal output in order to compute apparent total tract nutrient digestibility in future studies.

Diet Production

Diets were mixed as three separate batches and produced over three replicate processing days. After mixing, diets were added to an overhead bin with live bottom feeder which conveyed the mix to the preconditioner at a feed rate of 285.76 kg/h. In the preconditioner, moisture and thermal energy were added in the form of water and steam to begin the process of hydration and starch gelatinization, respectively. Water and steam inputs were recorded approximately every 20 minutes. Preconditioner paddles rotated at a speed of 165 rpm to effectively mix the matrix.

Material exited the preconditioner into the extruder at a temperature of 85°C and an average total mass flow (TMF) of 335 kg/h.

A small production scale single screw extruder (model E525; Extru-Tech, Sabetha, KS) was used for this experiment. The following processing parameters were recorded every 20 minutes: injection of water in kg/h, extruder RPM, die temperature, die pressure, percent openness of the mass restriction valve (MRV), bulk density out of the extruder (g/L), and percent load, which was calculated as the actual load in amps divided by the maximum extruder load (186 A). Material flowed through the extruder at an average TMF of 345 kg/h. This data was used to calculate the specific mechanical energy (SME) using the following equation (Equation 1).

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

Where τ is the percent torque, or motor load, τ_o is the no-load torque (18.71%), N is the screw speed in rpm, N_r is the rated screw speed (425 rpm), P_r is the rated motor power (111.85 kW), and m is the total mass flow in kg/s. The in-barrel moisture content (MC) was also calculated using the equation below (Equation 2).

$$MC = \frac{m_f \times X_f + m_{ps} + m_{pw} + m_{es} + m_{ew}}{m_f + m_{ps} + m_{pw} + m_{es} + m_{ew}} \tag{2}$$

Where m_f is the feed rate, X_f is the moisture content of the raw material, m_{ps} is the percentage of added steam in the preconditioner, m_{pw} is the percentage of added water in the preconditioner, m_{es} is the percentage of steam added into the extruder, and m_{ew} is the percentage of water added into the extruder. A moisture content of 10% was assumed for X_f. A 3.2 mm die was used for all diets to produce the food in an appropriate size for both dogs and cats. Knife speed was kept constant at 1,300 rpm. The MRV, which is located directly behind the die plate on the extruder, was used to aid in controlling the flow of material through the extruder by either increasing or decreasing constriction. This valve was utilized to aid in expansion of kibbles in order to achieve a similar bulk density out of the extruder (OE), which was used as a reference point for product consistency.

Kibbles were dried on aerated cookie sheets in a forced air convection oven at approximately 141°C. In process product moisture was determined by IR heat lamp (DSH-50-1; WANT Balance Instrument Co., Ltd., Changzhou, China) and kibbles were considered dry when moisture was less than 10%. Kibbles were separated into aliquots for dogs and cats and then coated with chicken fat (5.0%) fortified with antioxidant preservatives (0.03%), and species appropriate powdered flavor. Coated diets were stored in 9 kg poly-lined Kraft paper bags until fed. Within the bags replicates were composited. Feed samples were analyzed for nutrient composition at a commercial laboratory (Midwest Laboratories, Omaha, NE, U.S.A.). Analysis included moisture and dry matter (AOAC 930.15), organic matter and ash (AOAC 942.05), crude protein (AOAC 990.03), fat by acid hydrolysis (AOAC 954.02), and crude fiber (AOCS Ba 6a-05)

Kibble Analysis

Ten kibbles were collected for measurement at 20-minute intervals during each extrusion replicate. Digital calipers were used to measure the length and diameter of kibbles. The recorded diameter was an average of two diameter measurements taken by rotating the piece 90 degrees, as kibbles are often not symmetrical. Kibble weight was also recorded using an analytical scale with 0.1mg sensitivity (EX324N; OHAUS Corporation, Parsippany, NJ, U.S.A.). This information was used to calculate the sectional expansion index (SEI; mm^2/mm^2), specific length

(l_{sp}; mm/g), to measure radial and longitudinal expansion, as well as the volume (V_e; cm^3) and the piece density (ρ; g/cm^3), using the equations described below:

$$SEI = \frac{D_e^2}{D_d^2} \tag{3}$$

$$l_{sp} = \frac{l_e}{m_e} \tag{4}$$

$$V_e = \frac{\frac{\pi}{4} * D_e^2 * l_e}{1000} \tag{5}$$

$$\rho = \frac{m_e}{V_e} \tag{6}$$

where, D_e is the diameter of the extrudate in mm, D_d is the diameter of the die used (3.2 mm), l_e is the length of the extrudate in mm, and m_e is the mass of the extrudate in g.

Kibbles were analyzed for hardness and toughness using a TA-XT2 Texture Analyzer (Texture Technologies Corporation, Hamilton, MA, U.S.A.). The procedure used was modified from Dogan and Kokini (2007). A total of 20 kibbles per collection point per day were measured, amounting to 180 kibbles total per treatment. A 25 mm cylindrical probe was used for a compression test with a pre-test speed of 2 mm/s, a test speed of 1 mm/s, and a post-test speed of 10 mm/s. The strain level for the test was 50%. Hardness was considered to be the peak fracture force, or the maximum force at which a fracture occurs in each compression signature and was measured in kg. The toughness was considered to be the energy required to completely disintegrate the sample and was calculated as the total area under the curve in each compression signature in kg*mm.

Statistical Analysis

The study was organized as a 1-way treatment structure organized in a complete block design with day (1, 2, or 3) serving as the block. Each diet was produced in each block, providing 3 replicates per treatment. Data for kibble measurement and texture analysis were averaged within each block, giving three replicates for analysis. Means were analyzed using mixed models of the GLM with statistical software (SAS version 9.4; SAS Institute, Inc., Cary, NC, U.S.A.). The model statement for extrusion parameters contained preconditioner added water, preconditioner added steam, extruder added water, extruder rpm, die temperature, die pressure, percent openness of the MRV, percent load, SME, and bulk density as fixed variables. The model statement for kibble measurements contained length, diameter, weight, density, SEI, and specific length as fixed variables. The model statement for the texture analysis contained hardness and toughness as fixed variables. All model statements included production day as a random variable, and all means were separated using Fisher's LSD with a significant *F* (α = 0.05).

Results

The NG-DDG diet had more (37.93 kg/h; $P<0.05$) water injected into the preconditioner than the CGM diet (37.82 kg/h), with SBM being intermediate (37.89; Table 2.3). Steam addition into the preconditioner was similar among dietary treatments (average 53.46 kg/h). Similarly, water added to the matrix in the extruder (average 3.42%), extruder RPM (average 224.25 rpm), TMF (average 343.86 kg/h), die temperature (average 103.17°C), percent load (average 36.00%), SME (average 109.69 kJ/kg), and MC (average 19.62%) were also similar among treatments (Table 2.3). Die pressure was highest (2987.73 kPa; $P<0.05$) during the extrusion of the NG-DDG diet, followed by the SBM diet (2528 kPa), with the least amount of pressure recorded for the CGM diet (1666.26 kPa; Table 2.3). Moreover, the treatments also differed ($P<0.05$) in the percent openness of the MRV. The CGM diet had the largest opening at 60%, followed by SBM at 51.67% and NG-DDG at 40.00% (Table 2.3). There was no difference among treatments for bulk density out of the extruder (average 362.91 g/L).

The length of kibbles was similar between treatments, averaging 7.49 mm (Table 2.4). Conversely, the kibble diameter was larger ($P<0.05$) when CGM and SBM were added to the diet (5.66 and 5.60 mm, respectively) compared to the diet containing NG-DDG (5.18 mm; Table 2.4). Kibble mass (average 0.0899 g), volume (average 0.1772 cm^3), and piece density (average 0.5112 g/cm^3; Table 2.4) were similar among treatments. The similarities among treatments in kibble length and mass are reflected in the calculated specific length, which indicated no difference between treatments in longitudinal expansion (average 81.16 mm/g). Additionally, the differences ($P<0.05$) in diameter are mirrored in the SEI, with SBM having the largest expansion index of 3.07 mm^2/mm^2, NG-DDG kibbles having the least amount of radial expansion with an index of 2.62 mm^2/mm^2, and CGM kibbles intermediate to the extremes with an index of 3.13 mm^2/mm^2 (Table 2.4).

Analysis of kibble texture revealed no differences between hardness and toughness between all treatments (Table 2.5). The treatments averaged a hardness of 4.37 kg and an average toughness of 2,956.98 kg*mm.

Discussion

Processing parameters were attempted to be held constant throughout production to determine the effect of different ingredients on kibbles produced. However, in order to maintain some control over the final product and reach a similar bulk density exiting the extruder, some parameters needed to be adjusted. Ideally, all three treatments would have had similar amounts of water injected into the preconditioner. Differences in added water could affect the product moisture upon exiting the extruder, and result in a need for increased drying time, and drive up energy costs (Riaz and Rokey, 2012). Water and steam were added into the preconditioner to hydrate the matrix and add thermal energy. This begins the starch gelatinization process, which is largely responsible for the expanded, puffy texture for which kibbles are known (Rokey et al., 2010; Kannadhason et al., 2010). During the production, the NG-DDG treatment required more water in the preconditioner when compared to the CGM and SBM treatments. As material exits the extruder die, the drastic change in pressure causes the water trapped in the gelatinized starch matrix to vaporize, leaving an empty cell, and resulting in an expanded and puffy product (Kannadhason et al., 2010). During production of the NG-DDG ingredient, a large portion of the starch content is removed in the fermentation process. As a result, the expansion that occurs during extrusion is decreased (Chevanan et al., 2007; Stein and Shurson, 2009). Reported starch concentrations for traditional DDGS have been quite varied, with values ranging from 5.10-14.5% (Batajoo and Shaver, 1997; Belyea et al., 2004; Kim et al., 2008). Conversely, CGM, which originates from the same cereal as NG-DDG (corn), is well known for its high starch content and readily gelatinizes, decreasing the need for more restriction from the MRV (Belyea et al., 2004). Additionally, the CGM and SBM diets had corn starch added to make up for the difference in mass resulting from the various protein inclusions. This added starch may have further promoted kibble expansion in the CGM treatment (Table 2.2). High moisture and temperatures during extrusion allows for near complete gelatinization of starch (Tran et al., 2008). This may be why NG-DDG matrix required slightly more water to be added in the preconditioning step, in order to further promote the gelatinization of the remaining starch. Gelatinization is not only affected by the starch contents of ingredients used. Other nutritional aspects, such as fat or insoluble fiber contents, can have a decreasing effect on kibble expansion (Riaz, 2000). Distillers dried grains are known for having high levels of insoluble fiber (Widyarante and Zijlstra, 2006). A study by Jin et al. (1994) found that increasing the fiber content in materials resulted in increased dough viscosity. This can result in increased friction within the barrel and affect the bulk density of the final product (Jin et al. 1994). By adding more water, the matrix can be better hydrated, and friction can be managed. However, while there was more water required for the NG-DDG diet when compared to the CGM diet, numerically the difference was quite small, and the kibbles did not require any additional drying. Furthermore, the increase in added water did not result in a difference in bulk density out of the extruder or in piece density (Table 2.3, 2.4).

In addition to hydration, the water added in the extruder, as well as in the preconditioner, has a plasticizing effect (Ding et al., 2006). This changes the properties of the matrix to be glassy and slippery and can ultimately affect the retention time in the barrel of the extruder, which directly impacts the amount of mechanical energy added to the matrix (Rokey et al., 2010). Mechanical energy is an imperative function in this type of extrusion and is impacted by a variety of factors, including but not limited to screw configurations, retention time, and ingredient functionality. Extrudate properties are affected by the viscosity of the matrix as it moves through the barrel (Tran et al., 2008). If a material is overly plasticized, it will slip through the screws in the barrel, reducing friction and the application of mechanical energy to the mixture (Ding et al., 2006). This was observed in a study by Chevanan et al. (2007) in which the authors reported a 5% increase of moisture in the extruder barrel that decreased SME by over 150 J/g when extruding aquaculture diets containing 40% DDGS. High lipid content can also have this effect, decreasing the torque in the barrel, and resulting in decreased expansion due to insufficient barrel pressure (Riaz, 2000). Consequently, a lack of adequate gelatinization can decrease digestibility and result in a denser, less expanded kibble (Tran et al., 2008). Additionally, the ratio of thermal to mechanical energy can alter the physical characteristics of the extrudate (Tran et al., 2008; Koppel et al., 2014). Therefore, when attempting to create similar products with these three treatments, the lack of difference observed between the in-barrel MC is a positive attribute, as it implies the matrix within the barrel is behaving similarly and should have similar retention times and levels of mechanical energy applied within each treatment.

There were, however, a few variables that needed to be adjusted between treatments in order to create a similar product. As previously mentioned, NG-DDG have some compositional factors (i.e. low starch, high insoluble fiber) that may affect the expansion potential of the extrudate. By reducing the openness of the MRV, more friction is created behind the die plate, which reduces flow in the extruder barrel and translates to more mechanical energy. This increase in friction helps to further gelatinize the matrix (Riaz, 2000). This is further supported by the differences seen between the treatments in die pressure. As previously mentioned, high lipid content can have a detrimental effect on the build-up of pressure within the extruder barrel, reducing expansion and application of mechanical energy (Riaz and Rokey, 2010). The NG-DDG had the highest lipid content among treatments, which may be another reason why the treatment required a smaller opening at the MRV to create more pressure at the die. By controlling pressure within the barrel, it is possible to control the expansion of the extrudate, and ultimately create comparable products even with varying starch levels. While the radial expansion was not similar among all treatments as seen by the higher ($P<0.05$) values for the CGM and SBM treatments, the piece density did not differ, implying similar levels of expansion were achieved overall (Table 2.4). Furthermore, while these differences between treatments can result in altered mechanical energy, there was no difference in SME detected between treatments.

On the first day (replicate 1) of extrusion for the NG-DDG diet and the second day (replicate 2) of extrusion for the SBM diet, it was discovered that one of the die openings had become blocked. This resulted in increased pressure in the barrel and caused the kibbles produced during those runs to have a more elongated appearance. However, there was no difference detected between both the length of the kibbles and the longitudinal expansion overall. This situation amplifies the importance of replication in extrusion research. Without replication, any inference drawn from the results is likely inaccurate (Moonesinghe et al., 2007). Additionally, having replication allowed for a composite sample of the kibbles to be formed and normalized the sample pool to override the effects of any outliers. Similar longitudinal expansion values for extruded SBM were reported by Rosentrater, et al. (2015), supporting these findings. There were, however, differences between treatments in both the diameter and radial expansion of the kibbles. The lower radial expansion of the NG-DDG kibbles can be explained by the gelatinization of the matrix, further supporting the theory that the NG-DDG kibbles are less likely to expand on their own accord and reinforcing the need for minor changes in processing parameters, such as the openness of the MRV, to aid in this expansion. Studies by Hsieh et al. (1989, 1991) found that increasing fiber contents can result in a decrease in radial expansion. The NG-DDG had the highest fiber content among treatments, and the effects of this are seen in the SEI of the treatment (Table 2.4). These results are supported by the findings in a study by Kannadhason et al. (2010), in which they examined the use of graded levels of DDGS and their effects on processing parameters and final products in extruded aquaculture feeds. It was found that no matter the die size or screw speed, the addition of DDGS decreased the expansion ratio of the feed when compared to a control diet which did not include DDGS. A comparable study by Chevanan et al. (2004) also examined the use of DDGS in extruded aquaculture feeds and also found that the expansion of the extrudate decreased as inclusions of DDGS increased. Converse to the expansion, there were no differences in the mass, volume, or piece density of the kibble. Throughout production, the bulk density OE was measured periodically to provide a guideline for product consistency, and processing parameters were adjusted accordingly (Table 2.3). As gelatinization increases, bulk density decreases (Case et al., 1992). The ability to maintain similarities in piece density between treatments was achieved and further supports the conclusion that it is possible to create similar products with the treatments used with minimal changes to processing parameters.

There were no differences between treatments in terms of hardness and toughness when evaluating kibble texture. The hardness measurement is meant to simulate the force necessary to initially fracture the kibble, or a first bite for animals. The forces needed to fracture kibbles is related to the internal structure of the kibble, including attributes such as porosity, size of pores, and cell wall thickness (Dogan and Kokini, 2007). These attributes are all controllable through extrusion and can be affected by the ratio of thermal to mechanical energy and are directly related to the gelatinization of starch (Tran et al., 2008; Koppel et al., 2014). This analysis can potentially predict whether or not an animal will like the food (Hullar et al., 1998). For example, cats have been found to prefer a more mechanically cooked kibble, while dogs seem to prefer a product cooked with more thermal energy (Tran et al., 2008). A study by Koppel et al. (2015) examined the effects of fiber inclusions and fiber particle size on palatability of dry dog foods as well as the correlations between palatability and sensory attributes. Results from their study indicated that dogs preferred kibbles with higher fracturability and crispness. These results indicate that ingredient inclusion and processing parameters can affect texture, which in turn can affect the palatability of diets. The toughness of the kibbles is representative of the total force necessary to completely compress the kibble and may be reflective of the amount of force necessary for an animal to completely chew the kibble. However, while this information gives us a point of comparison between treatments, it does not provide as much of a practical application as the hardness. Both dogs and cats tend to not chew their food completely, as opposed to humans who completely masticate their food before swallowing, implying that the kibble toughness test may serve more of a quality and comparative purpose as opposed to one of practical application (Koppel et al., 2015).

Conclusion

Next generation-distillers dried grains show a strong potential for use in pet foods. In addition to being readily available, this high-protein ingredient is a more sustainable alternative to many of the commonly utilized ingredients which compete with the human food systems. As it relates to extruded pet food, at equivalent protein contributions, the NG-DDGs resulted in a similar kibble length, specific length, mass, volume, and piece density to the diets containing SBM and CGM. However, the diameter and SEI were lower relative to diets with CGM. The NG-DDG diets required slightly more water to be added in the preconditioner and had a need for more restriction in the extruder to achieve the target expansion for the kibbles. However, the treatments did not differ in SME in extrusion. The use of NG-DDG in exchange for SBM and CGM can be managed to produce a kibble of similar size, shape, and density appropriate for the pet food market.

Tables

Table 2.1. Nutrient composition of experimental ingredients* expressed on a dry matter basis.

Item, %	CGM*	SBM*	NG-DDG*
Moisture	10.17	12.33	6.71
Crude Protein	74.70	54.50	54.40
Crude Fat	1.76	1.28	4.18
Crude Fiber	n.d.**	3.63	4.43
Ash	1.11	6.96	4.57

*Corn gluten meal (CGM), Soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)
**Measured but below detection limit.

Table 2.2. Diet composition for experimental treatments.

Ingredient, %	CGM	SBM	NG-DDG
Base Ration	72.71	72.71	72.71
Corn	33.94	33.94	33.94
Chicken Meal, Low Ash	28.85	28.85	28.85
Beet Pulp	4.00	4.00	4.00
Fish Oil	0.14	0.14	0.14
Vitamins and Minerals	1.35	1.35	1.35
Natural antioxidant (dry)	0.04	0.04	0.04
CGM*	20.50	-	-
SBM*	-	24.75	-
NG-DDG*	-	-	25.00
Corn Starch	4.50	0.25	-
Titanium Dioxide	0.40	0.40	0.40
Chromium Sesquioxide	0.25	0.25	0.25
Chicken Fat + Antioxidant**	5.03	5.03	5.03
Flavor Powder**	1.00	1.00	1.00

*Corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)
**Indicates ingredient was applied topically after extrusion and drying.

Table 2.3. Processing parameters recorded in preconditioner and extruder

	Item	Diet CGM*	SBM*	NG-DDG*	SEM	p-Value
Preconditioner	Water (kg/h)	37.82[b]	37.89[ab]	37.93[a]	0.08	0.0375
	Steam (kg/h)	53.92	53.20	53.25	0.54	0.6223
Extruder	Water (kg/h)	10.90	11.32	10.51	0.68	0.5031
	RPM	245.00	225.83	209.17	33.19	0.7616
	TMF (kg/h)	345.47	345.14	345.07	0.55	0.5744
	Die Temperature (°C)	104.21	105.59	106.21	3.08	0.4598
	Die Pressure (kPa)	1666.26[c]	2528.08[b]	2987.73[a]	81.25	0.0008
	MRV (% open)	60.00[a]	51.67[b]	40.00[c]	0.96	<.0001
	Percent Load	36.55	35.92	35.55	1.45	0.4669
	SME (kJ/kg)	120.26	109.74	91.65	22.18	0.6144
	Bulk Density (g/L)	354.92	367.75	366.06	5.94	0.2811
	MC (%)	20.02	19.38	19.46	0.42	0.2768

[abc] indicates that within a row, unlike letters differ (P<0.05)

*Corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)

Table 2.4. Kibble measurements and calculations

Item	Diet CGM*	SBM*	NG-DDG*	SEM	p-Value
Length (mm)	6.66	8.11	7.69	0.73	0.4337
Diameter (mm)	5.66[a]	5.60[a]	5.18[b]	0.05	0.0040
SEI (mm^2/mm^2)	3.13[a]	3.07[a]	2.62[b]	0.23	0.0046
Specific Length (mm/g)	84.83	81.35	77.29	4.29	0.4910
Mass (g)	0.0793	0.1007	0.0897	0.01	0.4093
Volume (cm^3)	0.1682	0.2007	0.1626	0.02	0.2839
Piece Density (g/cm^3)	0.4760	0.5039	0.5537	0.01	0.4327

[abc] indicates that within a row, unlike letters differ (P<0.05)

*Corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)

Table 2.5. Texture analysis of kibbles using a TA-XT2 Texture Analyzer

Item	Diet CGM*	SBM*	NG-DDG*	SEM	p-Value
Hardness (kg)	3.79	4.53	4.80	0.32	0.1411
Toughness (kg*mm)	3223.38	2981.18	2666.39	781.10	0.8834

[abc] indicates that within a row, unlike letters differ (P<0.05)

*Corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)

CHAPTER 3 – EVALUATION OF NEXT-GENERATION DISTILLERS DRIED GRAINS AS A DIETARY INGREDIENT IN DOG AND CAT DIETS

Abstract

Trends in the pet food market have been focusing on grain-free diets, yet the majority of pet owners still buy foods that utilize plant-based ingredients and co-products. Corn and soy proteins have been used in pet foods for decades, and the use of new variations of co-products, such as next generation-distillers dried grains, should be explored. Twelve beagle dogs were fed diets utilizing three plant-based proteins: corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG). The study was conducted as a 3×3 replicated Latin Square design with 3 dogs randomly assigned to each of 3 treatments for each period. Diets were formulated to be isonitrogenous and were supplemented with titanium dioxide to serve as an external marker in order to estimate apparent total tract digestibility. Dogs were housed individually and fed twice daily, and water was available *ad libitum*. Feces were collected after feedings. Means were separated using Fisher's LSD using the GLIMMIX procedure in statistical analysis software (SAS 9.1). The diet produced with CGM was slightly more digestible ($P<0.05$) than NG-DDG and SBM in terms of dry matter, organic matter, crude protein, crude fat and gross energy. Dogs fed the diet containing NG-DDG had larger ($P<0.05$) fecal mass than those fed SBM and CGM. The NG-DDG diet also resulted in a higher fecal score ($P<0.05$) than those fed diets with CGM, and a similar score to those fed SBM. For palatability assessment, dogs had a preference ($P<0.05$) for CGM over SBM or NG-DDG, but cats showed a preference ($P<0.05$) for SBM and NG-DDG over CGM. Results indicate that NG-DDG is comparable to CGM and SBM in dog and cat diets.

Introduction

About two-thirds of U.S. households own pets, which economically translates into a pet industry worth $66.75 billion in 2016. Further, the pet food market in the U.S. is nearly half of this industry at $30 billion with expectations to reach more than $37 billion by 2020 (Packaged Facts, 2016). While there are many new pet food formats being introduced to the market (i.e. freeze dried, raw, etc.), dry foods consistently dominate the volume (Pet Food Industry, 2013). Humanization has been a major influence on market trends in the pet food industry. Today's "pet parents" want their animals to eat as well as they do and are beginning to seek options in ingredient composition that reflect their own purchases (Boya et al., 2015). According to Pet Food Industry magazine (2015), 55% of pet owners are concerned about the amount of "fillers," such as grains and meat by-products, in their pet's diets. However, despite these trends, 81% of dog foods and 85% of cat foods still utilize traditional ingredients like corn, wheat, and soy (Packaged Facts, 2016). Use of plant-based ingredients and co-products may prove economically beneficial in this rapidly growing market (Silva et al., 2016). In addition to lower costs, it has been found that there is less variation in nutritional content between plant proteins relative to animal protein meals (Clapper et al., 2001). Protein concentrates from corn and soy have been used successfully in pet foods for decades. Improvements in processing technology and co-products from distilling grains from ethanol production have created new variations in these base proteins. Therefore, new alternatives such as distillers dried grains from new processes (Next Generation-Distillers Dried Grains; NG-DDGS) should be considered.

Corn ethanol production is a rapidly growing renewable energy industry on a global scale (de Godoy et al., 2014). Processing of ethanol from corn can be divided into three categories:

wet and dry milling, and dry grinding (Kingsly et al., 2010). Distillers dried grains with solubles are a co-product resulting from the dry grinding process. After the fermentation of starch, the non-fermentable residues are dried and blended to create DDGS. Traditionally, the ingredient boasted an affordable cost and a high crude protein, fat, and fiber content (Lodge et al., 1997). While frequently utilized in livestock feed, the nutritional profile of this ingredient implies an application in companion animal foods in low cost diets or specialty foods, such as weight loss diets (Silva et al., 2016). Over 150 billion metric tons of corn were used for ethanol production in the month of October 2017, with almost 450,000 metric tons of DDG produced as a co-product (Renewable Fuels Association, 2017). New technology has been developed in the ethanol industry to improve these co-products that may make them more suitable to use in companion animal food. Next generation-distillers dried grains are produced using post-fermentation separation technologies to separate protein and yeast from fiber prior to drying. This process utilizes a series of screens and centrifuges to separate approximately twenty percent of total DDGS volume as the new high protein ingredient, while the remaining eighty percent is marketed as DDGS. These NG-DDG contain twice as much protein as traditional DDGs and may be a viable option for inclusion as a novel ingredient in pet foods. However, there is currently no research available describing the use of this product in companion animal diets. Therefore, it was the objective of this study to determine the effect of NG-DDG in pet diets on nutrient utilization (digestibility) and stool consistency in dogs, and palatability in dogs and cats.

Materials and Methods

Diet Formulation

Diets with three different plant protein sources [corn gluten meal (CGM; Fairview Mills, Seneca, KS, U.S.A.), soybean meal (SBM; Fairview Mills, Seneca, KS, U.S.A.), and next generation-distillers dried grain (NG-DDG; Flinthills Resources, Wichita, KS, U.S.A.)] were formulated to be isonutritional and to contain equal amounts of protein from test variable ingredients, with remaining mass made up of corn starch (Tables 3.2 and 3.3). Diets were formulated to meet nutritional requirements of both dogs and cats. Chromium sesquioxide (2.5 g/kg) and titanium dioxide (4.0 g/kg) were added to serve as external markers to estimate fecal output in order to compute apparent total tract nutrient digestibility; however, for this chapter, only the titanium results will be discussed.

Diet Production

Each diet was mixed and split into three batches and produced over three replicate days using a single screw extruder (model E525; Extru-Tech in Sabetha, KS). A 3.2 mm die was used to produce the food in an appropriate size for both dogs and cats. Processing parameters were recorded every 20 minutes. At three time periods (beginning, middle, and end of extrusion batch), 10 kibbles were collected and physical dimensions were recorded. As kibbles exited the extruder (wet) they were dried in a forced air convection oven. Kibble moisture was determined by infrared heat lamp and considered fully dried when the moisture was less than 10%. Kibbles were then separated into aliquots for dogs and cats and coated with chicken fat fortified with antioxidant preservatives, and the species appropriate powdered flavor. Coated diets were stored in poly-lined Kraft paper bags until fed. Within the bags replicates were composited.

Feeding Trial

Twelve intact beagle dogs (8 male, 4 female) with average weight of 10.99 ± 1.24 kg were individually housed in 1.83 m ×1.20 m cages with acrylic coated mesh flooring with a three-piece tray underneath to allow for separation of urine and feces. The study consisted of 3 periods comprised of 9 days diet adaptation, followed by 5 days of collection in a replicated Latin Square (3 x 3) experimental design. Wherein, each treatment was fed in each period over the 3 periods (6 weeks total). In this model, each animal served as its own control, and each treatment had 12 total observations. Dogs were housed at the Large Animal Research Center at Kansas State University in Manhattan, KS. All animal use was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC) prior to the beginning of the study and complied with the National Institutes for Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Animals were housed under temperature controlled environmental conditions and water was provided *ad libitum*. Lights were on a 12 h cycle with lights off from 1900 to 0700 each night. The food amounts were estimated to maintain body weight throughout the duration of the study using the NRC (2006) equations to estimate the ME of the food and food amounts as $130*BW^{0.75}$ for dogs. Dogs were weighed after each period and feeding amounts were adjusted accordingly. Feeding occurred twice daily at 0800 and 1600. Food was offered for 1 hour, and remaining orts were then collected and weighed.

Following the 9 days of adaptation, sample collection began starting at 0800 and extended for the next 120 hours. Feces were collected after meals and whenever observed throughout the period. Samples were scored on a 5-point scale created by Royal Canin: (1) completely liquid stool that can be poured, (2) very soft stool that takes the shape of its container, (3) soft stool that retains shape, (4) hard formed stool, and (5) hard dry pellets (Figure 3.1; Royal Canin, St. Charles, MO., USA). Samples were scored in 0.5 increments, and a score of 3.5-4 was considered ideal. Samples were placed in plastic bags (Whirl-pak, The Aristotle

Corporation, Stamford, CT, U.S.A.) labeled with dog name, period number, and day of study. Samples from each period were pooled and frozen for later analysis.

Digestibility Calculations

At the conclusion of the feeding assay, all feces were dried until no additional weight was lost (24-48 hr) in an electric oven (Cat 52755-20, Matheson Scientific, Morris Plains, NJ, U.S.A.) at 55°C. Dried samples were ground using a high-speed fixed blade rotor mill to pass through a 1 mm screen (ZM 200, Retsch, Verder Scientific, Haan, Germany). Titanium (Ti) concentration was measured in food and feces by use of a microplate reader (Synergy H1, Biotek, Winooski, VT, USA), described by Myers et al. (2004). Apparent total tract nutrient digestibility (ATTD) by Ti was determined by equation 1:

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

Nutrient Analysis

Feed and fecal samples were analyzed for nutrient composition at a commercial laboratory (Midwest Laboratories, Omaha, NE, U.S.A.). Analysis included moisture and dry matter (AOAC 930.15), organic matter (AOAC 942.05), crude protein (AOAC 990.03), and fat by acid hydrolysis (AOAC 954.02). Gross energy was measured using bomb calorimetry according to the methods defined in the Parr operating manual (1341 Oxygen Bomb Calorimeter, Parr Instrument Company, Moline, IL, USA).

Palatability Trial

Experimental treatments were evaluated for palatability by both dog and cat panels at a commercial kennel (Summit Ridge Farms, Susquehanna, PA). Each was conducted as a split-plate test, in which two stainless steel bowls each containing 400 g of food for dogs, and 100 g of food for cats were presented to animals for a total of 30 minutes before removal. Bowl positions were switched daily. Twenty animals were fed each day of the study, and each comparison trial was repeated for two days, providing a total of 40 observations for each species and paired comparison test. Preference was observed by the technicians who recorded the animals first choice when approaching the food bowls, and total food consumption. Data from consumption are presented as a ratio (Equation 2).

$$Intake\ Ratio = \left(\frac{consumption\ of\ Diet\ A}{total\ consumption\ Diet\ A + Diet\ B}\right) \quad (2)$$

Statistical Analysis

The digestibility experiment was conducted as a 3 × 3 replicated Latin Square design with 3 dogs randomly assigned to each of 3 dietary treatments for each period. Each of 12 dogs was randomly assigned to treatment and replicate according to the procedure of Kim and Stein (2009). Data was analyzed using the GLM for mixed models procedure in SAS (GLIMMIX; SAS version 9.4; SAS Institute, Inc., Cary, NC). The model statement contained nutrient digestibility, feed intake, fecal score, number of defecations, fecal weight, and diet as fixed variables. Period and dog were included as random variables. Means were separated using Fisher's LSD with a significant F ($\alpha = 0.05$). In the palatability experiments, the consumption ratio was analyzed using a 2-Way ANOVA, and the first-choice preference was analyzed using a Chi^2 test.

Results

Diet Analysis

Nutrient analysis of experimental ingredients and diets are presented in Tables 3.1 and 3.3.

Feeding Trial

Results for food intake, fecal output, fecal score, and fecal weight are presented in Table 3.4. There was no difference in food intake between all diets (P>0.05) as expected as food intake was controlled to maintain body weight and fed as a meal twice daily. Dogs were observed to be eager to consume food, and any orts recorded were generally the result of spilled food from a respective dog. Dogs fed the CGM diet had less daily defecations (P<0.05) than those fed the SBM or NG-DDG diets (2.03 vs average 2.41, respectively). Fecal output (dry) was lowest (P<0.05) for dogs fed the CGM (35.91 g/d) compared to both other treatments, with 20% greater fecal output for those fed SBM and nearly 55% greater fecal output for dogs fed the NG-DDG diet, respectfully. The dogs consuming CGM had a lower (P<0.05) fecal score to those fed the NG-DDG diet, with dogs fed the SBM diet being intermediate.

Apparent Total Tract Digestibility

The dry matter digestibility was lower (P<0.05) for dogs fed the NG-DDG diet versus both CGM and SBM diets (78.19 vs. 83.37 and 80.61%, respectively; Table 3.5). Organic matter (OM) digestibility was also lower for NG-DDG diets (P<0.05; 79.46%) relative to dogs fed CGM and SBM (86.17 and 83.13%, respectively). Crude protein digestibility did not vary between SBM and NG-DDG (average 82.40 %), and both were less digestible than CGM (P<0.05; 85.65%). The dogs fed the CGM had the highest (P<0.05) digestibility of gross energy (77.16%) when compared to both SBM and NG-DDG diets (75.48 and 69.89%, respectively).

Palatability Trial

On a consumption basis, the dogs preferred (P<0.05) CGM over NG-DDG, roughly 2:1 (Table 3.6). There was no difference between the CGM and SBM (IR of 0.432), or between SBM and NG-DDG (IR of 0.454). When evaluating which food was approached first by the dogs, there was no difference between DDG and CGM (17 vs. 23) or between SBM and DDG

(20 vs. 20). However, there was a difference between SBM and CGM, with 13 observations of an approach to the SBM first and 27 observations of an approach to the CGM first over the two-day trial.

The cats displayed different preferences to the diets than dogs (Table 3.7). In this case NG-DDG and SBM were preferred ($P<0.05$) over the CGM diet with an IR of 0.606 and 0.632, respectively. There was no difference between SBM and NG-DDG (IR of 0.456). No difference was seen in first approach between any of the paired comparisons over the two-day trial.

Discussion

The experimental diets were developed on a platform of 25% inclusion of the NG-DDG with corresponding quantities of protein from the CGM and SBM. During their development standard crude protein values for ingredients (CGM: 60%, SBM: 49.6%, NG-DDG: 49.2%) were used with an initial estimate of CP between 33 and 34%. However, the CP content of each of the individual ingredients and ration were greater than initially assumed with a final diet CP analysis that was higher than expected (Table 3.3). Likewise, the crude fat values did not meet the expected value of 12 to 13% among the diets. This was in part controlled by the external application of fat during the coating process. But, as a raw material the NG-DDG had more than double the fat content of the CGM and SBM, resulting in 3% more crude fat in the NG-DDG diet (Table 3.3). Otherwise, the experimental diets followed the initial experimental design and were produced in a fashion to yield similar products from a visual and physical perspective conducive to the study.

Dogs fed the SBM and NG-DDG diets had higher fecal mass and more daily defecations compared to the dogs fed the CGM diet. This may in part be explained by the higher crude fiber in the SBM and NG-DDG relative to the CGM. High levels of fiber may increase the rate of passage through the digestive system and decrease absorption, resulting in less overall digestion, and larger fecal mass (Yamka et al., 2003). Similar results were also found by Allen et al. (1981) with the addition of DDGS to dog diets. The fecal scores while differing between treatments, were all within the acceptable range, e.g. 3.0 – 4.0. The dogs consuming CGM had a slightly lower ($P<0.05$) fecal score to those fed the NG-DDG diet, with dogs fed the SBM diet intermediate and similar to both extremes. Fecal scores for SBM diets in this study were similar to those recorded by Bednar et al. (2000) and Clapper et al. (2001). Clapper et al. (2001) also explored levels of insoluble vs. soluble fiber in SBM and reported that SBM had nearly a 10:1 ratio of insoluble to soluble fiber. Insoluble fiber can aid in the formation of ideal, firm feces (Burkhalter et al., 2001). This is an area that should be explored more fully in the current work. Additionally, higher fiber levels can be supplemented in diets to aid in body weight control and promote regular defecation (de Godoy et al., 2009), suggesting possible benefits for these ingredients beyond simply providing protein.

Dry matter digestibility was highest in dogs fed the CGM diet, followed by SBM and NG-DDG. These results are corroborated by the findings of previous studies (Zuo et al., 1996; Bednar et al., 2000; Carciofi et al., 2009); wherein, a dry matter digestibility of 81% was reported for dogs fed a CGM diet, and an average DM of 78.3% was reported in dogs fed an SBM-based diet. Similarly, a series of studies by Allen et al. (1981) also found a comparable DM digestibility for dogs fed a diet with DDGS, reporting an ATTD of 79.9% when DDGS were included at 15.7%. The similar ATTD value (78.19% for NG-DDG; Table 3.5) of our study suggest that increasing the DDG inclusion over 15% does not negatively affect digestibility. This is supported by a study in which a diet containing 52.17% DDGS was fed to swine, and a DM digestibility of 78.04% was reported (Lei et al., 2017). Differences in organic matter digestibility were reflective of the ATTD of DM, with CGM having the highest digestibility, followed by SBM and NG-DDG. Similar values were reported by Youssef et al. (2008), who observed an OM digestibility 79.7% in broiler chicks fed a diet containing 15% DDGS. The digestibility values for both DM and OM met our expectations and, while differing between treatments, would all be considered acceptable for a commercial pet food.

Crude protein digestibility values observed in this study met expectations and were consistent with previous literature. The results agree with the data reported by Carciofi et al. (2009) in which they found cats fed a diet including 17.2% CGM had a crude protein digestibility of 84%. While a different species, these animals share enough similarities to extrapolate results from this type of evaluation. Additionally, the results from the NG-DDG at 25% of the formula concurs with the results reported by Silva et al. (2016) in which a CP digestibility of DDGS was 85% when included at 18%. Inclusion of NG-DDG at 25% did not negatively impact utilization. The digestibility of crude fat was slightly less for those fed the NG-DDG diet, however, numerically the treatments were quite similar, and the reported value is analogous to previously observed values (Silva et al., 2016). Again, while the experimental diets differed slightly in digestibility, all would be considered acceptable.

One could concede that the level of the three protein sources were higher than typically considered practical. Further, previous research has shown detrimental effects on animal utilization due to elevated levels of oligosaccharides from the SBM at 30% inclusion as an example. Oligosaccharides are non-starch polysaccharides (NSP) that are highly fermentable in the large intestine, and an excess can result in decreased digestibility, increased flatulence, and (or) loose stools (Félix et al., 2012). These high levels of NSP are present not only in SBM, but in DDG as well. An increased presence of NSP could explain the decrease in GE digestibility in both SBM and NG-DDG treatments, as the added fibers could act as a caloric diluent. Both Félix et al. (2012) and Silva et al. (2016) have examined the use of these ingredients at graded levels in dog foods along with the addition of enzyme complexes to aid in digestion to diminish the effect of NSPs. Both studies reported increased digestibility by the dogs fed foods with added enzymes, and a decrease in digestibility with increasing levels of added protein sources (at levels up to 18% DDG and 30% SBM). Different from these previous studies, the elevated level chosen for the current research was intended to test the ingredients as a major contributor of protein in the diet and to increase the chances of identifying sensory differences. In this case, the NG-DDG's proved to be slightly less digestible than CGM and equal to SBM. No overt sign of negative effects on stools or digestibility were observed. Thus, it appears the elevated level included could be acceptable in the face of high protein diet (exceeding requirements by 25%) and (or) for production of feline diets that typically exceed 30% CP.

While the CGM diet was preferred over the NG-DDG diet, the dogs did not reject the NG-DDG diet. Silva et al. (2016) found that the addition of DDG at levels up to 180g/kg enhanced palatability when compared to diets with no DDG inclusion. The results of this trial were unexpected. Dogs have a high preference for fats (Li et al., 2017) and a dog's food selection is highly driven by smell. Houpt et al. (1978) found that when dogs were presented with a bland diet supplemented with a meat odor, they preferred it over a control diet with no odor. Oils and short chain fatty acids are recognized in a special section of the olfactory bulb, and this strong odor may be a driving factor behind a dog's liking (Manabe et al., 2010). As seen in Table 3.3, the NG-DDG diet had a crude fat content 3% higher than both CGM and SBM, so it was surprising that it was not preferred by the dogs.

The cats displayed different preferences to the diets than dogs (Table 3.9). In this case NG-DDG and SBM were preferred ($P<0.05$) over the CGM diet with an IR of 0.606 and 0.632, respectively. There was no difference between SBM and NG-DDG (IR of 0.456). No difference was seen in first approach between any of the paired comparisons over the two-day trial. The aversion to the CGM diet was unexpected, as cats have a high preference for amino acids (White and Boudreau, 1975). Given the CGM diet was almost 4% units higher in protein relative to the SBM diet, and slightly higher than the NG-DDG diet (Table 3.3) it would have been hypothesized to be preferred. However, the higher affinity to the NG-DDG diet for cats, in comparison to preferences shown by dogs, may be due to the high levels of yeast present in NG-DDG. As DDG are a product of fermentation, much of their protein content can be attributed to yeast (Belyea et al., 2004). Yeast has been observed as highly palatable to cats, most likely due to the high presence of nucleotides (Swanson and Fahey, 2006; White and Boudreau, 1975). In a publication by Li et al. (2017) it was observed in a ranking test that dogs have a liking for corn over other sources of grain. This would support the overall acceptability of the diets in this study. One might consider rice as the starch source in a control for future work so as to not confound the evaluation of the proteins and serve as a blander base ingredient to evaluate the various corn and soy derived protein concentrates. More studies should be done with both species to evaluate palatability of NG-DDG to confirm these initial results. In spite of this, the quantity of each should have been effective to determine if any unwanted sensory attributes were present in the protein sources. The results support they were well liked and effective for use in pet foods.

Conclusion

Next Generation-Distillers Dried Grain is a viable alternative protein source for extruded dog and cat foods. Dogs fed NG-DDG had high quality stools, that were higher in mass than dogs fed both SBM and CGM. Additionally, dogs fed the NG-DDG diet had more defecations per day than those fed the CGM diet. Digestibility assessment in dogs indicated that the NG-DDG diet was less digestible than SBM and CGM. However, when used in a well-balanced diet, all ingredients would be viable options for pet foods. Further, though less digestible than SBM and CGM, NG-DDG meets the nutrient requirements established by the National Research Council for adult dogs in maintenance (NRC, 2006). Both dogs and cats found the food to be palatable and showed no signs of refusal. In preference testing, dogs preferred CGM over NG-DDG and SBM, while cats preferred NG-DDG or SBM over CGM. Future work should explore practical inclusion levels of NG-DDG and the resulting impact on digestibility, as well as the value derived from the yeast levels on extra-nutritional value and their influence on palatability.

Tables and Figures

Table 3.1. Nutrient composition of experimental ingredients* expressed on a dry matter basis.

Item, %	CGM*	SBM*	NG-DDG*
Moisture	10.17	12.33	6.71
Crude Protein	74.70	54.50	54.40
Crude Fat	1.76	1.28	4.18
Crude Fiber	n.d.**	3.63	4.43
Ash	1.11	6.96	4.57

*Corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)
**Measured but below detection limit.

Table 3.2. Diet composition for experimental treatments.

Ingredient, %	CGM*	SBM*	NG-DDG*
Base Ration	72.71	72.71	72.71
Corn	33.94	33.94	33.94
Chicken Meal, Low Ash	28.85	28.85	28.85
Beet Pulp	4.00	4.00	4.00
Fish Oil	0.14	0.14	0.14
Vitamins and Minerals	1.35	1.35	1.35
Natural antioxidant (dry)	0.04	0.04	0.04
CGM	20.50	-	-
SBM	-	24.75	-
NG-DDG	-	-	25.00
Corn Starch	4.50	0.25	-
Titanium Dioxide	0.40	0.40	0.40
Chromium Sesquioxide	0.25	0.25	0.25
Chicken Fat + Antioxidant**	5.03	5.03	5.03
Flavor Powder**	1.00	1.00	1.00

*Corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)
**Indicates ingredient was applied topically after extrusion and drying.

Table 3.3. Nutrient composition of experimental diets expressed on a dry matter basis.

	Diet		
Nutrient, %	CGM*	SBM*	NG-DDG*
Moisture	5.56	7.02	3.74
Crude Protein	39.90	36.20	39.20
Crude Fat	12.40	12.50	15.30
Crude Fiber	2.19	3.24	3.08
Ash	6.18	7.29	7.07

*Corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)

Table 3.4. The effect of experimental diets on daily defecations, fecal score, and dry fecal weight

	Diet				
Item	CGM*	SBM*	NG-DDG*	SEM	p-Value
Food Intake (g/d)	221.06	223.80	225.68	4.31	0.5130
Daily Defecations (no.)	2.03[b]	2.43[a]	2.38[a]	0.13	0.0124
Wet Fecal Weight (g/d)	108.31[b]	151.36[a]	154.29[a]	4.46	<0.0001
Dry Fecal Weight (g/d)	35.91[c]	43.25[b]	55.65[a]	7.17	<0.0001
Fecal Score**	3.27[b]	3.43[ab]	3.63[a]	0.09	0.0074

[abc] means within a row with unlike letters differ (P<0.05)
*Corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)
**Samples were scored on a 5-point scale: (1) completely liquid stool that can be poured, (2) very soft stool that takes the shape of its container, (3) soft stool that retains shape, (4) hard formed stool, and (5) hard dry pellets

Table 3.5. The effect of experimental diets on apparent total tract digestibility as determined by titanium concentration.

	Diet				
Item, %	CGM*	SBM*	NG-DDG*	SEM	p-Value
Dry Matter	83.37[a]	80.61[b]	78.19[c]	0.36	<0.0001
Organic Matter	86.17[a]	83.13[b]	79.46[c]	0.38	<0.0001
Crude Protein	85.65[a]	82.59[b]	82.21[b]	0.45	<0.0001
Crude Fat	91.41[a]	91.72[a]	90.16[b]	0.25	<0.0001
Crude Fiber	20.98[a]	19.58[a]	-0.25[b]	6.17	0.0004
Ash	31.57[b]	39.57[a]	35.61[ab]	1.79	0.0150
Gross Energy	77.16[a]	75.48[b]	69.89[c]	0.63	<0.0001

[abc] means within a row with unlike letters differ (P<0.05)
*Corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)

Table 3.6. The effect of experimental diets on palatability assessed by dogs

Diet Comparison* (A vs. B)	FC, n[1]	IR of diet A[2]
NG-DDG vs. CGM	17	0.365**
SBM vs. CGM	13**	0.432
SBM vs. NG-DDG	20	0.454

*Corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)
**Comparison differs P<0.05
[1]First Choice (FC): number of first visits to bowl A (40 observations)
[2]IR of diet A = intake (g) of diet A/total intake (g) of diets A+B

Table 3.7. The effect of experimental diets on palatability assessed by cats

Diet Comparison (A vs. B)	FC, n[1]	IR of diet A[2]
NG-DDG vs. CGM	22	0.606*
SBM vs. CGM	19	0.632**
SBM vs. NG-DDG	20	0.456

*Corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)
**Comparison differs P<0.05
[1]First Choice (FC): number of first visits to bowl A (40 observations)
[2]IR of diet A = intake (g) of diet A/total intake (g) of diets A+B

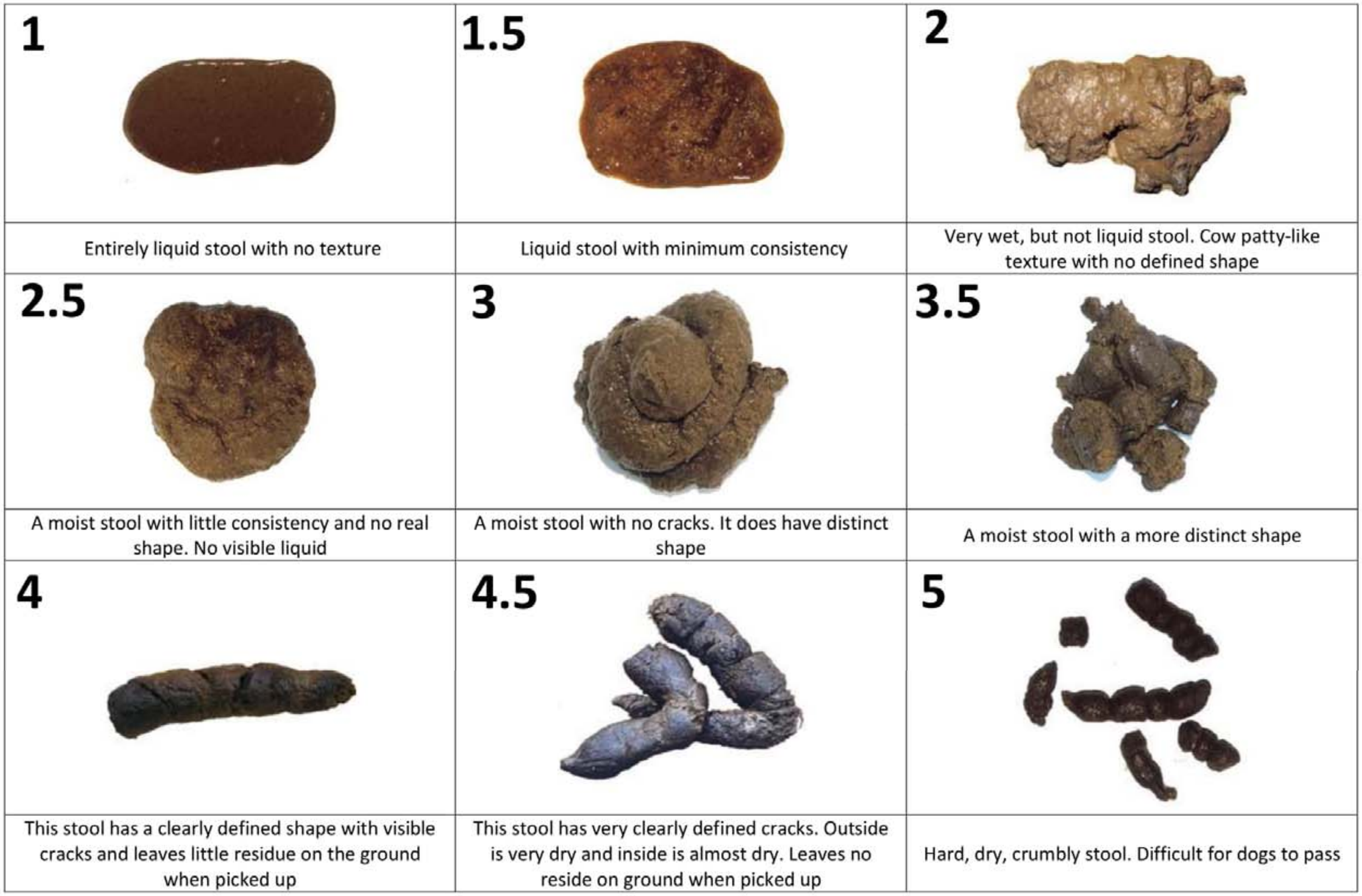

Figure 3.1. Scoring chart used to evaluate feces (Royal Canin).

CHAPTER 4 – AN EVALUATION OF THE AMINO ACID PROFILE AND PROTEIN QUALITY OF NEXT-GENERATION DISTILLERS DRIED GRAINS FOR USE IN COMPANION ANIMAL DIETS

Abstract

Introduction of novel protein sources has had a considerable influence in new product growth in the pet food industry, but knowledge of their shortcomings are limited. The objective of this study was to evaluate the protein quality of next-generation distillers dried grains in comparison to traditional pet food ingredients through a chick growth assay. Experimental diets contained 10% crude protein from ingredients [spray dried granulated egg (SDG), corn gluten meal (CGM), next generation-distillers dried grains (NG-DDG), and soybean meal (SBM)]. The SDG treatment served as a positive control, and a nitrogen-free diet was also fed to serve as a negative control (NEG). Chicks were fed the experimental diets for 10 days. There were 6 chicks per pen with 4 pens per treatment, and the collective pen weight was relatively equal for all treatments (912.4 ± 5.7 g). The protein efficiency ratio (PER) of each treatment was calculated as weight gain (g) per protein intake (g). The PER of SDG was higher ($P<0.05$) than all other treatments with NG-DDG and CGM having the lowest of all treatments ($P<0.05$). While all treatments ranked inferior to the positive control all would be acceptable as supplemental protein sources.

Introduction

Approximately two thirds of U.S. homes own a pet, resulting in a pet food industry worth more than $30 billion (APPA, 2016; Packaged Facts, 2016). Humanization has been a driving factor behind today's market trends, which is reflected in pet owners purchasing decisions. Today's owners are seeking pet foods similar to their own dinner plates and are more likely to seek and purchase foods with higher meat quantity and quality (Okin, 2017). Although companion animal consumption of animal proteins only accounts for a small percentage of global protein consumption, these protein sources are also potential food sources for humans, resulting in increased competition and, consequently, less animal proteins available to pet food companies without a major increase in cost (Fiacco et al., 2018). This has led to an avoidance of more traditional ingredients, such as meat or plant by-products that are not utilized by the human food systems, as a result many nutritionally adequate co-products are going to waste (Swanson et al., 2013).

In order to increase sustainability, it may be necessary to shift towards plant-based or other novel proteins that are not currently being utilized by the human food systems. The pet food industry has been practicing sustainability for decades by utilizing co-products from plant derived sources, such as soybean meal or corn gluten meal (Alonzo, 2017). Despite these trends towards higher inclusion of animal-sourced proteins, over 80% of dog and cat foods still utilize traditional ingredients, such as corn gluten meal or soybean meal (Packaged Facts, 2016). Additionally, novel and somewhat controversial ingredients such as distillers grains from ethanol production have been considered for potential pet food ingredients. Further, improvements in processing technology and co-products from distillers grains from ethanol production have created new variations in these base proteins. Therefore, new alternatives such as distillers dried grains from new processes (Next Generation-Distillers Dried Grains; NG-DDGS) should be considered. While some studies have examined the digestibility of traditional distillers dried grains in companion animal diets with some success, there remains a distinct gap in knowledge of the protein quality of these ingredients due to the tendency of the pet food industry to formulate diets with an excess of nutrients (Swanson et al., 2013; Silva et al., 2016). Therefore, it is necessary to examine the protein quality of these individual ingredients to truly understand their potential for use in the pet food industry.

One method for examining the quality of a protein source is to evaluate the amino acid profile of the ingredient. Both canines and felines have a dietary requirement for amino acids. However, the protein source (plant vs. animal) does not matter as long as the ingredient fulfills the animal's nutritional requirements. In addition to the complete amino acid content of ingredients, the bioavailability of amino acids must also be considered. The bioavailability of a protein sources is directly related to its digestibility and the ability of an animal to utilize its amino acids. Ingredient processing can result in changes to the protein that are positive or negative. For example, cooking the ingredient will denature the protein, increasing digestibility. However, if the ingredient is overheated, damage can occur to the protein as a result of transformations such as aggregation and Maillard reactions, making them less usable by the animal (Hodge, 1953; Cromwell et al., 1993; van Rooijen et al., 2013; Salazar-Villanea et al., 2016). Therefore, it is necessary to employ the use of a secondary evaluation method to fully assess the quality of a particular protein source.

Several researchers have used the chick protein efficiency ratio (PER) model as a method to evaluate various protein sources for use in a pet food diet (Wang and Parsons; 1998, Godoy et al., 2014). While it may seem indirect to evaluate pet food through an avian model, there have been proven similarities between the digestion of amino acids between canines and poultry (Fiacco, et al., 2018). Further, while many studies have chosen to use rats as a model, rats and chicks have been found to produce similar results (Hayward and Hafner, 1941). These PER assays can be used to obtain quick and meaningful data which may be used to rank protein ingredients by an animal's ability to fulfill their nutrient requirements and utilize the ingredients.

Therefore, it was the objective of this study to examine the protein quality of next-generation distillers dried grains, a protein ingredient novel to the pet food industry, compared to other common protein ingredients being used in pet food today through amino acid analysis and protein efficiency ratio.

Materials and Methods

Experimental Ingredients and Amino Acid Analysis

Experimental ingredients [spray dried granulated egg (SDG; Isonova Technologies LLC, Springfield, MO, USA), corn gluten meal (CGM), soybean meal (SBM; Fairview Mills, Seneca, KS, USA), and next generation-distillers dried grains (NG-DDG; Flinthills Resources, Wichita, KS, USA)] were acquired prior to study. A 200g sample of each ingredient was collected and analyzed for nutrient composition at a commercial laboratory (Midwest Laboratories, Omaha, NE, USA). Analysis included dry matter (AOAC 930.15), organic matter and ash (AOAC 942.05), crude protein (AOAC 990.03), fat by acid hydrolysis (AOAC 954.02), and crude fiber (AOCS Ba 6a-05). Additionally, samples were sent to a university laboratory (University of Missouri Agriculture Experiment Station chemical laboratory, Columbia MO) to obtain a complete amino acid profile, including tryptophan and cysteine, of each ingredient according to AOAC standard methods (AOAC 2012a, 2012b).

Protein Efficiency Ratio

A basal diet was formulated to meet all of the nutrient requirements of the chicks except for crude protein and amino acids (Nutrient Requirement of Poultry, 1994; Table 4.1). Protein sources were added to the nitrogen-free basal diet to achieve 10% crude protein in all diets, at the displacement of the 2:1 corn starch and dextrose mixture (Johnson et al., 1998). Additionally, the nitrogen-free basal diet was use to serve as a negative control (NEG) for the experiment (Table 4.2).

All animal use was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC) prior to the beginning of the study. Day old male broiler chicks (n=120; CobbxCobb; Cobb Vantress, Siloam Springs, AR), were housed in groups of 10 in Petersine starter batteries with raised wire floors. Chicks had *ad libitum* access to feed and water. For the first 7 days of life, chicks were fed a starter diet (Table 4.2). Feed was removed at 0030 on the morning of the 8th day. Beginning at 0900, chicks were individually weighed and sorted into one of 20 pens in groups of 6 to achieve an average pen weight of 912.4 ± 5.7g. Treatments were assigned to pen in a randomized complete block design with each battery (n=4) serving as a block. At 0300, experimental feeds were provided to the chicks and remained available *ad libitum* for the next 10 days. At 0900 on study day 10, feed was removed and weighed to record total feed consumption of each pen. Chicks were withheld from feed until 0300, at which time they were weighed by pen to record weight gain.

Feed weight and weight gain were recorded to calculate protein efficiency ratio (PER) and net protein ratio (NPR). The following equations were used to calculate these values:

(1) $PER = \frac{BWG}{CPI}$

(2) $NPR = \frac{(BWG)-(N-Free\ BWG)}{CPI}$

Wherein, BWG represents body weight gain per chick in g, CPI represents crude protein intake per chick in g, and N-Free BWG represents body weight gain per chick of chicks fed the nitrogen-free basal diet in g.

Statistical Analysis

Data was analyzed as a randomized complete block design using the GLM for mixed models procedure of SAS (SAS version 9.4; SAS Institute, Inc., Cary, NC). The experimental unit for each treatment was a pen, and each treatment had 4 replicates. The block (n=4) was the battery and was set as a random variable. Means were separated using Fisher's LSD with a significant F ($\alpha = 0.05$).

Results

Ingredient Analysis

Nutrient analysis of experimental ingredients is presented in Table 4.3. Diets were formulated to include ingredients at a 10% protein inclusion based on as-is values. Analyzed crude protein values for each ingredient were within expected ranges. Essential amino acid profile, non-essential amino acid profile, and summary of amino acids are reported in Tables 4.4-4.6.

Protein Efficiency Ratio

Feed intake and body weight gain was highest ($P<0.05$) for birds fed SDG and SBM, followed by NG-DDG (Table 4.7). Corn gluten meal and NEG had the lowest ($P<0.05$) feed intake and body weight gain and did not differ between treatments. This pattern is also reflected in the crude protein intake per bird. In terms of feed efficiency, which was expressed as g of body weight gain per g of feed intake, SDG had the highest ratio ($P<0.05$; 0.5163) followed by SBM and NG-DDG, which did not differ (average 0.3179). Corn gluten meal had the poorest G:F compared to all other treatments ($P<0.05$). Spray dried granulated egg had the highest PER value ($P<0.05$; 5.16) followed by SBM (PER=3.76). Both NG-DDG and CGM had lower PER values ($P<0.05$), with no differences between treatments (average PER=1.17). When examining the PER values as a percentage of the SDG, all other protein sources had reduced ($P<0.05$)

efficiency. Soybean meal was 71.20% as efficient as SDG, followed by NG-DDG and CGM, which did not differ from each other (34.45 and 10.71%, respectively).

Discussion

Analyzed nutrient values for proximate analysis and amino acid profile were consistent with previous findings, with NG-DDG having an altered nutrient profile compared to traditional DDGS as expected (Yamka et al., 2003; Yamka et al., 2004; Fastinger et al., 2006; Stein et al., 2006; Godoy et al., 2014). The values for NG-DDGS were different than traditional DDGS as the production process for the NG-DDG involves a clarifying and protein decanting step, which concentrates the remaining nutrients. Spray dried granulated egg was used as a reference protein for this experiment, therefore all amino acid contents of experimental ingredients were compared to the SDG. By examining the amino acid profiles, it is possible to determine changes to protein during processing. These changes can be both positive, by initiating denaturation and increasing digestibility, or negative, causing aggregation or Maillard reactions (Salazar-Villanea et al., 2016). Production methods such as heating, rendering, and drying can initiate reactions like Maillard reactions, which alter and degrade amino acids and, consequently, can affect their bioavailability (van Rooijen et al., 2013). These changes in bioavailability can decrease the nutritional value of ingredients (Sgarbieri et al., 1973). While all amino acids are susceptible to this reaction, certain amino acids, such as lysine, are especially reactive (van Rooijen et al., 2013). This is reflected in the lysine availability of the experimental ingredients. As seen in Table 4.6, all ingredients used have a lysine availability over 90%, indicating little, if any, heat damage. While plant proteins generally require less processing than animal sourced proteins and are therefore less susceptible to damage, it is not impossible. This was demonstrated in a study done by Klopfenstein (1996), who found that feed efficiency decreased as DDGS underwent further processing via drying.

As well as being the most susceptible to damage, lysine has been reported to be the first limiting amino acid for dogs (Longnecker and Hause, 1959). Meaning, the animal must achieve its requirements for lysine before being able to utilize any other amino acids present. Dogs and cats have lysine requirements of 3.5 and 3.4%, respectively (NRC, 2006). As seen in Table 4.44, only the control ingredient meets these requirements. Added with the decreased PER values reported in Table 4.7, it is apparent these ingredients could not stand as a sole source of protein in companion animal diets. However, it is unlikely any commercial pet food would formulate a diet utilizing one single ingredient as the protein source from the animal. Additionally, previous studies have examined the use of these ingredients as a primary source of protein in companion animal diets when supplemented with a secondary protein source successfully and no adverse effects to the animals (Allen et al., 1981; Bednar et al., 2000; Carciofi et al., 2009; Silva et al., 2016). Additionally, the cat has a metabolic requirement for taurine for which a deficiency can result in serious health consequences such as dilated cardiomyopathy and retinal degeneration (Spitze et al., 2003). Typically, this amino-sulfone can be found in high concentrations in animal viscera, such as the brain, heart, and eyes, making it unfeasible for a cat to fulfill this requirement through a plant-based diet (Novotny et al., 1991). However, it is not uncommon to see trace amounts of this nutrient in plant-based materials, as was seen in the work done by Spitze et al. (2003).

Body weight gain and feed intake was highest for chicks fed the SDG and SBM diets, followed by NG-DDG, and CGM and NEG diets which were not different. This was to be expected, as chicks have been recorded to self-adjust their feed intake based on amino acid deficiencies, which were present in all experimental diets (Piccard et al., 1993). Dogs have also been observed to reflect this behavior (Romsos and Ferguson, 1983), while cats have not (Cook et al., 1985). It was unsurprising that the treatment ranked the worst (aside from the NEG control) was CGM, as corn derived by-products have been reported to be an unbalanced and incomplete source of amino acids (Belyea et al., 2004). Corn has a very low lysine content, which is the first limiting acid in both poultry and companion animals. This shortcoming is reflected in this data, as the birds showed decreased growth throughout the experimental period. The CGM also had the lowest ratio of essential to non-essential amino acids, indicating that the majority of its protein content is not made up of the crucial amino acids needed by the animal. These trends are reflected in the feed efficiency as well.

The PER assay proved to be an effective tool for ranking protein sources. While all birds were fed an identical amount of protein (10%) the results illustrate the importance of the amino acid profile in the animal's ability to utilize a given protein. In terms of the test treatments, SBM was the most efficient source of protein compared to NG-DDG and CGM. While differing in specific PER values, a study by Godoy et al. (2014) also found that SBM ranked higher than DDGS and CGM in a chick growth assay. Additionally, similar feed efficiency and PER values were reported by Parsons et al. (1983) for SBM and DDGS when including the ingredients at a crude protein level of 9 and 12% respectively. This indicates that even in increased levels of protein inclusion, the NG-DDG would not perform at a higher level. This is due to the limiting effects of its amino acid profile. It is not surprising that SBM would perform more efficiently compared to both NG-DDG and CGM, as both are by-products of corn, which as previously mentioned is typically low in lysine. Additionally, the work done by Parsons et al. (1983) determined that tryptophan and arginine were the next two limiting amino acids in the makeup of

DDGS. This is supported by our work as both the NG-DDG and CGM ingredients had lower, and nearly identical, TRP and ARG contents when compared to SBM and the SDG control. Conversely, work done by Baker (2009) found that the most limiting amino acid in SBM is the sulfurous amino acids, such as methionine and cysteine. This explains the higher PER values for SBM. Because the lysine requirements were first met, the bird was able to utilize a portion of the protein before being hampered by the decreased methionine and cysteine concentrations, as opposed to the birds fed NG-DDG and CGM who were at a disadvantage from the start due to decreased levels of lysine. Further, it has been well established that spray-dried egg is a highly bioavailable protein for chicks that can be readily utilized and promotes growth at maximum efficiency (Johnson and Parsons, 1997). By expressing the PER values as a percentage of the SDG treatment, it is easy to see how the protein sources rank in comparison to an industry standard. Again, these values further illustrate the superiority of SBM in comparison to NG-DDG and CGM in this assay. Results from NPR, which factored in the resulting weight loss for maintenance as calculated by the NEG treatment, gave an identical ranking to the PER values.

Conclusion

The PER assay allowed for further insight into the amino acid concentration of CGM, NG-DDG, and SBM, and allowed for an empirical ranking of the ingredients. Further, while none of the ingredients used in this experiment could meet an animals amino acid requirements on its own, it is unlikely that a companion animal diet would be formulated using a single source of protein. When supplemented with a complimentary protein source, any of the proteins used in this experiment would be acceptable for inclusion in a dog or cat diet. This assay contributed valuable information for both novel and traditional ingredients utilized by the pet food industry and validated their use in diets with complimentary protein sources and formulation.

Tables

Table 4.1. Composition of starter diet

Ingredient	Percentage (as fed)
Corn	55.250
Soybean meal	37.150
Ground Limestone	1.450
Monocalcium phosphate	1.700
Salt	0.370
DL-Methionine	0.325
Lysine-HCl	0.132
L-Theronine	0.044
Sodium bicarbonate	0.220
Vitamin/Mineral Premix	0.250
Soybean oil	3.100

Table 4.2. Composition of nitrogen-free basal diet

Ingredient	Percentage (as fed)
Corn Starch*	59.567
Dextrose*	29.727
Mineral Pre-mix	5.365
Soybean Oil	5.365
Choline	0.220
Vitamin Pre-mix	0.203

*Protein sources added in experimental diets replaced a portion of 2:1 cornstarch to dextrose mix to achieve 10% crude protein

Table 4.3. Proximate analysis of experimental ingredients expressed on a dry matter basis

	Percentage				
Ingredient	Crude Protein	Moisture	Ash	Fiber	Fat
SDG*	53.30	5.20	4.58	0.83	39.2
CGM*	74.70	10.10	1.00	n.d.	1.76
NG-DDG*	54.40	6.71	4.57	4.43	4.18
SBM*	54.50	12.33	6.96	3.63	1.28

*Spray dried granulated egg (SDG), corn gluten meal (CGM), next-generation distillers dried grain (NG-DDG), soybean meal (SBM)

Table 4.4. Essential amino acid content of experimental ingredients expressed as a percentage of total amino acid content

	Percentage											
Ingredient	ARG**	CYS**	HIS**	ILE**	LEU**	LYS**	MET**	PHE**	THR**	TRP**	TYR**	VAL**
SDG*	6.08	2.40	2.44	5.59	8.64	7.74	3.27	5.91	4.65	1.56	3.98	6.93
CGM*	3.07	1.70	1.87	4.06	15.55	1.62	2.26	6.05	3.18	0.57	4.86	4.43
NG-DDG*	4.94	2.07	2.83	4.53	12.00	4.12	2.23	5.45	4.12	0.88	4.36	5.80
SBM*	7.26	1.47	2.67	5.03	7.96	6.55	1.41	5.29	3.95	1.39	3.66	5.20

*Spray dried granulated egg (SDG), corn gluten meal (CGM), next-generation distillers dried grain (NG-DDG), soybean meal (SBM)
**Arginine (ARG), cysteine (CYS), histidine (HIS), isoleucine (ILE), leucine (LEU), methionine (MET), phenylalanine (PHE), threonine (THR), tryptophan (TRP), tyrosine (TYR), valine (VAL)

Table 4.5. Non-essential amino acid content of experimental ingredients expressed as a percentage of total amino acid content

	Percentage										
Ingredient	ALA**	ASP**	GLU**	GLY**	HYLYS**	HYPRO**	LAN**	ORN**	PRO**	SER**	TAU**
SDG*	5.73	9.88	11.64	3.39	0.06	0.00	0.00	0.12	3.68	6.26	0.04
CGM*	8.24	5.74	20.40	2.67	0.06	0.00	0.00	0.01	8.81	4.76	0.08
NG-DDG*	7.11	7.27	15.32	3.99	0.10	0.00	0.47	0.08	7.90	4.30	0.14
SBM*	4.42	11.43	17.93	4.31	0.13	0.09	0.00	0.09	5.46	4.10	0.20

*Spray dried granulated egg (SDG), corn gluten meal (CGM), next-generation distillers dried grain (NG-DDG), soybean meal (SBM)
**Alanine (ALA), asparagine (ASP), glutamine (GLU), glycine (GLY), hydroxylysine (HYLYS), hydroxyproline (HYPRO), lanthionine (LAN), ornithine (ORN), proline (PRO), serine (SER), taurine (TAU)

Table 4.6. Summary of amino acid composition of experimental ingredients.

Ingredient	Available Lys	Total Lys	Lys Availability	Total AA	EAA[1]	NEAA[2]	EAA:NEAA
SDG*	3.67	3.81	96.33%	49.21	29.13	20.08	1.45
CGM*	1.10	1.16	95.83%	71.62	35.26	36.36	0.97
NG-DDG*	1.89	2.01	94.03%	48.83	26.03	22.80	1.14
SBM*	2.92	3.02	96.69%	46.12	23.91	22.21	1.08

[1]Essential amino acids (EAA; Arg, Cys, His, Ile, Leu, Lys, Met, Phe, Thr, Tyr, and Val)
[2]Nonessential amino acids (NEAA; Ala, Asp, Glu, Gly, HyLys, HyPro, Lan, Orn, Pro, Ser, Tau)
*Spray dried granulated egg (SDG), corn gluten meal (CGM), next-generation distillers dried grain (NG-DDG), soybean meal (SBM)

Table 4.7. Results from protein efficiency ratio.

Treatment	Feed Intake (g)	Weight Gain (g)	G:F**	CPI (g) **	PER**	PER % of SDG**	NPR**
SDG*	366.37[a]	189.29[a]	0.5163[a]	36.63[a]	5.16[a]	100.00[a]	5.95[a]
CGM*	141.58[c]	8.36[d]	0.0553[c]	14.16[c]	0.55[c]	10.71[c]	2.23[c]
NG-DDG*	231.25[b]	45.21[c]	0.2685[b]	23.13[b]	1.78[c]	34.45[c]	2.82[c]
SBM*	330.29[a]	121.29[b]	0.3673[b]	33.03[a]	3.67[b]	71.20[b]	4.39[b]
NEG*	127.46[c]	-23.58[d]	---	---	---	---	---
SEM	14.86	11.10	0.040	1.61	0.44	8.43	0.43
P-Value	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.0002	0.0007

[abc] means within a row with unlike letters differ (P<0.05)
*Spray dried granulated egg (SDG), corn gluten meal (CGM), next-generation distillers dried grain (NG-DDG), soybean meal (SBM), nitrogen-free basal diet (NEG)
**Feed efficiency (G:F), crude protein intake (CPI), protein efficiency ratio (PER), PER expressed as a percentage of spray dried granulated egg (PER % of SDG), net protein ratio (NPR)

REFERENCES

Allen, S. E., G. C. Fahey Jr., J. E. Corbin, J. L. Pugh, and R. A. Franklin. 1981. Evaluation of byproduct feedstuffs as dietary ingredients for dogs. J. Ani. Sci., 53:1538-1544.

Alonzo, A. PetfoodIndustry.com. 2017. How to use alternative, plant-based proteins in pet food. Available from: https://www.petfoodindustry.com/articles/6384-how-to-use-alternative-plant-based-proteins-in-pet-food

Anderson, K., E. F. Crowder and P. Woodruff. 1964. The isolation of Salmonellae from kangaroo meat sold as pet food. Med. J. Aus. 688-689.

APPA. 2016. Pet industry market size and ownership statistics. American Pet Products Association, Inc. Greenwich, CT.

Baker, D. H. 2009. Advances in protein-amino acid nutrition of poultry. Amino Acids. 37:29-41.

Batal, A. B. and N. M. Dale. 2006. True metabolizable energy and amino acid digestibility of distillers dried grains with solubles. J. Appl. Poul. Res. 15:89-93.

Batajoo, K. K. and R. D. Shaver. 1998. In situ dry matter, crude protein, and starch degradabilities of selected grains and by-product feeds. J. Anim. Feed. Sci. and Tech. 71:165-176.

Beauchamp, G. K., O. Maller and J. G. Rogers Jr. 1977. Flavor preferences in cats. J. of Comparative and Physiological Psychology, 91:1118-1127.

Bednar, G. E., S. M. Murray, A. R. Patil, E. A. Flickinger, N. R. Merchen, and G. C. Fahey Jr. 2000. Selected animal and plant protein sources affect nutrient digestibility and fecal characteristics of ileally cannulated dogs. Arch. Anim. Nutr., 53:127-140

Belyea, R. L., K. D. Rausch, and M. E. Tumbleson. 2004. Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. J. Bioresearch Tech., 94:293-298.

Bobeck, E. A., N. A. Nachtreib, A. B. Batal and M. E. Persia. 2014. Effect of xylanase supplementation of corn-soybean meal-dried distiller's grain diets on performance, metabolizable energy, and body composition when fed to first-cycle laying hens. J. Appl. Poult. Res. 23:174-180.

Boya, U. O., M. J. Dotson and E. M. Hyatt. 2015. A comparison of dog food choice criteria across dog owner segments: an exploratory study. Int. J. of Consumer Studies. 39: 74-82.

Brandyberry, S. D., R. C. Cochran, E. S. Vanzant and D. L. Harmon. 1991. Technical note: effectiveness of different methods of continuous marker administration for estimating fecal output. J. Anim. Sci. 69:4611-4616.

Burkhalter, T. M., N. R. Merchen, L. L. Bauer, S. M. Murray, A. R. Patil, J. L. Brent Jr., and G. C. Fahey Jr. 2001. The ratio of insoluble to soluble fiber components in soybean hulls affects ileal and total-tract nutrient digestibilities and fecal characteristics of dogs. J. Nutr., 131: 1978-1985.

Camire, E. M., Camire, A., and Krumhar, K. (2009). Chemical and nutritional changes in foods during extrusion. Critical Reviews in Food Science and Nutrition. 29:1, 35-37.

Carciofi, A. C., L. D. de-Oliveira, A. G. Valério, L. L. Borgoes, F. M. de Carvalho, M. A. Brunetto, and R. S. Vasconcellos. 2009. Comparison of micronized whole soybeans to common protein sources in dry dog and cat diets. J. Ani. Feed Sci., 151: 251-260.

Case, S. E., D. D. Hamann and S. J. Schwartz. 1992. Effect of starch gelatinization on physical properties of extruded wheat- and corn-based products. Cereal Chem. 69:401-404.

Chevanan, N., K. A. Rosentrater and K. Muthukumarappan. 2004. Twin-screw extrusion processing of feed blends containing distillers dried grains with solubles (DDGS). Cereal Chem. 84:428-436.

Chevanan, N., K. Muthukumarappan, K. A. Rosentrater and J. L. Julson. 2007. Effect of diet dimensions on extrusion processing parameters and properties of DDGS-based aquaculture feeds. Cereal Chem. 84:389-398.

Chevanan, N., K. A. Rosentrater and K. Muthukumarappan. 2008. Effect of DDGS, moisture content, and screw speed on physical properties of extrudates in single screw extrusion. Cereal Chem. 85:132-139.

Clapper, G. M., C. M. Grieshop, N. R. Merchen, J. C. Russett, J. L. Brent Jr., and G. C. Fahey Jr. 2001. Ileal and total tract digestibilities and fecal characteristics of dogs as affected by soybean protein inclusion in dry, extruded diets. J. Anim. Sci., 79: 1523-1532.

Cook, N. E., E. Kane, Q. R. Rogers and J. G. Morris. 1985. Self-selection of dietary casein and soy-protein by the cat. Physiol. Behav. 34:583-594.

Cromwell, G. L., K. L Herkelman and T. S. Stahly. 1993. Physical, chemical, and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. J. Anim. Sci. 71:679-686.

De Godoy, M. R. C., L. L. Bauer, C. M. Parsons, and G. C. Fahey Jr. 2009. Select corn coproducts from the ethanol industry and their potential as ingredients in pet foods. J. Ani. Sci. 87:189-199.

Diehl, J. 2004. All in good taste: creating natural savory flavorings from yeast. Nutritional biotechnology in the feed and food industries. 257-264.

Ding, Q., P. Ainsworth, A. Plunkett, G. Tucker and H. Marson. 2006. The effect of extrusion conditions on the functional and physical properties of wheat-based expanded snacks. J. Food Eng. 73:142-148.

Dogan, H. and J. L. Kokini. 2007. Psychophysical markers for crispness and influence of phase behavior and structure. J. Texture Studies. 38:324-354.

Fastinger, N. D., J. D. Latshaw and D. C. Mahan. 2005. Amino acid availability and true metabolizable energy content of corn distillers dried grains with solubles in adult cecectomized roosters. Poul. Sci. 85:1212-1216.

Fiacco, D. C., J. A. Lowe, J. Wiseman and G. A. White. 2018. Evaluation of vegetable protein in canine diets: assessment of performance and apparent ileal amino acid digestibility using a broiler model. J. Anim. Physiol. a. Anim. Nutr. 102:422-449.

Hayward J. W. and F. H. Hafner. 1941. The supplementary effect of cysteine and methionine upon the protein of raw and cooked soybeans as determined with chicks and rats. Proceedings. Poultry Sci. Annual Meeting. 139-150.

Hodge, J. E. 1953. Chemistry of browning reactions in model systems. Ag. And Food Chem. 1.15:928-943.

Houpt, K. A., Hintz, H. F., and Shepherd, P. 1978. The role of olfaction in canine food preferences. Chem. Sense. Flavour 3:281-290.

Hsieh, F., S. J. Mulvaney, H. E. Huff, S. Lue and J. Brent Jr. 1989. Effects of dietary fiber and screw speed on some extrusion processing and product variables. Lebensm. Wiss. Technol. 22:204.

Hsieh, F., H. E. Huff, S. Lue and L. Stringer. 1991. Twin-screw extrusion of sugar beet fiber and corn meal. Lebensm. Wiss. Technol. 24:495Hullar, I., S. Fekete and Z. Szocs. 1998.

Effect of extrusion of the quality of soybean-based catfood. J. Anim. Physiol. a. Anim. Nutr. 80:201-206.

Johnson, M. L. and C. M. Parsons. 1997. Effects of raw material source, ash content, and assay length on protein efficiency ratio and net protein ratio values for animal protein meals. Poul. Sci. 76:1722-1727.

Johnson, M.L., and C.M. Parsons, G.C. Fahey, Jr., N.R. Merchen, and C.G. Aldrich. 1998. Effects of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally cannulated dogs. J. Anim. Sci. 76:1112-1122.

Jin, Z., F. Hsieh and H. E. Huff. 1994. Extrusion cooking of corn meal with soy fiber, salt, and sugar. Cereal Chem. 71:227-234.

Kannadhason, S., K. A. Rosentrater, K. Muthukumarappan and M. L. Brown. 2010. Twin screw extrusion of DDGS-based aquaculture feeds. J. World Aquaculture Society 41:1-15.

Kim, B. G. and H. H. Stein. 2009. A spreadsheet program for making a balanced latin square design. Rev. Colomb. Cienc. Pecu. 22:591-596.

Kim, Y., N. S. Mosier, R. Hendrickson, T. Ezeji, H. Blaschek, B. Dien, M. Cotta, B. Dale and M. R. Ladish. 2008. Composition of corn dry-grind ethanol by-products: DDGS, wet cake, and thin stillage. Bioresource Tech. 99:5165-5176.

Kingsly, A. R. P., K. E. Ileleji, C. L. Clementson, A. Garcia, D. E. Maier, R. L. Stroshine, and S. Radcliff. 2010. The effect of process variables during drying on the physical and chemical characteristics of corn dried distillers grains with solubles (DDGS) – plant scale experiments. J. Bior. Tech. 101:193-199.

Klopfenstein, T. 1996. Distillers grains as an energy source and effect of drying on protein availability. J. Anim. Feed Sci. a. Tech. 60:201-207.

Koppel, K. M. Gibson, S. Alavi and G. Aldrich. 2014. The effects of cooking process and meat inclusion on pet food flavor and texture characteristics. Animals. 4:254-271.

Koppel, K., M. Monti, M. Gibson, S. Alavi, B. Di Donfrancesco and A. C. Carciofi. 2015. The effects of fiber inclusion on pet food sensory characteristics and palatability. Animals. 5:110-125.

Lei, X. J., S. I. Lee, S. T. Hong, Y. S. Jang and I. H. Kim. 2017. Ileal and total tract apparent crude protein, dry matter, gross energy and amino acids digestibility of soybean meal, solvent-extracted canola meal, and corn distillers dried grains with solubles for growing pigs. Indian J. Anim. Sci. 87:1251-1254.

Li, H., S. Smith, G. Aldrich and K. Koppel. 2017. Preference ranking procedure proposal for dogs: a preliminary study. J. Sens. Stud. E12307.

Lodge, S. L., R. A. Stock, T. J. Klopfenstein, D. H. Shain and D. W. Herold. 1997. Evaluation of corn and sorghum distillers byproducts. J. Anim. Sci. 75:37-43.

Longnecker, J. B. and N. L. Hause. 1959. Relationship between plasma amino acids and composition of the ingested protein. Arch of Biochem and Biophys. 84: 46-59.

Manabe, Y., Matsumura, S., and Fushiki, T. 2010. Preference for high-fat in animal foods. Fat Detection: Taste, Texture, and Post Ingestive Effects. Boca Raton, FL. Chapter 10.

McCay, C. M., T. C. Huang, W. Ramseyer, A. Stevens and K. M. Palmer. 1957. The use of corn distillers solubles in dog foods. Proc. Dist. Feed Conf. 12:28.

Monti, M., M. Gibson, B. A. Loureiro, F. C. Sa, T. C. Putarov, C. Villaverde, S. Alavi and A. C. Carciofi. 2016. Influence of dietary fiber on macrostructure and processing traits of extruded dogs foods. Anim. Feed Sci. and Tech. 220:93-102.

Moonsinghe, R., M. J. Khoury, A. Cecile and J. W. Janssens. 2007. Most published research findings are false: but a little replication goes a long way. PloS Med. 4:218-221.

Myers, W. D., P. A. Ludden, V. Nayigihugu, and Hess, B.W. 2004. Technical Note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. Journal of Animal Science, 82:179-183.

Nutritional Research Council. 200. Nutrient requirements of poultry. Washington, DC: The National Academies Press.

National Research Council. 2006. Nutrient requirements of dogs and cats. Washington, DC: The National Academies Press.

Nijsse, R., L. Mughini-Gras, J. A. Wagenaar and H. W. Ploeger. 2014. Coprophagy in dogs interferes in the diagnosis of parasitic infections by faecal examination. Vet. Parasitology 204:304-309.

Novotny, M. J., P. M. Hogan, D. M. Paley and H. R. Adams. 1991. Systolic and diastolic dysfunction of the left ventricle induced by dietary taurine deficiency in cats. Amer. Phys. Soc. H121-H127.

Okin, G. S. 2017. Environmental impacts of food consumption by dogs and cats. Plos One. 12(8): e0181301.

Overland, M., A. Krogdahl, G. Shurson, A. Skrede and V. Denstadli. 2013. Evaluation of distiller's dried grains with solubles and high protein distiller's dried grains in diets for rainbow trout. Aquaculture. 201-208.

Packaged Facts 2016. Pet Food in the U.S., 12th Edition. March 2016.

Parsons, C. M. and D. H. Baker. 1983. Distillers dried grains with solubles as a protein sources for the chick. Poultry Sci. 62:2445-2451.

PetfoodIndustry.com. 2013. Market Data. Available from: http://www.petfoodindustry.com/pet-food-market-data/US-dog-food-sales-trend-by-type-2010-13

PetfoodIndustry.com. 2015. Humanization driving trends in pet food. Available from: https://www.petfoodindustry.com/articles/5241-humanization-driving-trends-in-pet-food

Plattner, B. PetfoodIndustry.com. 2007. Extruding for palatability. Available from: http://www.petfoodindustry.com/articles/553-extruding-for-palatability

Piccard, M.I., G. Uzu, E.A. Dunnington and P.B. Siegel. 1993. Food intake adjustments of chicks: short term reactions to deficiencies in lysine, methionine and tryptophan. Brit. Poul. Sci, 34:737–746.

Renewable Fuels Association. 2017. Industry Statistics. Available from: http://www.ethanolrfa.org/resources/industry/statistics/#1461259793696-ff278874-f140

Rho, Y., C. Zhu, E. Kiarie and C. G. M. de Lange. 2017. Standardized ileal digestible amino acids and digestible energy contents in high protein-distiller's dried grains with soluble fed to growing pigs. J. Anim. Sci. 95:3591-3597.

Riaz, M. N. 2000. Extruders in food applications. Boca Raton, FL: Taylor and Francis Group, LLC.

Riaz, M. N. and G. J. Rokey. 2012. Extrusion problems solved: food, pet food, and feed. Philadelphia, PA: Woodhead Publishing.

Rokey, G. J., B. Plattner and E. M. de Souza. 2010. Feed extrusion process description. R. Bras. Zootec. 39:510-518.

Rokey, G. J. Petfood Technology. Edited by J. L. Kvamme and T. D. Phillips, Watt Pub., 2003.

Romsos, D. R. and D. Ferguson. 1983. Regulation of protein intake in adult dogs. J. Am. Vet. Med. Assoc. 182:41-43.

Rosentrater, K. A., T. L Richard, C. J. Bern and R. A. Flores. 2005. Small-scale extrusion of corn masa by-products. Cereal Chem. 82:436-446.

Sa, F. C., R. S. Vasconcellos, M. A. Brunetto, F. O. R. Filho, M. O. S. Gomes and A. C. Carciofi. 2013. Enzyme use in kibble diets formulated with wheat bran for dogs: effects on processing and digestibility. J. Anim. Physiol. a. Anim. Nutr. 97:51-59.

Salzar-Villanea, S., E. M. A. M. Bruininx, H. Gruppen, W. H. Hendriks, P. Carre, A. Quinsac and A. F. B. van der Poel. 2016. Physical and chemical changes of rapeseed meal proteins during toasting and their effects on in vitro digestibility. J. Anim. Sci. and Biotech. 7:62.

Schaafsma, G. 2005. The protein digestibility-corrected amino acid score (PDCAAS)—a concept for describing protein quality in foods and food ingredients: a critical review. J. AOAC Int. 88:988-994.

Sgarbieri, V. C., J. Amaya, M. Tanaka and C. O. Cichester. 1973. Nutritional consequences of the maillard reaction. Amino acid availability from fructiose-leucine and fructose-tryptophan in the rat. J. Nutr., 103:657-663.

Silva, J. R., T. T. Sabchuk, D. C. Lima, A. P. Félix, A. Maiorka, and S. G. Oliveira. 2016. Use of distillers dried grains with solubles (DDGS), with and without xylanase, in dog food. J. Ani. Feed Sci., 220:136-142.

Singh, V., R. A. Moreau, K. B. Hicks, R. L. Belyea and C. H. Staff. 2002. Removal of fiber from distillers dried grains with solubles to increase value. Transactions of the ASAE. 45:389-392.

Spitze, A.R., D.L. Wong, Q.R. Rogers and A.J. Fascetti. 2003. Taurine concentrations in animal feed ingredients; cooking influences taurine content. J. Anim. Physiol. a. Anim. Nutr. 87, 251-262.

Stein, H. H. and G. C. Shurson. 2009. The use and application of distillers dried grains with solubles in swine diets. J. Anim. Sci. 87:1292-1303.

Stein, H. H., M. L. Gibson, C. Pederson and M. G. Boersma. 2006. Amino acid and energy digestibility in ten samples of distillers dried grain with solubles fed to growing pigs. J. Anim. Sci. 84:853-860.

Swanson, K. S., and G. C. Fahey Jr., 2004, The role of yeasts in companion animal nutrition. Nutritional biotechnology in the feed and food industries. 475-484.

Swanson, K. S., R. A. Carter, T. P. Yount, J. Arentz and P. R. Buff. 2013. Nutritional sustainability of pet foods. Adv. Nutr. 4:141-150.

Swiatkiewicz, S., M. Swiatkiewicz, A. Arczewska-Wlosek and D. Jozefiak. 2016. Efficacy of feed enzymes in pig and poultry diets containing distillers dried grains with solubles: a review. J. Anim. Physiol. a. Anim. Nutr. 100:15-26.

Tran, Q. D., W. H. Hendriks and A. F. B. van der Poel. Extrusion processing: effects on dry canine foods. 2008. Thesis. Wageningen University and Research Centre.

Twomey, L. N., J. R. Pluske, J. B. Rowe, M. Choct, W. Brown and D. W. Pethick. 2003. The replacement value of sorghum and maize with or without supplemental enzymes for rice in extruded dog foods. J. Anim. Feed Sci. and Tech. 108:61-69.

Van Rooijen, C., G. Bosch, A. F. B. van der Poel, P. A. Wierenga, L. Alexander and W. H. Hendriks. 2013. The Maillard reaction and pet food processing: effects on nutritive value and pet health. Nutrition Research Reviews. 26:130-148.

Wall, T. Petfood Industry. 2018. Bison: sustainable and novel pet food protein. Available from: https://www.petfoodindustry.com/articles/6879-bison-sustainable-and-novel-pet-food-protein

Wang, X. and C. M. Parsons. 1998. Effect of raw material source, processing systems, and processing temperatures on amino acid digestibility of meat and bone meals. Poultry Sci. 77:834-841.

Wanner, R. L., C. M. McCay, K. M. Palmer and A. C. Stevens. 1958. Reproduction of beagles fed commercial feed containing corn distillers dried solubles. Proc. Dist. Feed Conf. 13:27.

White, T. D., and J. C. Boudreau. 1975. Taste preferences of the cat for neurophysiologically active compounds. Physiological Psychology, 3:405-410.

Whitney, M. H. and G. C. Shurson. 2004. Growth performance of nursery pigs fed diets containing increasing levels of corn distiller's dried grains with solubles originating from a modern Midwestern ethanol plant. J. Anim. Sci. 82:122-128.

Widyaratne, G. P. and R. T. Zijlstra. 2006. Nutritional value of wheat and corn distiller's dried grain with solubles: digestibility and digestible contents of energy, amino acids and phosphorus, nutrient excretion and growth performance of grower-finisher pigs. Can. J. Anim. Sci. 87:103-114.

Yamka, R. M., U. Jamikorn, A. D. True and D. L. Harmon. 2003. Evaluation of soyabean meal as a protein source in canine foods. J. Anim. Feed Sci. and Tech. 109:121-132.

Yamka, R. M., S. E. Kitts, A. D. True, and D. L. Harmon. 2004. Evaluation of maize gluten meal as a protein source in canine foods. J. Anim. Sci. Feed. A. Tech. 116:239-248.

Yang, Y., E. Kiarie, B. A. Slominski, A. Brûlé-Babel and C. M. Nyachoti. 2014. Amino acid and fiber digestibility, intestinal bacterial profile, and enzyme activity in growin pigs fed dried distillers grains with solubles-based diets. J. Anim. Sci. 88:3304-3312.

Yen, G.C, Lee, T.-C, and Chichester, C. O., Effect of maillard browning reaction on the chemical properties of various proteins, in Protein Quality and the Effects of Processing, Phillips, R. D. and Finley, J. W., Eds., Marcel Dekker, New York, 1988.

Zuo, Y., G. C. Fahey Jr., N. R. Merchen, and N. L. Bajjalieh. 1996. Digestion responses to low oligosaccharide soybean meal by ileally-cannulated dogs. J. Anim. Sci., 74: 2441-2449.

APPENDIX A – ADDITIONAL MEASURES OF ATTD

Table A-1. Apparent total tract digestibility of experimental diets as determined by total fecal collection

	Diet				
Item, %	CGM*	SBM*	NG-DDG*	SEM	p-Value
Dry Matter	84.25[a]	81.07[b]	77.27[c]	0.46	<.0001
Organic Matter	88.09[a]	84.53[b]	82.25[c]	0.57	<.0001
Crude Protein	86.90[a]	83.36[b]	82.92[b]	0.44	<.0001
Crude Fat	92.15[a]	91.72[a]	90.16[b]	0.25	<.0001
Crude Fiber	29.30[a]	23.20[a]	3.67[b]	6.80	<.0001
Ash	37.39[b]	42.50[a]	38.12[ab]	1.49	0.0531
Energy	79.06[a]	76.62[b]	71..06[c]	0.62	<.0001

*Corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)

Table A-2. Apparent total tract digestibility of experimental diets as determined by chromic oxide

	Diet				
Item, %	CGM*	SBM*	NG-DDG*	SEM	p-Value
Dry Matter	81.13	78.78	79.90	0.88	0.1323
Organic Matter	85.26[a]	82.28[b]	83.00[b]	0.82	0.0153
Crude Protein	83.76[a]	80.98[b]	83.67[a]	0.73	0.0103
Crude Fat	90.26	90.91	90.94	0.42	0.4467
Crude Fiber	10.02	12.52	7.53	7.54	0.7107
Ash	22.13[b]	34.27[a]	40.86[a]	2.90	0.0006
Energy	73.99	73.26	72.27	1.13	0.5557

*Corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)

Table A-3. Apparent total tract digestibility of experimental diets as determined by acid insoluble ash

	Diet				
Item, %	CGM*	SBM*	NG-DDG*	SEM	p-Value
Dry Matter	86.72[a]	80.70[b]	80.21[b]	0.78	<.0001
Organic Matter	89.61[a]	84.01[b]	83.40[b]	0.72	<.0001
Crude Protein	88.53	87.45	86.92	0.50	0.0895
Crude Fat	93.14[b]	94.03[a]	92.75[b]	0.28	0.0072
Crude Fiber	36.97	25.08	25.52	10.31	0.3212
Ash	45.49	50.91	53.64	2.83	0.1430
Energy	81.78[a]	82.26[a]	77.75[b]	0.90	0.0004

*Corn gluten meal (CGM), soybean meal (SBM), and next-generation distillers dried grains (NG-DDG)