

An assessment of starch composition and gelatinization in traditional and non-traditional dog food formulations

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KEY WORDS: dog food, starch, novel ingredient, starch gelatinization

Abstract

Gelatinization of starch content in pet foods can be impacted by several factors including moisture, retention time, and ingredients used. Starch gelatinization has been associated with digestibility but isn't well studied using ingredients common in non-traditional canine diets. The objective of this research was to examine the impacts of dietary ingredient profile (traditional vs non-traditional) and assess impacts to total starch content and starch gelatinization. Traditional diets (n = 10) utilizing meat-based ingredients including chicken, chicken by-product meal, meat and bone meal and plant-based ingredients including rice, barley, oats, and corn were examined in comparison with non-traditional diets (n = 10) utilizing meat-based ingredients including alligator, buffalo, venison, kangaroo, squid, quail, rabbit, rabbit and salmon along with plant-based ingredients including tapioca, peas, chickpeas, lentils, potato, and pumpkin. Representative samples were collected via grab sample technique (5 samples/diet) and were assessed for total starch content as well as percent starch gelatinization. Difference between ingredient type was assessed using a Students t-test in SAS 9.4. Significance was set at $P < 0.05$. Distribution of total starch content based on ingredient type (traditional vs non-traditional) revealed that mean total starch content was higher in traditional diets as compared to non-traditional diets ($P < 0.0001$). Conversely, starch gelatinization was found to be higher in non-traditional diets ($P < 0.0001$). Total starch content and total gelatinized starch had a strong negative correlation ($P < 0.01$) in traditional diets, though no correlation was observed in non-traditional diets. This negative correlation indicates a decrease in total gelatinized starch associated with increased total starch content. These novel data reveal important differences between starch content and gelatinization and could impact manufacturing processes for

ingredient types as well as feeding recommendations. Unpredicted variation between ingredient formulations could potentially lead to decreased digestibility and absorption and may result in nutrient deficiencies.

1. Introduction

Non-traditional diets with grain-free formulations for canines have become increasingly popular in recent years. These diets exclude traditional cereal grains but frequently contain carbohydrates such as legumes or potatoes. Legumes are high in lysine and low in methionine, allowing the use of these ingredients as complementary proteins (NRC, 2006; Mansilla et al., 2019). Yamka et al. (2003) reported adverse impacts to dry matter (DM) and crude protein (CP) digestibility in the small intestine for diets formulated with increasing levels of soybean meal despite little effect on total tract. This may be problematic as amino acids broken down in the large intestine are not available for utilization. Mansilla et al. (2019) reported that current diets grain-free formulations frequently contain a legume content greater than 40%. Fiber composition, amino acid content, and data on anti-nutritional factors is necessary in order to assess proper inclusion levels (Yamka et al., 2003)

Other recent work has reported changes in palatability, total dietary fiber and fecal output for grain-free diets when compared to ancient grain formulations (Pezzali and Aldrich, 2019). Authors attribute these impacts to changes that occur as a result of formulation differences during extrusion. These factors as well as retention time, ingredients used, particle size, physical structure, and carbohydrate interaction can all affect the degree of gelatinization in products (Bazolli et al., 2015; Lewis et al., 2015; Pezzali and Aldrich, 2019).

Starch gelatinization in pet food kibble occurs during the extrusion process. By altering the temperature and moisture, the structure of starch deteriorates, and starch granules begin to swell (Inal et al., 2017). Starch gelatinization is believed to improve the digestibility of some starches, generate viscosity, and allow adequate expansion of kibble (van Rooijen et al., 2013; Lankhorst et al., 2007). Because the crystalline structure of the amylose is disrupted during production, digestive enzymes have greater accessibility to the cellular structure which may improve digestion (Gibson and Sajid, 2013).

Unfortunately, digestibility studies are not plentiful in diets formulated with non-traditional ingredients. Although some studies have been done identifying digestibility potential for individual protein sources (Deng et al., 2016; Reilly et al., 2018) in poultry, more work is needed on non-traditional diets compared to traditional formulations.

Characterization studies have examined total starch, resistant starch, and starch cook in commercially available pet foods (Corsato Alvarenga and Aldrich, 2020). Similarity was reported for resistant starch but grain-free diets revealed higher starch cook. Studies investigating gelatinization could improve our understanding of digestibility for grain-free diets as compared to formulations utilizing more common grain sources.

The objective of this research was to measure starch content in both traditional and non-traditional formulations of dog foods. Researchers hypothesized that non-traditional diet formulations with high inclusion of legumes and potatoes will have differences measured in gelatinization and total starch content when compared to traditional diets utilizing cereal grains.

2. Materials & methods

2.1 Diets

Twenty (n = 20) commercially available, extruded dog foods were purchased from two pet food suppliers (www.chewy.com and www.amazon.com) during March, 2019. Traditional diets (n = 10) utilizing meat-based ingredients including chicken, chicken by-product meal, meat and bone meal and plant-based ingredients including rice, barley, oats, and corn were examined in comparison with non-traditional diets (n = 10) utilizing meat-based ingredients including alligator, buffalo, venison, kangaroo, squid, quail, rabbit and salmon along with plant-based ingredients including tapioca, peas, chickpeas, lentils, potato, and pumpkin. All diets selected were manufactured for all life stages or adult maintenance (Table 1).

2.2 Sample analysis

Each product was inspected upon receipt and five sub-samples (50 g each) were collected. Samples were shipped to a commercial analytical laboratory (Midwest Laboratories, Omaha, Nebraska; accreditation certificate number 2853.02). Total starch content (as a percentage of total diet) was measured using a method adapted from AOAC 996.11 and YSI Application Note 319. Starch gelatinization (as a percentage of total starch content) was measured using a method adapted from AOAC 996.11 (AOAC, 2016). Calculations for total gelatinized starch were conducted using the below equation:

$$\frac{\text{Starch gelatinization} \times 100}{\text{Total starch content}}$$

2.3 Statistical analysis

Data were analyzed using SAS Studio (SAS Institute Inc., Cary, NC). Student's t-test was used to analyze total starch content, starch gelatinization, and total gelatinized starch in non-traditional and traditional ingredient formulations for maintenance and all life stage diets. Linear

regression and correlation were used to determine the relationship between total starch content and total gelatinized starch in traditional and non-traditional diets. Values are expressed as mean (\pm SD). Significance was set at $P < 0.05$.

3. Results

Total starch content (%) was higher in traditional (35.2 ± 4.0) as compared non-traditional diets (22.3 ± 8.0) for diets formulated as adult maintenance ($P = 0.0032$) (Table 2). Similarly, total starch content in diets for all life stages was also higher using traditional ingredient (36.0 ± 7.8) formulations as compared to non-traditional ingredients (19.9 ± 3.4) ($P = 0.0128$).

When starch gelatinization was examined across both categories of ingredients, traditional diets were higher than non-traditional diets (30.2 ± 2.4 and $21.4 \pm$, respectively) formulated for maintenance ($P = 0.0165$). Once again, a similar pattern emerged for higher starch gelatinization in diets formulated for all life stages with traditional diets (30.9 ± 5.9) compared to non-traditional diets (19.4 ± 3.4) ($P = 0.0220$).

Calculations for total gelatinized starch indicate a similar relationship in both adult maintenance and all life stage formulations according to ingredient profile. Traditional diets at maintenance were similar to traditional diets at all life stages (86.1 ± 3.8 and 86.3 ± 4.0 , respectively). A similar result was observed in non-traditional diets when comparisons for life stage were examined with total gelatinization calculated at 95.4 ± 2.1 and 97.6 ± 2.3 for maintenance and all life stages, respectively.

When total starch content and total gelatinized starch were examined, a strong negative correlation ($r = -0.78$) was demonstrated ($P < 0.01$) in traditional diets as shown in Figure 1A. However, no correlation ($r = 0.28$) was demonstrated ($P = 0.43$) in non-traditional diets as shown

in Figure 1B. Despite having higher total starch content, traditional diets did not have higher total gelatinization when compared to non-traditional diet formulations.

4. Discussion

Extruded dog foods were estimated to make up approximately 95% of dry pet foods in 2008 (Tran et al., 2008). Extrusion is a quick process that utilizes high temperatures to produce extrudates. This process results in the denaturation of proteins, oxidation of lipids, gelatinization of starches, and the Maillard Reaction, all of which can change characteristics of the nutrients and digestibility of raw products (Tran et al., 2008; van Rooijen et al., 2013).

Prior work has demonstrated a direct relationship between percent gelatinization and digestibility for some ingredients. Wolter et al. (1998) found that gelatinization improved the digestibility of tapioca starch but had no effect on the digestibility of wheat starch. Carciofi et al. (2008) found that peas and lentils had lower digestibility as compared to grains; however, these results were assessed from individual ingredients rather than a complete diet. Other work comparing grain-free and ancient grain diets demonstrated full digestion of starch even with lower starch gelatinization for the ancient grain diet (Pezzali and Aldrich, 2019; Pacheco et al., 2018).

Pezzali and Aldrich (2019) analyzed carbohydrate sources and impacts associated with extrusion parameters, nutrient utilization, and starch gelatinization. Grain-free diets were found to have lower crude fiber, higher total dietary fiber (TDF), higher starch content, and a lower degree of cook. This conflicts with the findings in the present study and is likely due to the utilization of different carbohydrate sources.

Many factors may affect total gelatinization including amount of moisture, retention time, temperature, ingredient profile, and ingredient interactions (Pezzali and Aldrich, 2019;

Lewis et al., 2015). Likely, the different ingredients (including carbohydrate sources) and various ingredient interactions arising from their use, contributed to the results presented here.

Prior work has reported higher TDF digestibility in grain-free diets with authors theorizing differences related to soluble fibers in legumes (Bednar et al., 2001; de-Oliveira et al., 2012; Pezzali and Aldrich, 2019). While soluble fibers cannot be digested by the gastrointestinal tracts of dogs, they can be fermented. These end products from fermentation can result in an increased TDF digestibility. Higher levels of dietary soluble fiber may result in higher starch gelatinization. Pezzali and Aldrich (2019) reported higher levels of fiber digestibility for the grain-free diets as compared to the ancient grain diets compared but that may be attributed to differences in formulation. Those results are consistent with the data from the current study which found higher starch gelatinization in non-traditional diets. Sandri et al. (2020) reported changes in microbial taxa for dogs offered raw meat diets with varying starch sources. Other differences such as impacts to pH and N-NH₃ were theorized to have been related to variation in nutrient uptake. The authors suggest that the lower digestibility coupled with higher levels of resistant starch changed the starch substrate available to the large intestine which resulted in a shift of the microflora. Future studies should evaluate differences related to starch gelatinization, fiber content, and fiber digestibility.

5. Conclusions

Differences in retrogradation potential associated with varying ingredient profiles, is likely associated with the differences observed. Higher levels of amylopectin which are typical of pea and potato-based ingredients, likely have higher retrogradation potential which may impact starch digestibility (Cornejo-Ramírez et al., 2018). Future work should investigate retrogradation differences in commercially available diets formulated with varying ingredient profiles.

Prior work has established significant differences associated with manufacturing conditions (Alvarenga & Aldrich, 2020; Alvarenga et al., 2021) with impacts starch gelatinization and resistant starch. Future studies should include *in vitro* digestibility trials should be conducted to determine the relationship between starch content, starch gelatinization, and digestibility for pet food ingredients commonly utilized in formulations for grain-free diets. Ultimately, comprehensive digestibility trials utilizing AAFCO approved standards should be completed in order to identify impacts to canine health associated with ingredient variation and interactions (AAFCO, 2015).

Author contributions: Conceptualisation, EBP; methodology, EBP, AAV, and GM; data curation and formal analysis, EBP, AAV, JMF, and GM; writing, EBP, and AAV; review and editing; EBP, AAV, JMF, and GM. All authors have read and agreed to the published version of the manuscript.

Funding: Funding for this work was provided by Southern Illinois University.

Data availability statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of interest: The authors declare no conflicts of interest.

Table 1. Primary ingredients of diets for maintenance and all life stages.

<u>Diet</u>	<u>Treatment</u>	<u>Life Stage</u>	<u>Ingredient 1</u>	<u>Ingredient 2</u>	<u>Ingredient 3</u>	<u>Ingredient 4</u>	<u>Ingredient 5</u>
1	Non-traditional	All life stages	Chicken	Chicken meal	Sweet potatoes	Pea protein	Pea flour
2	Non-traditional	All life stages	Deboned alligator	Menhaden fish meal	Tapioca starch	Peas	Pea protein
3	Non-traditional	All life stages	Kangaroo	Kangaroo meal	Peas	Chickpeas	Pea flour
4	Non-traditional	All life stages	Rabbit	Salmon meal	Menhaden fish meal	Chickpeas	Canola oil
5	Non-traditional	Maintenance	Buffalo	Lamb meal	Chicken meal	Sweet potatoes	Peas
6	Non-traditional	Maintenance	Chicken	Peas	Pea starch	Chicken by-product meal	Lentils
7	Non-traditional	Maintenance	Deboned venison	Turkey meal	Pork meal	Chickpeas	Lentils
8	Non-traditional	Maintenance	Quail	Chickpeas	Peas	Potatoes	Turkey meal
9	Non-traditional	Maintenance	Squid	Chickpeas	Pumpkin	Sunflower oil	Flaxseed
10	Non-traditional	Maintenance	Venison meal	Dried potatoes	Lentils	Chickpeas	Canola oil
11	Traditional	All life stages	Chicken	Chicken meal	Whole grain brown rice	Cracked pearled barley	Pea flour
12	Traditional	All life stages	Chicken meal	Brown rice	Rice	Chicken fat	Olive oil
13	Traditional	All life stages	Chicken meal	Grain sorghum	Peas	Whole grain millet	Whole grain brown rice
14	Traditional	Maintenance	Chicken	Brewers rice	Corn gluten meal	Whole grain corn	Poultry by-product
15	Traditional	Maintenance	Chicken	Brown rice	Brewers rice	Cracked pearled barley	Chicken meal
16	Traditional	Maintenance	Chicken	Chicken by-product meal	Corn meal	Ground whole grain sorghum	Brewers rice
17	Traditional	Maintenance	Chicken	Organic barley	Organic oats	Organic peas	Chicken meal
18	Traditional	Maintenance	Chicken meal	Ground barley	Ground oats	Ground brown rice	Chicken fat
19	Traditional	Maintenance	Ground whole grain corn	Meat & bone meal	Corn gluten meal	Animal fat	Soybean meal
20	Traditional	Maintenance	Whole ground brown rice	Dehydrated chicken	Coconut	Sun-cured alfalfa	Whole ground flaxseed

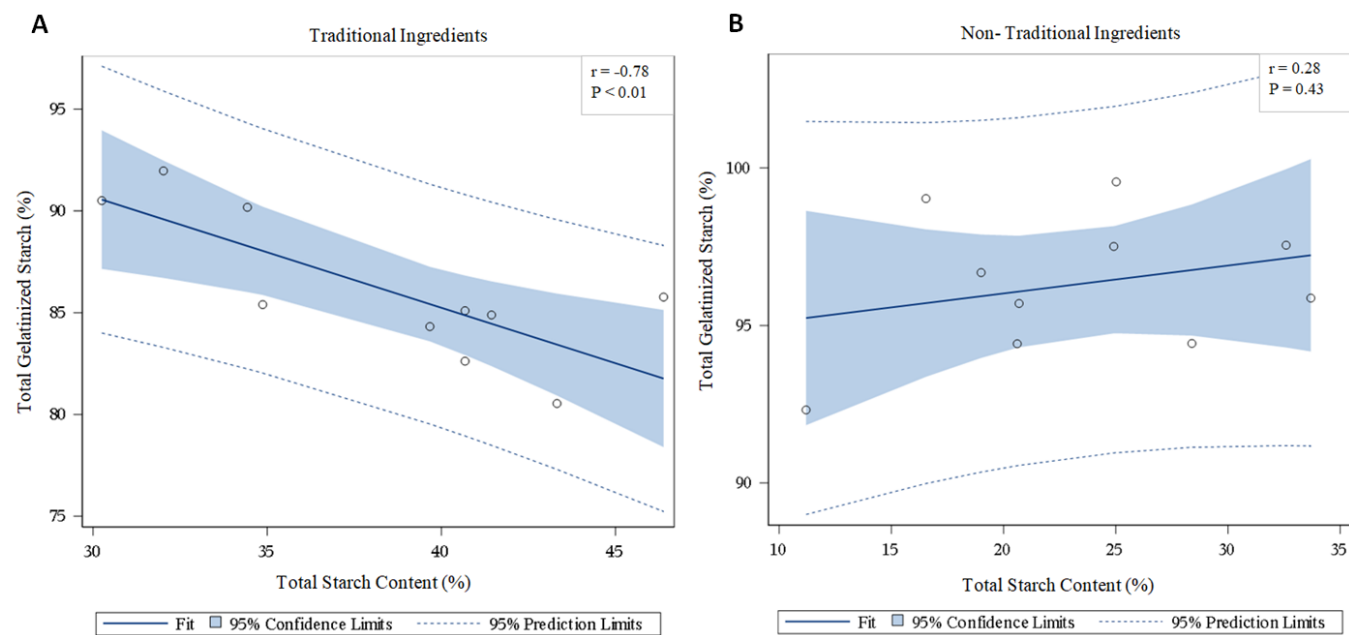
Table 2. Starch composition in non-traditional and traditional ingredient diets formulated for maintenance and all life stages.

	Non-traditional	Traditional	P-value
Maintenance			
Total starch (% of total diet)	22.3 (±8.0)	35.2 (±4.0)	0.0032*
Starch gelatinization (% total starch)	21.4 (±7.8)	30.2 (±2.4)	0.0165*
Total gelatinized starch	95.4 (±2.1)	86.1 (±3.8)	0.0002*
All life stages			
Total starch (% total diet)	19.9 (±3.4)	36.0 (±7.8)	0.0128*
Starch gelatinization (% total starch)	19.4 (±3.4)	30.9 (±5.9)	0.0220*
Total gelatinized starch	97.6 (±2.3)	86.3 (±4.0)	0.0049*

Mean (±SD)

* = mean values differ P < 0.05

Figure 1. Correlation between starch content and total gelatinization in diets formulated with A) traditional and B) non-traditional ingredients.



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