The Acute Effects of Starch Sources on Glycemic Index, Glycemic Response, Insulinemic Response and Satiety-Related Hormones in Dogs

by

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THE ACUTE EFFECTS OF STARCH SOURCES ON GLYCEMIC INDEX, GLYCEMIC RESPONSE, INSULINEMIC RESPONSE AND SATIETY-RELATED HORMONES IN DOGS

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This thesis investigated the glycemic index (GI) of single starch sources in dogs, including cooked white rice, cooked green lentils and white bread, as compared to a glucose control. In addition, commercial extruded dog foods featuring different categories of starch sources (traditional, whole grain, grain-free and vegan) were investigated for their glycemic response, insulineic response, GI and satiety-related hormones (ghrelin, leptin, GLP-1, GIP, PP, and PYY). There were no differences in GI between the single starch sources tested. There were no differences in glycemic response, insulineic response, GI or the satiety-related hormones measured between the commercial diets tested. Further research is necessary to investigate and modify canine GI methodology so that it may become reliable for future use, in addition to investigating the physiological benefits of low GI foods for dogs. Further research is also needed to investigate the correlation of satiety-related hormones and other measures of satiety in dogs.
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LIST OF ABBREVIATIONS

GI: Glycemic index
VFA: Volatile fatty acid
SCFA: Short chain fatty acid
RDS: Rapidly digestible starch
SDS: Slowly digestible starch
RS: Resistant starch
SGLT1: Sodium-dependent glucose transporter-1
ATP: Adenosine triphosphate
GLUT2: Glucose transporter-2
DM: Diabetes mellitus
AAFCO: Association of American Feed Control Officials
FEDIAF: European Pet Food Industry Federation
AUC: Area under the curve
SEM: Standard error of the mean
BCS: Body condition score
BMI: Body mass index
ROS: Reactive oxygen species
GLP-1: Glucagon-like peptide-1
GIP: Glucose-dependent insulinotropic polypeptide
PP: Pancreatic polypeptide
PYY: Peptide tyrosine-tyrosine
g: Gram(s)
BW: Body weight
CBC: Complete blood count
kg: Kilogram(s)
AUP: Animal utilization protocol
Wt/vol: Weight/volume
AOAC: Association of Official Analytical Chemists
IV: Intravenous
mL: Milliliter(s)
h: Hour(s)
ANOVA: Analysis of variance
nd: Non-determinable
Av CHO: Available carbohydrates
DMB: Dry matter basis
NSERC: Natural Sciences and Engineering Research Council
CRD: Collaborative Research and Development
AOCS: American Oil Chemist Society
CV: Coefficient of variation
ME: Metabolizable energy
GE: Gross energy
CHAPTER 1: LITERATURE REVIEW

1.0 Introduction

The large majority of pet owners consider their dogs as members of their family (Cohen 2002), and are therefore highly invested in their dog’s health. Considering that nutrition has an important association with the prevention of chronic disease (Gropper et al. 2009), this concern for health has become reflected in the pet food industry. Currently, there are a very large number of pet foods that boast various claims, often mimicking trends seen in human foods (Agriculture and Agri-Food Canada 2013). At present, consumers are debating the health effects of carbohydrates for themselves, as well as for their dogs. Commercial extruded dog food often contains a large proportion of carbohydrates, in order to provide an economical source of dietary energy (Spears and Fahey 2004), and to support the structural integrity of the kibble during processing (Crane et al. 2014). As has been shown in humans (Jenkins et al. 1981; Wolever and Bolognesi 1996), the type and amount of carbohydrate within the diet also affects postprandial glycemic responses in dogs (Carciofi et al. 2008).

The glycemic index (GI) was created as a way to compare glycemic responses between carbohydrate sources in humans (Jenkins et al. 1981). The chronic consumption of low GI foods in humans has been linked to decreased risk of various diseases, including diabetes and obesity (Ludwig 2002; Barclay et al. 2008). Both of these nutritional disorders are affecting an increasing proportion of companion animals globally (Guptill et al. 2003; Fracassi et al. 2004; German 2006). GI has recently become of interest in the pet food industry. Dog foods containing carbohydrate sources known to be low GI in humans, such as lentils, are often marketed as being beneficial to dogs as well. However, there is limited research investigating GI in dogs.
specifically, as well as the effects of different carbohydrate sources in commercial dog foods on glycemic response.

This literature review will examine carbohydrate metabolism in dogs, the use of carbohydrates in commercial dog foods, the GI and its associations with health in humans, our current knowledge of the GI in dogs and its potential for future use in canine nutrition, and lastly, our knowledge of obesity and satiety in dogs.

1.1 Classification of Carbohydrates

Carbohydrates are one of the macronutrients responsible for providing dietary energy to animals (Nelson and Cox 2008; Gropper et al. 2009). They are biological molecules made up of carbon, hydrogen and oxygen, and can be represented by the formula \((\text{CH}_2\text{O})_n\). Carbohydrates are categorized into one of two classes, simple or complex, based on chemical structure and degree of polymerization (Figure 1.1). Simple carbohydrates are further divided into monosaccharides and disaccharides, containing one and two sugar units, respectively. Complex carbohydrates consist of oligosaccharides and polysaccharides, made up of 3-10 or more than 10 sugar units respectively (Nelson and Cox 2008).

Monosaccharides (glucose, fructose and galactose), the simplest form of carbohydrates, cannot be broken down further by enzymatic digestion to produce smaller compounds. The monosaccharide of highest nutritional importance is glucose, as it is the major fuel for cells in the body. When two monosaccharide units are joined together via glycosidic bonds, they are referred to as disaccharides (maltose, lactose, sucrose, trehalose).
Figure 1.1. Classification of carbohydrates into simple and complex, based on chemical structure and degree of polymerization. Simple carbohydrates are comprised of monosaccharides and disaccharides, containing one and two sugar units respectively. Complex carbohydrates include oligosaccharides and polysaccharides, containing 3-10 or more than 10 sugar units respectively.
Unlike monosaccharides, which are readily absorbed by the small intestine, disaccharides need to be broken down by intestinal enzymes (disaccharidases) prior to being absorbed (Nelson and Cox 2008). The properties of polysaccharides vary, as it is dependent on the monosaccharides they are composed of, the bonds between them, and branching within the molecule itself. Polysaccharides can be further categorized according to digestibility. Polysaccharides that can undergo enzymatic digestion are referred to as starches; whereas complex carbohydrates that resist enzymatic digestion, and are fermented via intestinal microbes, are known as dietary fibers (Nelson and Cox 2008).

1.1.1 Dietary Fiber

Dietary fibers are complex carbohydrates that resist enzymatic digestion in the mammalian small intestine, due to the β-glycosidic bonds between the sugar units, and are instead fermented by microbes in the colon (Nelson and Cox 2008). The fermentation of fiber in the colon produces volatile fatty acids (VFAs), also known as short chain fatty acids (SCFAs), which include, but are not limited to: propionate, acetate and butyrate. These VFAs serve many purposes, including stimulating the growth of beneficial bacteria within the colon, and stimulating the proliferation of cells within the gastrointestinal tract (Gropper et al. 2009). Dietary fibers are derived from plant cell wall polysaccharides and are further classified into soluble or insoluble, based on their rate of fermentation and solubility. Cellulose and hemicellulose, the more slowly fermentable fibers, are considered insoluble fibers. In contrast, pectins and gums are more rapidly fermentable, and are therefore considered soluble fibers. Additionally, soluble fibers are more viscous, and produce more VFAs within the colon than insoluble fibers (Gropper at al. 2009).
1.1.2 Starch

Starch is the main storage polysaccharide, and most abundant digestible carbohydrate found in plants (Nelson and Cox 2008). Dry pet diets often contain a large volume of starch, as it provides an economical way of achieving ample dietary energy (Bednar et al. 2001). The starch in commercial dog foods commonly originates from cereal grains, and is found to be highly digestible by dogs (Spears and Fahey 2004). Starch is bound together by \(\alpha\)-glycosidic bonds and can therefore undergo enzymatic digestion by \(\alpha\)-amylase in the small intestine of dogs (Nelson and Cox 2008; Gropper et al. 2009; Hoenig 2014). Additionally, starch is made up of two glucose polymers: amylose and amylopectin (Nelson and Cox 2008). The ratio of amylose to amylopectin affects several properties of starch, namely digestibility. Starches containing a higher content of amylose are considered less digestible, whereas starches high in amylopectin are digested and absorbed much more quickly. As a result, the digestibility of different starch ingredients varies (Biliaderis 1991). This difference in digestibility stems from the variation in structure between the two glucose polymers. Amylose is a linear glucose polymer, whereas amylopectin is branched-chain (Nelson and Cox 2008). The variation in amylose to amylopectin ratio within starches also affects other properties such as gelatinization and retrogradation. As a result, the quality and shelf-life of starch products will vary (Biliaderis 1991). Starch is made up of numerous subtypes, classified by their rate of digestion into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst et al. 1992).

Unlike RDS and SDS, RS cannot be digested in the small intestine, and therefore goes on to be fermented in the large intestine (Englyst et al. 1992). As a result, RS becomes a source of VFAs as opposed to a source of glucose (Bednar et al. 2001). Although RS is present to some
degree in all ingredients containing starch, the amount varies based on many factors, including the quantity of starch, how the food was processed and stored, and the size of the starch granules (Annison and Topping 1994). Namely, RS will be increased in starches that have not been cooked, those that have cooled after cooking, and those with larger particle sizes (Annison and Topping, 1994). Foods that regularly contain a large RS content include pulses, high amylose corn, bananas and raw potatoes (Murphy et al. 2008). Resistant starch has been associated with a significant number of health benefits in humans, such as increased fecal bulk, lowered postprandial insulinemic and glycemic responses, lowered cholesterol levels, and increased satiety (Willis et al. 2009; Behall et al. 1989; Klosterbuer et al. 2012). Similarly, in pigs, RS has been shown to lower postprandial glucose and insulin, and increase satiety (Souza da Silva et al. 2014). Studies in dogs have been very limited. A study by Murray et al. (1998) found that the incorporation of RS in enteral formulas fed to ileal-cannulated dogs resulted in an increased fecal bulk in dogs.

1.2. Carbohydrate Digestion, Absorption and Metabolism in Dogs

1.2.1 Carbohydrate Digestion

Carbohydrates are broken down into smaller units, and ultimately into glucose, through various enzymatic processes in the digestive tract (Nelson and Cox 2008). Digestion in the dog starts in the mouth, with the mechanical breakdown of complex carbohydrates. Unlike in humans, enzymatic digestion in dogs starts in the small intestine as opposed to the mouth. This is due to the very limited production of salivary α-amylase in the dog (Contreras-Aguilar et al. 2017). The various physiological traits of carbohydrate digestion in the canine gastrointestinal tract are summarized in Figure 1.2. The pancreas secretes pancreatic α-amylase, which enters the
lumen of the small intestine, and allows for the efficient break down of polysaccharides into disaccharides and oligosaccharides (Bednar et al. 2001). The activity of canine pancreatic α-amylase will adapt to the quantity of starch consumed, increasing with increased dietary starch concentrations (Kienzle and Kienzie 1993; Kienzle 1988). Digestive enzymes known as brush border hydrolases, located on the outer membranes of the epithelial cells in the small intestine, catabolize disaccharides and oligosaccharides into their monosaccharide units, primarily glucose (Nelson and Cox 2008).

1.2.2 Carbohydrate Absorption

Once digestion occurs, monosaccharides are actively transported via intestinal sodium-dependent glucose transporter-1 (SGLT1) across the mucosal brush border of the small intestine. This is considered the rate-limiting step in glucose absorption (Gropper et al. 2009). As dogs express the T1R2/T1R3 heterodimer within their sweet receptor, necessary for the regulation of SGLT1, it is suggested that dogs are able to upregulate SGLT1 in response to the level of dietary carbohydrates consumed (Batchelor et al. 2011). Dogs have been reported to have a glucose absorption rate and level similar to that of humans (Pagliassotti et al. 1996). Once taken up into the bloodstream, monosaccharides are circulated to various tissues and organs in the body. Galactose and fructose are converted into glucose molecules within the liver (Nelson and Cox 2008; Gropper et al. 2009).
Figure 1.2. Physiological traits of carbohydrate digestion in the canine gastrointestinal tract. Dogs are able to efficiently digest, absorb and metabolize carbohydrates. Due to the minimal production of salivary α-amylase, enzymatic digestion will start in the small intestine. VFA = volatile fatty acids. (Adapted from Bosch et al. 2015; Verbrugghe et al. 2012)
1.2.3 Carbohydrate Metabolism

The metabolic fate of glucose will depend on the body’s energy needs at that time. Once absorbed, glucose can be used for adenosine triphosphate (ATP) production through glycolysis, can be stored in the liver and skeletal muscles as glycogen, or converted to fat and stored as adipose tissue (Nelson and Cox 2008; Gropper et al. 2009). Glucose is absorbed via glucose transporter-2 (GLUT2) within the beta cells of the pancreas. The first rate-limiting step of glycolysis involves the production of glucose-6-phosphate through the phosphorylation of glucose by glucokinase (Nelson and Cox 2008; Gropper et al. 2009). In dogs, glucose oxidation is upregulated postprandially, through the increased activity of canine glucokinase (Hornichter et al. 1967). Glycolysis continues in the mitochondria, where two molecules of ATP are produced for every glucose-6-phosphate. Excess dietary glucose can be stored in the liver and skeletal muscles as glycogen, which are vital for maintaining the homeostasis of blood glucose (Nelson and Cox 2008).

1.2.4 Hormonal Control of Glucose Homeostasis

The metabolic fate of glucose is determined by the ratio of insulin to glucagon. Insulin, an anabolic hormone, has an important role in the regulation and maintenance of blood glucose homeostasis. Insulin is released by the pancreas in a fed state and is stimulated by an increase in postprandial blood glucose. Insulin functions to decrease circulating blood glucose levels by promoting the uptake of glucose into adipose tissue and muscle, and the synthesis of glycogen within the liver and muscle (Nelson and Cox 2008).
In comparison, the breakdown of glycogen is important for restoring and maintaining blood glucose concentrations when they fall, such as in a fasted state. The breakdown of glycogen, known as glycogenolysis, allows the body to quickly replenish glucose concentrations. Glucagon is the catabolic hormone responsible for increasing glycogen breakdown within the liver. Glucagon is released in response to low blood glucose concentrations. The release of glucagon will inhibit glycolysis (Nelson and Cox 2008).

1.2.5 Diabetes Mellitus

Diabetes mellitus (DM) is a common endocrine disorder in which a state of prolonged hyperglycemia occurs. Diabetes can occur either because of a lack of insulin production (Type 1 DM), or due to the inability of target tissues to respond to the insulin that is produced (Type 2 DM), also referred to as insulin resistance (Nelson and Cox 2008; Gropper et al. 2009). Diabetes mellitus is a common disease in dogs, cats and humans (Fall et al. 2007; Centers for Disease Control and Prevention 2017; Lederer et al. 2009; McCann et al. 2007). The prevalence of DM in dogs has increased over the last 30 years (Guptill et al. 2003), and it is estimated that approximately 1.2% of dogs will develop DM before they turn 12 years of age (Fall et al. 2007). The development of DM in dogs is believed to be multifactorial, and can be influenced by a variety of environmental and genetic factors, such as breed and sex (Fall et al. 2007; Hess et al. 2000; Marmor et al. 1982). Although obesity may lead to insulin resistance in dogs (Verkest et al. 2011), and has previously been proposed as a potential risk factor in the development of DM (Klinkenberg et al. 2006; Pöppl et al. 2017); it is believed that obesity rarely leads to overt clinical signs of DM in dogs as Type 2 DM is not commonly observed in dogs (Rand et al. 2004; Verkest et al. 2011).
The autoimmune destruction of beta cells, as seen in Type 1 DM in humans, is a common feature of DM in dogs. Unlike humans and cats, that mostly develop Type 2 DM as a result of chronic hyperglycemia, this does not appear to be of concern with dogs. As a result, there have been no studies observing the relationship of carbohydrate intake to the development of canine DM. However, obese dogs have been shown to develop insulin resistance (Gayet et al. 2004; German et al. 2009). Carbohydrates must be considered in the treatment of DM, as they will influence blood glucose and insulin levels. Several dietary modifications are of consideration when treating a dog with DM, including feeding starches as opposed to simple sugars, and the addition of dietary fiber in the diet (Klinkenberg et al. 2006). Starch, unlike simple sugars, needs to be broken down and digested, before it can be absorbed. As a result, the postprandial glucose and insulin responses after the consumption of these complex carbohydrates will be reduced in both rate and quantity (Nelson and Cox 2008; Jenkins et al. 1981).

The role of dietary fiber in the management of canine DM has been extensively researched. In humans, increased proportions of dietary fiber have been shown to be able to decrease postprandial blood glucose and insulin concentrations (Riccardi et al. 1984; Thorsdottir et al. 1998). The viscosity of fiber allows it to impair and slow the absorption of dietary glucose into the blood, resulting in reduced glycemic responses (Brenelli et al. 1997; O’Connor et al. 1981). In dogs, research has shown similar results (Karr-Lilienthal et al. 2002). Dogs, both diabetic and healthy, have shown reduced fluctuations and concentrations of blood glucose and insulin, when fed a higher percentage of dietary fiber (Graham et al. 2002; Holste et al. 1989; Blaxter et al. 2018; Nelson et al. 1991).
1.3. Carbohydrates in Commercial Dog Food

Dogs do not have a dietary requirement for carbohydrates; however, they do have a metabolic requirement for glucose. Certain tissues and cells, such as the brain and red blood cells, rely on glucose for their energy needs (Gropper et al. 2009). Carbohydrates are primarily added to pet foods as an economic way of supplying energy, as 1 gram will supply on average 3.5 kcal of energy (Gropper et al. 2009). Surveys estimate that the vast majority of dog owners feed commercial extruded dry food, due to its value, variety and convenience (Laflamme et al. 2008; Connolly et al. 2014; Michel et al. 2008). Extruded kibble for adult dogs may contain up to 76.5% dry matter as carbohydrates, in order to allow for the minimum protein and fat percentages established by The Association of American Feed Control Officials (AAFCO) (2017) and The European Pet Food Industry Federation (FEDIAF) (2017). Apart from being a valuable and affordable source of energy (Spears and Fahey 2004), carbohydrate ingredients are also necessary to create the desired structure and shape of extruded kibble (Crane et al. 2014). Wet foods, such as those in cans and pouches, have also remained popular in the pet food market. Similar to kibble, wet food must contain carbohydrates in order to achieve their desired texture and softness. The structure of wet foods can be attributed to the gelling agents added to these formulas. These gelling agents are required for their abilities to form a gel upon processing, adding to the texture and gelatinization of the finished product (Karr-Lilienthal et al. 2002). Additionally, the starch-protein interactions that occur during processing not only assist in overall consistency and texture of wet foods, but may also contribute to palatability (van Boekel et al. 2010).
1.3.1 Effects of Processing on Starch

When exposed to the high temperatures of extrusion or canning, starch granules will swell and rupture, and the content of amylose and amylopectin will be hydrolyzed (Cheftel 1986). This disruption in chemical structures causes the starch granules to undergo gelatinisation (Tran et al. 2008). Gelatinisation increases starch hydrolysis by amylase, allowing starches that would otherwise be resistant to undergo enzymatic digestion in the small intestine (Lankhorst et al. 2007). As a result, the digestibility of starch increases after extrusion and canning, and the content of resistant starch will decrease (Spears and Fahey 2004).

However, gelatinised starches can start to recrystallize and re-associate once they have cooled; a process known as retrogradation (Kendall et al. 2004). Retrogradation of amylose is thought to happen almost immediately, while amylopectin needs a much longer storage time (Lii et al. 2004). As a result, the cooling of cooked products can result in a higher content of resistant starch. To date, there appears to be very limited knowledge on the specific effects extrusion and canning have on the quality and nutrient modification of pet foods.

1.3.2 Glycemic and Insulinemic Response in Dogs

In humans, the glycemic and insulinemic responses to foods will vary based on the type of carbohydrates present and their digestibility (Jenkins et al. 1981). Starch sources that are digested and absorbed more rapidly will result in bigger spikes in postprandial blood glucose and insulin curves, leading to higher glycemic and insulinemic responses. Similarly, research in dogs has demonstrated that the starch sources present within extruded kibble will affect both postprandial glucose and insulin responses. Notably, Carciofi et al. (2008) measured
postprandial glucose and insulin responses after feeding dogs an extruded diet that incorporated one of the following six starch sources as its exclusive source of starch: corn, brewer’s rice, sorghum, peas, lentils or cassava flour. The diets were fed once per day in volumes that met maintenance requirements in the dogs. Diets containing sorghum, lentils and peas produced a delayed and lengthened glycemic and insulinemic response, when compared to the diets containing brewer’s rice, corn, and cassava flour. Similarly, Adolphe et al. (2015) compared acute feeding of extruded diets containing rice or peas on postprandial glucose and insulin concentrations in dogs, and found no significant differences in glycemic or insulinemic responses between the diets. When both diets were fed long-term for 12 weeks, insulin sensitivity improved in the dogs fed the pea diet, following an oral glucose tolerance test. It is important to note that these studies may not be representative of commercial pet foods which typically contain numerous carbohydrate sources. Nguyen et al. (1998) investigated postprandial glucose and insulin responses of a variety of commercial foods, with varying macronutrient compositions in healthy dogs. Although the authors found that the starch content of the diet impacted postprandial glucose, the starch sources within the diets were unfortunately not reported. In contrast, protein and fat seemed to have the most impact on postprandial insulin.

1.4 Glycemic Index

In order to effectively rank the glycemic responses to various carbohydrate-containing foods, Jenkins et al. (1981) introduced the concept of the GI. The GI is measured by comparing the postprandial glucose response to equal amounts of available carbohydrate in different food sources compared to a reference food, either glucose or white bread (Wolever et al. 1991) (Figure 1.3).
Figure 1.3. Examples of postprandial glycemic responses from two starch sources, as compared to a glucose solution. Starch sources classified as high GI, such as potato, will produce a higher overall postprandial blood glucose curve, along with a bigger peak in postprandial glycemic response. Low GI foods, such as pulses, will produce a smaller overall postprandial blood glucose curve, and a more delayed and lengthened glycemic response (Adapted from Ramdath 2016).
This cross-over approach makes it possible to compare glycemic responses between different individuals, as each participant functions as their own control. Although the storage and processing conditions that a food has been exposed to can influence GI, the rate of starch digestibility plays a prominent role (Brouns et al. 2005). Rapidly digestible starches, and those containing more amylopectin, produce a greater postprandial glycemic response, due to their rapid rate of digestion and absorption (Englyst et al. 1992; Donduran et al. 1999). The GI was originally developed to help diabetic individuals with blood glucose control (Jenkins et al. 1981). Low GI foods, such as pulses, are considered to be beneficial to individuals with diabetes as they minimize postprandial glucose response, effectively stimulating less insulin secretion than high GI foods. Research suggests a positive correlation between the habitual consumption of low GI foods and reduced risks of both diabetes and obesity in healthy individuals (Bhupathiraju et al. 2014; Livesey et al. 2008; Lan-Pidhainy and Wolever 2011; Venn and Green 2007).

1.4.1 Glycemic Index Methodology in Humans

In humans, GI testing is done by feeding individuals portion sizes containing 50 g of available carbohydrate, and measuring their postprandial blood glucose response (Jenkins et al. 1981; Wolever et al. 1991). Capillary or venous blood may be sampled, with samples on healthy subjects being taken at 15, 30, 45, 60, 90, and 120 minutes post meal feeding. This value is compared to 50 g of available carbohydrate of a reference food, being either glucose or white bread (Wolever et al. 1991). Available carbohydrate is defined as carbohydrates that can be digested in the small intestine, such as RDS and SDS. Dietary fiber and RS are not classified as available carbohydrates. The quantity of available carbohydrates within a food can be
calculated indirectly through difference (Equation 1), or determined directly (Equation 2) (Food and Agriculture Organization of the United Nations 2003).

**Equation 1:**

\[
Available\ carbohydrate = 100 - [weight\ in\ grams\ \{protein + fat + water + ash + alcohol + \]
\[
dietary\ fiber\}\ in\ 100\ g\ of\ food] 
\]

**Equation 2:**

\[
Available\ carbohydrate = weight\ in\ grams\ \{monosaccharides + disaccharides + 
\]
\[
oligosaccharides + [polysaccharides-fiber]\} 
\]

The GI of an individual test food is defined as the area under the postprandial blood glucose curve (AUC) of the food, expressed as a percentage of the AUC of the reference food (Equation 3) (Wolever et al. 1991).

**Equation 3:**

\[
GI_{test\ food} = \frac{AUC_{test\ food}}{mean\ AUC_{reference\ food}} \times 100
\]
Glycemic index is measured on a scale from 1 to 100, with the reference food being assigned a value of 100. Foods that have a GI below 55 are considered to be low glycemic, whereas those with a score above 70 are considered to be high GI (Ramdath 2016). High GI foods will produce a greater spike in postprandial blood glucose (Figure 1.3). The GI of a meal is calculated based on the GI values of the ingredients, and their proportions (Wolever et al. 1991; Brouns et al. 2005). However, this method has caused controversy, as several studies have demonstrated no association between the measured GI of a meal and that of its calculated value (Laine et al. 1987; Henry et al. 2008; Flint et al. 2004).

1.4.2 Glycemic Index Testing in Dogs

Despite the growing interest in low GI dog foods from pet owners and manufacturers, very limited research has been done to investigate GI testing in dogs. Currently, dog food containing starch sources considered low GI in humans, is often marketed as low GI for dogs as well. However, given species differences in carbohydrate metabolism, the GI of these starch sources could differ between dogs and humans. Currently, there are only two studies investigating the GI of various ground and hydrated starch sources in dogs (Adolphe et al. 2012; Briens 2018). Collectively, these studies investigated the GI of pulses, including lentils, peas, and faba beans, compared to more traditional starch sources, including barley, corn, rice, tapioca and wheat. In both studies, GI was tested by providing 10g of available carbohydrates compared to a glucose control in a research dog population consisting of beagles. However, only Adolphe et al. (2012) saw significant differences in AUC and GI. Adolphe et al. (2012) reported the GI of peas as 29 ± 5 (mean ± SEM). In comparison, Briens (2018) reported the GI values of peas, lentils and faba beans as 49 ± 15, 47 ± 10, and 46 ± 17, respectively. As extruded dog foods
undergo elevated temperature and pressure conditions during extrusion, the glycemic responses
to these ingredients may change when utilized in a pet food, due to potential changes in
digestibility and starch structure. Both studies cited above followed up their initial GI research
by investigating the GI of extruded dog foods made of different starch sources. Each extruded
diet contained one starch source. When Adolphe et al. (2015) compared the GI of a pea diet to
that of a rice diet, there were no statistical differences observed as the GI of the pea diet was only
slightly lower at 56 ± 12 (mean ± SEM), compared to the rice diet which had a GI of 63 ± 9.
Similarly, Briens (2018) found that whole extruded diets containing either pea, lentil or faba
bean, had GI values of 55 ± 20, 37 ± 11, and 48 ± 11, respectively. In comparison, the cornstarch
diet had a GI of 65 ± 15. Although the diets containing pulses still had lower GI values, it is
evident in both studies that the majority of GI values increased when the starch sources were
incorporated into an extruded diet, suggesting an effect of processing. The high pressure and
temperature conditions of extrusion processing can cause partial gelatinization of starch granules
(Nayak et al. 2014). Gelatinization disrupts the intermolecular bonds and structures of these
granules, causing them to become more susceptible to enzymatic degradation. As a result,
gelatinization will assist in the digestion and absorption of these starches within the
gastrointestinal tract, resulting in an increased postprandial glycemic response and subsequent GI
(Brand and Nicholson 1985). However, these test diets may not be representative of commercial
pet foods that contain various starch sources, as opposed to a single source. Overall, limited
research has been done on investigating GI testing and methodology in dogs; apart from these
studies, no other GI testing has been done on dogs.
1.5. Canine Obesity

Obesity is the most common nutritional disorder in companion animals, and a serious health condition that affects both the quality of life and overall life expectancy (German 2006; German et al. 2010; Kealy et al. 2002; Crane 1991). The prevalence of obesity in dogs has been reported to be anywhere between 17 - 44 %, depending on the location of the study and the criteria used (Jerico and Scheffer 2002; Edney 1974; Robertson 2003; Lund et al. 1999; Vandendriessche et al. 2017; Montoya-Alonso et al. 2017; Mao et al. 2013; Lund et al. 2006; McGreevy et al. 2005). Obesity can be defined as an accumulation of excess adipose tissue in the body, which can contribute to low-grade inflammation and negative health consequences (German 2006). Dogs are considered clinically obese when their body condition score (BCS) is classified as an eight or greater on the nine-point scale (Laflamme 1997), or when their current body weight is at least 20% greater than their ideal body weight (Laflamme 2006).

The main reason for the development to obesity is positive energy balance; meaning that the level of energy intake is higher compared to energy utilization (German 2006; German et al. 2010; Kealy et al. 2002; Crane 1991). Although certain diseases and pharmaceuticals may also contribute to the development of obesity, a study by Bland et al. (2010) observed that 97% of veterinary practices attributed owner specific factors, such lack of exercise and overfeeding, as the causes of canine obesity. Additionally, research has demonstrated a positive correlation in the BCS of dogs and their owners (Nijland et al. 2010; Mason 1970; Kienzle et al. 1998).
1.5.1 The Health Effects of Obesity

Excess body fat in humans and dogs is associated with increased risk and exacerbation of numerous co-morbidities, including orthopaedic diseases, respiratory diseases, cardiac disease, cancer, and DM (German 2006). The increased risk for Type 2 DM is a result of the changes in glucose metabolism and abnormal hormone function, caused by obesity. In humans, obesity is a major risk factor for both insulin resistance and hyperinsulinemia. Concentrations of plasma insulin, as well as the proportion of insulin resistant tissue, have both been shown to increase in correlation to increasing body mass index (BMI) (Pittas et al. 2004; Call et al. 2004). Although dogs are commonly diagnosed with DM resembling Type 1 DM in humans, obese dogs have been shown to develop insulin resistance (Gayet et al. 2004; German et al. 2009). Additionally, the excess body fat in obese individuals has also been linked to the development of oxidative stress and low-grade inflammation, resulting from the increased production of reactive oxygen species (ROS) and inflammatory adipokines (German et al. 2010; Manco et al. 2006; Furukawa et al. 2004).

In addition to impacting the quality of the dog’s life, excess body fat can also impact lifespan. Research by Kealy et al. (2002) demonstrated that lean dogs, who were restricted to 75% of the food given to an overweight control group, lived on average two years longer. In addition, these lean dogs also had delayed developments of chronic diseases, including cancer and osteoarthritis. Although the control group was not obese, this study was able to show the negative effects that even a small amount of excess body fat can have on both morbidity and mortality.
1.5.2 Satiety and Hormonal Regulators of Appetite

Satiety refers to postprandial events that affect the interval to the next meal, thereby regulating meal frequency. In comparison, satiation refers to the feeling of fullness that promotes meal termination, and limits meal size (Blundell and Halford 1994). Although there are many hormones that have a role in the regulation of appetite and satiety, the major hormones include ghrelin, leptin, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), pancreatic polypeptide (PP), and peptide tyrosine-tyrosine (PYY) (Austin and Marks 2008). Most of these hormones act as appetite regulators, and are released in response to a meal, acting to reduce appetite and stimulate satiety. The only known circulating orexigenic hormone is ghrelin, which has been found to stimulate hunger and increase food ingestion in humans and rodents (Austin and Marks 2008; Cummings et al. 2001). The production sites of these hormones and their respective actions are summarized in Table 1.1.

Although limited studies have been done investigating ghrelin in dogs, canine ghrelin immunoreactive cells appear to be similar to those in humans (Tomasetto et al. 2001). Circulating levels of ghrelin appear to be increased during fasting, and decline shortly after nutrient ingestion (Cummings et al. 2001; Cummings et al. 2004; Kojima and Kangawa 2005; Asakawa et al. 2001). The exogenous administration of ghrelin to both mice and humans has been reported to increase body weight through changes in food intake, energy expenditure and nutrient utilization (Wren et al. 2001a; Wren et al. 2001b; Tschöp et al. 2000). Additionally, research in humans has found ghrelin concentrations to increase after weight loss in obese individuals (Tschöp et al. 2001; Shiiya et al. 2002).
<table>
<thead>
<tr>
<th>Hormone</th>
<th>Function</th>
<th>Production Site</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghrelin</td>
<td>Hunger</td>
<td>Stomach</td>
<td>↑ peptides that act directly on the hypothalamus to stimulate feeding</td>
</tr>
<tr>
<td>PYY</td>
<td>Satiety</td>
<td>Ileum, colon, and rectum</td>
<td>↓ gastric motility ↓ secretion pancreatic enzymes</td>
</tr>
<tr>
<td>PP</td>
<td>Satiety</td>
<td>Pancreas</td>
<td>↓ gastric emptying ↓ Ghrelin</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Satiety</td>
<td>Ileum and colon</td>
<td>↓ gastric emptying ↓ gastric motility ↓ glucagon secretion ↑ insulin secretion</td>
</tr>
<tr>
<td>GIP</td>
<td>Satiety</td>
<td>Stomach, duodenum and jejunum</td>
<td>↓ gastric motility ↑ insulin secretion ↑ energy storage</td>
</tr>
<tr>
<td>Leptin</td>
<td>Satiety</td>
<td>Adipose Tissue</td>
<td>↓ peptides that act directly on the hypothalamus to stimulate feeding ↑ peptides that act directly on the hypothalamus to inhibit feeding</td>
</tr>
</tbody>
</table>

Table 1.1. Commonly investigated hormonal regulators of appetite, responsible for hunger or satiety, through their respective actions on the gastrointestinal tract, enzymatic secretions and/or the hypothalamus (Adapted from Austin and Marks 2008).
Similarly in dogs, research has reported decreased ghrelin concentrations in obese versus lean dogs, suggesting that ghrelin may be down-regulated as a consequence of excess stored energy (Jeusette et al. 2005). In both humans and dogs, ghrelin does not appear to be altered by the nutritional compositions of the diets, and it has therefore been suggested that ghrelin concentrations may be influenced by overall energy intake and meal volume instead (Callahan et al. 2004; Gibbons et al. 2013; Bosch et al. 2009; Lubbs et al. 2010).

Ghrelin also appears to be responsible for stimulating the release of PP (Austin and Marks 2008). This satiety peptide, along with PYY, is part of the pancreatic polypeptide fold peptide family. Research done in dogs and humans showed that these satiety peptides are released in response to food intake, and their concentrations appear to be proportion to energy intake (Lin and Chey 2003; Track et al. 1980). Bosch et al. (2009) investigated postprandial PYY concentrations in response to a low fibre and high fiber diet in dogs. The reported results indicated that PYY concentrations did not differ between diets, regardless of fibre content. In obese and lean individuals, the administration of PYY significantly reduced energy intake, and overall food intake (Batterham et al. 2003a). Similarly, the administration of PP to healthy individuals reduced appetite and overall energy intake (Batterham et al. 2003b). At this time, there does not appear to be any research on the administration of either of these peptides and energy intake in dogs.

Several appetite regulators can also be classified as incretin hormones, as they play a role in glucose homeostasis by enhancing glucose-stimulated insulin secretion. These hormones include GLP-1 and GIP (Austin and Marks 2008). In both obese and normal individuals, the
infusion of GLP-1 has been shown to dose-dependently reduce food intake and increase feelings of satiety (Flint et al. 1998; Verdich et al. 2001; Näslund et al. 1999). Moreover, research in dogs by Lubbs et al. (2010) showed that acute postprandial concentrations of GLP-1 were most affected by carbohydrate and fat content, and not protein. Additionally, research in dogs has reported higher concentrations of GLP-1 in response to diets with a higher fiber inclusion, although these differences were not found to be significant (Bosch et al. 2009).

Unlike the other satiety regulators that are secreted in amounts proportional to caloric load, leptin appears to be secreted in proportion to the body’s fat mass (Maffei et al. 1995). Research has demonstrated that BCS in dogs and their leptin concentrations appear to be positively correlated (Ishioka et al. 2002; Ishioka et al. 2007; Mazaki-Tovi et al. 2010; Jeusette et al. 2005). Leptin is an adipokine that appears to play a large role in the regulation of food consumption and regulation of body weight by suppressing appetite (Austin and Marks 2008). In mice, the administration of leptin has been shown to induce fat loss (Campfield et al. 1995; Maffei et al. 1995; Pelleymounter et al. 1995), while the absence of leptin in both humans and mice has been shown to lead to severe obesity (Zhang et al. 1994; Farooqi et al. 2002; Sinha and Caro 1998).

1.6 Conclusion

The purpose of this literature review was to investigate the canine metabolism of carbohydrates, the use of carbohydrates in commercial dog foods, satiety in dogs, the GI in humans, and lastly, our current knowledge of GI in dogs, and its potential for future use in canine nutrition. In humans, the habitual consumption of low GI foods has been linked to decreased
risk of chronic diseases, including diabetes and obesity (Ludwig 2002; Barclay et al. 2008). At this time, there is very little research investigating the use of GI methodology in dogs, or the potential physiological relevance of low GI foods for dogs. Future research should build on current knowledge by applying human GI methodology to test not only various individual starch sources, but also commercial extruded foods containing different starch sources in dogs. Canine GI methodology must also be tested on a larger breed of dog than beagles, in order to establish correct servings of available carbohydrates for testing in breeds of different sizes. Furthermore, to understand the effects of satiety that these foods may provide to dogs, research investigating the effects on postprandial appetite-related gut hormones should be done. Investigating the effects of different carbohydrate sources on not only insulin and glucose responses in dogs, but also satiety, could be paramount to providing dietary modifications that may address the increasing epidemics of diabetes and obesity in dogs.
1.7 Thesis Objectives and Hypotheses

The research objectives of this thesis are as follows:

1. To further develop GI methodology in dogs by using a larger dog breed.
2. To investigate the GI of cooked green lentils and cooked white rice in dogs, low and high GI foods in humans respectively; as well as the GI of white bread, to assess the use of GI methodology as an assessment tool in dogs.
3. To determine the GI, glycemic response, and insulinemic response of four extruded commercial dog foods containing different starch sources in healthy dogs.
4. To determine the type and amount of starch fractions in the four extruded commercial test foods, and examine the relationship between the content of starch and the GI in healthy dogs.
5. To determine the effect of the different starch sources in the four commercial extruded dog foods on various appetite-related gut hormones in healthy dogs.

It was hypothesized that:

1. Cooked green lentils would have the lowest GI due to their higher level of RS than white rice or white bread, and that white bread would be a suitable replacement for glucose as a control food in canine GI testing.
2. The grain-free commercial dog food containing pulses would have the largest content of RS, and therefore produce the lowest GI, as well as a delayed and lengthened glycemic and insulinemic response.
3. Postprandial concentrations of the various appetite-related gut hormones would be greatest after the consumption of the grain-free commercial dog food containing pulses.
1.8 References


Agriculture and Agri-Food Canada. 2016. *Pet Food Sales in Canada*.


CHAPTER 2: POSTPRANDIAL EFFECTS OF FEEDING SINGLE STARCH SOURCES ON GLYCEMERIC INDEX AND GLYCEMERIC RESPONSE IN DOGS

2.1 Abstract

Obesity and diabetes are leading nutrition-related disorders in pets. The perceived health benefits of low glycemic index (GI) foods in humans makes them of interest in the treatment and prevention of these conditions, though limited scientific data is available to support GI methodology to back up pet food claims. The objectives of this pilot study were to further develop GI methodology in dogs, and to examine the GI of several starch sources (white bread, cooked white rice, and cooked green lentils) as compared to a glucose control in healthy dogs. Each starch source was tested once in 6 adult Siberian Huskies, and the control, 50mL 20% w/vol glucose solution, was tested twice per dog in a randomized cross-over design. The dietary treatments were provided in 10 grams (g) of available carbohydrate. Whole blood glucose concentrations were measured preprandial and at 15, 30, 45, 60, 90, 120 and 150 minutes postprandial. There was no significant difference in GI between dietary treatments (p=0.569). Rice presented the highest GI of 71 ± 14 (mean ± SEM). Lentils and bread had GI values of 60 ± 20 and 47 ± 11. Incremental area under the glycemic response curve (AUC), peak glucose concentrations, and time to peak were not influenced by starch source (p>0.05). Bread, considered to be high GI in humans, presented the lowest GI in this study. Increasing the quantity of available carbohydrates fed may be necessary for future studies using large breed dogs. More research is needed to validate GI methodology in dogs, and to document GI values for common starch sources used in pet food formulations.
2.2 Introduction

Pet owners are increasingly treating their companion animals as members of their family. As such, they are looking for healthful ingredients that will benefit the quality of their pets’ lives (Agriculture and Agri-Food Canada 2013). The trend of grain-free diets has remained popular with pet owners in recent years (Agriculture and Agri-Food Canada 2016), who view these products as being healthier for their pets, despite a lack of evidence. This movement has encouraged pet food companies to switch from traditional starch ingredients, such as corn and rice, to novel, grain-free starch sources in their formulations (Buff et al. 2014). As a result, the use of pulses, such as lentils and peas, are of interest to pet food formulators.

Pulses have become a food of interest in human health and nutrition due to their low glycemic index (GI) value. The GI was created to rank foods based on their acute postprandial glycemic response in comparison to a control food, either a glucose solution or white bread (Jenkins et al. 1981). This approach makes it possible to compare glycemic responses of different foods using a cross-over design as each subject’s glucose response is standardized to the control. Glycemic index has been extensively studied in humans, and the regular consumption of low GI foods in humans has been linked to numerous health benefits, such as decreased levels of cholesterol and triglycerides, and decreased risk of obesity, diabetes and cardiovascular disease (Barclay et al. 2008; Ramdath 2016; Wolever et al. 1991). Common starch-containing pet food ingredients such as corn and rice have been shown in humans to have higher mean GI values of 54 and 56, respectively (Foster-Powell et al. 2002). In comparison, lentils have demonstrated lower GI values, between 18-52, depending on the form and source of the pulse (Foster-Powell et al. 2002). Obesity and diabetes are among the most common nutrition-related disorders seen in
companion animals. As such, the perceived health benefits of low GI foods in humans makes them of interest in both the treatment and prevention of these diseases in pets.

Although research has demonstrated that the type of carbohydrate plays a role in determining postprandial glycemic responses in dogs (Carciofi et al. 2008), GI methodology has not been validated for use in companion animals, and very limited work has been done investigating the use of GI in dogs (Adolphe et al. 2012; Adolphe et al. 2015; Briens 2018). Canine GI research done by Adolphe et al. (2012) and Briens (2018) has investigated feeding 10 g of available carbohydrates to beagles in the form of uncooked single starch sources. Adolphe et al. (2012) investigated the GI of rice, barley, corn and peas, while Briens (2018) looked at tapioca, wheat, rice, modified and unmodified cornstarch, peas, lentils, faba beans and potato. Both studies found that the pulse ingredients (peas, lentils and faba beans) produced lower GI values compared to the other starch sources. However, the later study by Briens (2018) did not find these differences to be significant.

It is apparent that GI methodology has only been tested in a smaller breed of dog (beagles) in a laboratory setting. There has been no research done to determine if 10 g of available carbohydrate can still be considered an ideal quantity in canine GI testing with a bigger breed of dog, or in client-owned dogs. Additionally, there has been no research done to examine the GI values of cooked starch sources in dogs. Therefore, the objectives of this pilot study were to further develop GI methodology in dogs. The study used client-owned, Siberian Husky dogs to determine the GI for cooked green lentils and cooked white rice, low and high GI foods in humans, respectively. In addition, these foods were chosen to test one grain (white rice) and one
non-grain starch (lentils) source. Additionally, we investigated the glucose response to white bread to assess its use as a control food instead of glucose for GI testing in dogs as is done in human GI testing. We hypothesized that green lentils would have the lowest GI due to their increased level of RS and that white bread would be a suitable replacement for glucose as a control food in GI testing. This research will further contribute to the development of canine GI testing methodology. By developing this methodology, future research will be able to assess the potential of chronic low GI consumption on health in dogs, and allow the pet food industry the opportunity to develop low GI commercial foods for dogs.

2.3 Materials & Methods

Animals

Six neutered, adult, client-owned Siberian husky dogs (n=3, male, neutered; n=3, female, neutered) were enrolled in the study. Body condition scores (BCS) for the dogs were between 4 and 6 (on a 9-point-scale (Laflamme 1997)), with a mean (± SEM) body weight (BW) of 24.94 ± 0.99 kg (range 21.51-28.72 kg), and a mean age of 5.63 ± 0.23 years (range 5.4-6.8 years). Prior to the study, all dogs enrolled were deemed healthy based on a medical and diet history, physical examination, complete blood count (CBC), and serum biochemistry profile. Dogs that had received medications six months prior to enrolment, had abnormalities on their physical examination, complete blood count or serum biochemistry, or were younger than one year of age, were not enrolled in this study. All dogs remained with their owner throughout the course of the study. The dogs were housed together in group housing. On study days, the dogs were separated and individually handled. All dogs were transitioned onto the same diet (GO! FIT + FREE™ Adult Dog Food, Petcurean Pet Nutrition, Chilliwack, BC, CA) two weeks prior to the start of the postprandial response tests. Initially, dogs were fed an amount determined to maintain
an optimal BCS, based on previous energy intake from their diet history. Body weight and BCS were recorded at each study visit, and food was adjusted to maintain stable BW. Dogs continued to eat the background diet throughout the entire study period. All experimental procedures for this study were approved by the University of Guelph Animal Care Committee (AUP#3650), and were in accordance with national and institutional guidelines for the care and use of animals.

Dietary Treatments and Analyses

Three starch-rich foods were tested: white bread (Wonder Bread®, Interstate Brands Companies, Kansas City, MO, USA); cooked white long grain rice (Selection®, St. Paul, MN, USA); and cooked Eston green lentils (AGT Food and Ingredients, Regina, SK, CA). A 20% (wt/vol) glucose solution was used as the control. Using a randomized, cross-over design, each dog tested each food once, except for the glucose control which was tested twice, with a minimum washout period of two days between study visits. Test foods and glucose were fed in amounts that provided 10 g of available carbohydrate (Adolphe et al. 2012, 2015), as determined through total starch and free sugar content of the foods (McCance and Lawrence 1929). The total and resistant starch content of each food was determined enzymatically using commercially available assay kits (Megazyme International, Wicklow, Ireland; AOAC Method 996.11 and 2002.02, respectively). Total and resistant starches were calculated as a percentage of the total dry matter. Free sugar content was determined by extracting and analysing monosaccharides and disaccharides, as described by Brummer et al. (2015).
**Meal Response Test**

Dogs underwent an overnight (14h) fast prior to each postprandial response test. On test days, dogs were weighed, BCS was recorded, and a 20Ga IV catheter (Becton Dickinson Canada Inc. Insyte-W 20GA x 1.1) was placed into a cephalic or saphenous vein. Catheter patency was maintained by flushing with 0.1 mL of 4.0% sodium citrate and 2.0 mL of 0.9% sodium chloride after each sample. Before collection, the catheter was flushed once more with 0.5 mL of the sodium chloride, and 0.5 mL of blood was withdrawn and discarded to avoid any dilution. Two baseline blood samples (0.5 mL) were taken before the test meal was given. Postprandial blood samples (0.5 mL) were taken at 15, 30, 45, 60, 90, 120 and 150 min after the start of the meal (Wolever et al. 1991). Time was started immediately when the dog started eating the meal or drinking the glucose solution. Each dog consumed all dietary treatments in less than 5 minutes. Collected whole blood was immediately tested for glucose using a handheld blood glucose monitor (AlphaTRAK 2®, Abbot Laboratories, North Chicago, IL, USA), validated for use in dogs (Kang et al. 2016). Each blood sample was tested twice for glucose. If results varied by more than 0.3 mmol/L, additional testing was done until two results that were 0.3 mmol/L apart or less were obtained. Glycemic index was calculated as the post-prandial incremental area under the curve (AUC) for glucose of each test diet, divided by the AUC of the glucose control for the same dog (Wolever et al. 1991). The AUC of each treatment was calculated as described by Brouns et al. (2005).

**Statistical Analysis**

Statistical analyses were performed using the MIXED procedure of SAS software (SAS® Studio, Version 9.4, SAS Institute Inc., Cary, NC, USA). Prior to analysis, data was explored for
normality using Q-Q plots, box plots, and the Kolmogorov-Smirnov test. A one-way analysis of variance (ANOVA) model was used to compare AUC, peak glucose concentration, time to peak, and GI, between dietary treatments. Dog was defined as a random effect, and treatment as the fixed effect. Tukey's post-hoc test was performed for all multiple comparisons. Results are expressed as mean ± SEM. A p-value < 0.05 was considered significant.

2.4 Results

Body weight and BCS were kept constant for all dogs throughout the study period. As shown in Table 1, the results from the total starch and free glucose assays found that the bread, rice, and lentils contained 42.27, 75.00, and 39.79 g of available carbohydrate per 100 g of food, respectively. As a result, in order to provide 10 g of available carbohydrates, 23.7 g of bread, 13.3 g of rice, and 25.1 g of lentils were fed. The nutrient composition for each starch source is listed in Table 2.

The post-prandial glycemic responses to the dietary treatments and glucose control are presented in Fig. 1 and Table 3. The order of GI for the starch sources, from highest to lowest, was observed as: rice > lentils > bread, with no significant differences being noted between treatments (p = 0.569). The overall AUC was higher for the glucose solution compared to the starch sources.
Table 2.1. In vitro starch and free sugar content (dry matter basis) of three single starch sources and their individual 10g available carbohydrate portion sizes (as fed basis) fed in a meal response test to six client-owned Siberian huskies.

<table>
<thead>
<tr>
<th></th>
<th>Lentil</th>
<th>Rice</th>
<th>Bread</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>9.25 ± 0.09</td>
<td>10.45 ± 0.21</td>
<td>37.65 ± 0.26</td>
</tr>
<tr>
<td>Total Starch (% DMB)</td>
<td>41.87 ± 0.15</td>
<td>75.00 ± 0.11</td>
<td>42.21 ± 0.26</td>
</tr>
<tr>
<td>Resistant Starch (% DMB)</td>
<td>2.72 ± 0.05</td>
<td>0.11 ± 0.02</td>
<td>0.82 ± 0.01</td>
</tr>
<tr>
<td>Galactose (% DMB)</td>
<td>0.018 ± 0.001</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Glucose (% DMB)</td>
<td>0.017 ± 0.002</td>
<td>0.020 ± 0.002</td>
<td>0.87 ± 0.013</td>
</tr>
<tr>
<td>Sucrose (% DMB)</td>
<td>1.25 ± 0.012</td>
<td>0.18 ± 0.001</td>
<td>nd</td>
</tr>
<tr>
<td>Fructose (% DMB)</td>
<td>nd</td>
<td>nd</td>
<td>1.74 ± 0.025</td>
</tr>
<tr>
<td>Av CHO (% DMB)</td>
<td>39.79</td>
<td>75.00</td>
<td>42.27</td>
</tr>
<tr>
<td>Portion Size for 10g Av CHO (g As Fed)</td>
<td>25.13</td>
<td>13.33</td>
<td>23.66</td>
</tr>
</tbody>
</table>

Values equal to means ± SEM; n = 3 in duplicate; nd, not determinable; Av CHO, available carbohydrate; DMB, dry matter basis; As Fed, as fed basis
Table 2.2. Nutrient composition (as fed basis) of single starch sources for their respective 10 g available carbohydrate portion sizes fed in a meal response test to client-owned Siberian huskies (n=6). ¹

<table>
<thead>
<tr>
<th></th>
<th>Lentil</th>
<th>Rice</th>
<th>Bread</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g)</td>
<td>6.02</td>
<td>0.89</td>
<td>1.90</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.71</td>
</tr>
<tr>
<td>Total Dietary Fiber (g)</td>
<td>7.0</td>
<td>0.30</td>
<td>0.90</td>
</tr>
</tbody>
</table>

¹Determined from the United States Department of Agriculture (USDA) Food Composition Databases
Figure 2.1. Mean increases in measured concentration of whole blood glucose from baseline (mmol/L) following acute feedings of single starch sources and a glucose control (20% wt/vol). Treatments were given as 10 g of available carbohydrates to fasted client-owned Siberian huskies (n=6). Values expressed as mean ± SEM.
Table 2.3. Postprandial glycemic responses to acute feedings of 10 g of available carbohydrates of a glucose solution (20% wt/vol) and single starch sources in fasted client owned Siberian huskies (n=6).

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>Lentil</th>
<th>Rice</th>
<th>Bread</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak Concentration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>7.24 ± 0.22</td>
<td>6.58 ± 0.29</td>
<td>7.23 ± 0.57</td>
<td>6.73 ± 0.20</td>
<td>0.175</td>
</tr>
<tr>
<td><strong>Time to Peak</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(min)</td>
<td>56.25 ± 8.28</td>
<td>87.50 ± 20.65</td>
<td>92.50 ± 16.62</td>
<td>60.00 ± 15.00</td>
<td>0.149</td>
</tr>
<tr>
<td><strong>AUC 0-150 min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol/L min)</td>
<td>144.23 ± 20.72</td>
<td>75.77 ± 23.73</td>
<td>92.86 ± 24.37</td>
<td>73.98 ± 25.20</td>
<td>0.0429</td>
</tr>
<tr>
<td><strong>AUC ≤ 30 min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.54 ± 2.66a</td>
<td>8.43 ± 2.34b</td>
<td>6.01 ± 2.70 b</td>
<td>9.34 ± 3.39 b</td>
<td>0.0024</td>
<td></td>
</tr>
<tr>
<td><strong>AUC ≥ 30 min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>123.69 ± 20.79</td>
<td>67.34 ± 22.54</td>
<td>86.85 ± 22.82</td>
<td>64.63 ± 22.22</td>
<td>0.110</td>
<td></td>
</tr>
<tr>
<td><strong>GI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 ± 20</td>
<td>71 ± 14</td>
<td>47 ± 11</td>
<td>0.569</td>
<td></td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM; n=6; Values in a row with superscripts without a common letter differ; p < 0.05, One-way ANOVA with Tukey-Kramer post-hoc test. AUC = area under the curve of postprandial glycemic response curve.
However, there were no significant differences noted between any of the treatments, including the glucose control, for the peak glucose concentration (p = 0.175) or time to peak (p = 0.149). For overall AUC (0 – 150 min), although there was a significant overall difference observed (p=0.0429), there were no significant differences between dietary treatments following a Tukey-Kramer post-hoc test. Immediate post-prandial glycemic response (AUC ≤ 30 min) was significantly greater for the glucose control compared to the other dietary treatments (p = 0.0024). Amongst the starch sources alone, bread produced the greatest immediate response (AUC ≤ 30 min), although not significant. Later glycemic response (AUC ≥ 30 min) was again greater for the glucose solution, although similarly, not significantly greater compared to any of the other dietary treatments (p = 0.110). Overall, there were no significant differences noted between the starch sources themselves for overall AUC, nor for the immediate (AUC ≤ 30 min) or later responses (AUC ≥ 30 min).

2.5 Discussion

Although there have been limited studies investigating GI in dogs, our results differed from those previously published. Currently, to our knowledge, there are only two studies investigating the GI of various individual uncooked starch sources in dogs (Adolphe et al. 2012; Briens 2018). These studies tested canine GI by feeding 10 g of available carbohydrates to beagles in a laboratory setting. Both Adolphe et al. (2012) and Briens (2018) reported the GI of rice in dogs as 55, however in both studies, the rice was ground and mixed with water, as opposed to cooked. It is therefore possible that the temperature of the cooking process utilized in this study may have contributed to the gelatinization of starch within the rice, resulting in a higher content of digestible starch, and therefore the increased GI of 71 ± 14. Briens (2018) reported a GI of 47 ± 10 after feeding dogs a slurry composed of ground and hydrated lentils.
Similar to the white rice, our research calculated a higher GI of 60 ± 20 for the cooked green lentils, which may have again been attributed to the cooking process. At this time, we are unaware of any research investigating GI of cooked green lentils and cooked white rice in dogs.

In humans, the GI of cooked white rice and cooked green lentils have been reported to be 56 ± 2 and 30 ± 4 respectively, with glucose as the control (Foster-Powell et al. 2002). Therefore, both the GI of 71 ±14 for the white rice, and the GI of 60 ± 20 for the green lentils observed in the dogs were notably higher than the reported values in humans (Foster-Powell et al. 2002). Similarly, the low GI of 47 ± 11 calculated for the white bread does not agree with human research. White bread is considered a high GI food in humans, with a reported GI of 73 ± 2 using glucose as the control (Foster et al., 2002). Although GI is influenced by a number of factors, including moisture content, processing and cooking methods, and temperature storage (Ramdath 2016), it is unclear what may have caused these unexpected GI values in the dogs, especially the low GI value for the bread. Due to the low AUC of the bread, the glucose solution was used to calculate the GI values of the other dietary treatments. Using white bread to calculate the GI values of the other treatments would have resulted in GI values over 100. Based on these results, a glucose solution may be a better and more reliable control than white bread for future GI testing in dogs.

Although test conditions were kept the same between dogs and dietary treatments, the variability in glycemic responses noted between the dogs was quite large, and may have also played a role in these outcomes. Based on the study by Adolphe et al. (2012), six dogs should have been sufficient to demonstrate significant differences in GI. However, given the variability
noted in our study, a sample size of 82 would have been necessary to demonstrate significant differences, with a power of 0.80. However, Matthan et al. (2016) noted that increasing both sample size, as well as increasing the number of repeated tests for both control and test foods in human GI testing, did not affect GI value, or reduce the variability between and within individuals. Therefore, it is possible that this variability in GI would have still have remained, even with a larger sample size. The variability in GI for the same foods between different individuals is a known factor of GI testing in humans. A study investigating the inter-variability between GI values of white bread in 63 individuals calculated a mean GI value of 62 ± 15 (mean ± SD), with an inter-individual variability of 25% (Matthan et al. 2016). The authors found that the individual GI values of the white bread for the study population ranged from 35-103, ranging across all three GI classification categories (low, medium and high). The individual GI values for white bread observed in our dogs ranged from 17-84, similarly spanning all three categories. However, this study observed larger inter-individual variability between the GI values of white bread in the dogs at 58%. Although variation between individuals is a known factor of GI testing in humans (Wolever and Bolognesi. 1996; Tabassum et al. 2013), previous studies investigating both GI and glycemic response in dogs have also demonstrated notable variability amongst dogs (Nguyen et al. 1994, Carciofi et al. 2008, Adolphe et al. 2015, Briens 2018). As a result, the variability in glycemic responses observed in this study, as well as previous research, suggest that GI testing may not be a reliable tool for use in dogs.

Additionally, increasing the quantity of available carbohydrates fed to the dogs may have been warranted for this study. Feeding 10 g of available carbohydrates was based on previous GI canine studies (Adolphe et al 2012, 2015). In both studies, 10 g of available carbohydrates was
fed to beagles of normal body weight, with a reported mean weight of 9.8 kg in the more recent study (Adolphe et al. 2015). Briens (2018) similarly fed 10 g of available carbohydrates to beagles of normal weight. Although the mean weight of the dogs was not reported, the author reported feeding 1 g of available carbohydrate per kilogram BW. In contrast, human GI research is based on portions of 50 g of available carbohydrates (Wolever et al. 1991). This dose has been proposed as the standard, due to curvilinear dose-response curve, and the trend for blood glucose concentrations to plateau as intake becomes greater than 50 g available carbohydrates (Jenkins et al. 1981; Brouns et al. 2005). Similarly, intakes of 10-25 g have demonstrated insignificant or only slight increases in blood glucose concentrations (Brouns et al. 2005). A suggested equation for the quantity of available carbohydrates fed in canine GI testing was proposed by Nguyen et al. (1998) as 2 g per metabolic kilogram (2 g/kg BW\(^{0.75}\)). As a result, 20-25 g as opposed to 10 g may have been more appropriate, in order to see the postprandial glycemic responses we were expecting in the Siberian Huskies, which are significantly larger than beagles. Additionally, although these dogs were not being actively raced throughout this study, it is possible that Siberian Huskies trained for sled dog racing may possess differences in carbohydrate and fat oxidation (Miller et al. 2017), as well as glucose transport activity (Davis et al. 2014), compared to more sedentary pet dogs. These differences may affect their GI results, and might potentially make them not an ideal breed for canine GI testing.

In humans, lentils have been a health food of interest due to their relatively high protein content for a plant-based food, as well as the delayed and lengthened glycemic response that they produce (Faris and Attlee 2016). Moreover, low GI foods have also been known to assist in the prevention and treatment of diabetes and obesity in humans (Ludwig 2002). Starch sources that
produce a delayed and lengthened glycemic response, with minimal fluctuations in blood glucose concentrations, may be beneficial for maintaining glycemic control in dogs with glucose intolerance resulting from obesity and diabetes (Graham et al. 1994). In this regard, the present study demonstrated that lentils might be advantageous over rice in these dogs, even though the GI was not significantly lower. Similar to our research, when Carciofi et al. (2008) fed an experimental diet containing solely lentils as the starch source to dogs, the total AUC of glucose for this diet was not lower or statistically different from the others; however, the lentil diet did produce a more lengthened and delayed glycemic response.

Overall, more research is needed to further improve and streamline GI testing in dogs, so that it may be used to investigate low-GI foods and their potential effects on health in dogs, particularly related to the prevention and treatment of both diabetes and obesity. The present study observed that the GI values between the three starch sources (white bread, cooked green lentils, and cooked white rice) did not demonstrate statistically significant differences, and moreover, did not agree with human GI results. Although there have been no studies in dogs looking specifically at cooked green lentils and white rice, previous canine GI studies investigating uncooked lentils and rice did not agree with our results. Moreover, our results indicated that further studies are warranted before white bread can be considered a good and reliable control for future canine GI testing. Additionally, further studies are necessary to investigate the GI of various starch sources in dogs, and how they may compare to the values reported in humans.
2.6 Acknowledgements

We would like to thank all student volunteers for all their help with animal handling and blood collections, as well as Dr. Sarah Dodd for assisting with catheter placement. Also a special thank you to Aileen Hawke for her assistance in the lab, and Michelle Edwards for her assistance with the statistics involved in this study. Additionally, we would like to extend a special thank you to Rajenn Siberian Huskies for allowing us to use his dogs for this research.

2.7 Funding

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2.8 References


Agriculture and Agri-Food Canada. 2016. “Pet Food Sales in Canada.”


3.1 Abstract

The glycemic index (GI), a measure of post-prandial blood glucose response, has been shown in humans to significantly impact glucose control, insulin sensitivity, weight management, and chronic disease risk. As a result, low GI foods have become of interest to both pet owners as well as pet food manufacturers. This study aimed to investigate the postprandial glycemic and insulinemic responses and subsequent calculation of a GI in dogs consuming four commercial extruded dog foods containing different starch sources, with similar levels of moisture, protein, fat and ash. The four test diets were classified based on the various starch sources they contained: traditional grains (corn, wheat), whole grains (oats, rye), grain-free (peas, lentils) or vegan (no animal ingredients). Each diet was tested once and the control (50% w/vol glucose solution) was tested twice in 11 healthy adult Siberian Huskies in a randomized cross-over design. Each meal and control provided 25g of available carbohydrate. Pre- and postprandial (15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, 420 & 480 minutes) blood samples were collected to measure whole blood glucose concentrations using a handheld blood glucose monitor. The grain-free diet had the lowest GI of 41 ± 6 (mean ± SEM), while the traditional grain diet had the highest GI of 83 ± 17. The whole grain diet and vegan diet had intermediate GI values of 56 ± 8 and 65 ± 15, respectively. Although numerical differences were observed, there were no significant differences detected in GI when compared using an ANOVA (p=0.1537). Due to the limited statistical power of this study, more research is needed to clarify
whether human GI methodology is suitable for dogs, and to provide more insight into whether the reported GI of various starchy foods in humans differs in dogs.

3.2 Introduction

In North America, the majority of pet dogs consume extruded kibble because of its convenience and value (Agriculture and Agri-Food Canada 2016; Agriculture and Agri-Food Canada 2013; Packaged Facts 2017). Extruded dry dog foods often contain a large proportion of starch (Spears and Fahey 2004). Starch is the primary digestible carbohydrate found in plants and is an economical source of dietary energy for both humans and pets (Bednar et al. 2001). Commercial pet foods typically contain a mixture of high starch carbohydrate sources, which fall into three broad categories: traditional grains (e.g. wheat, corn), novel whole grains (e.g. barley, oatmeal, rye), and non-grain carbohydrates (e.g. peas, lentils, tapioca, potato). Pet food companies often market their products based on these categories of starch sources, with the grain-free category remaining very popular with consumers over the past few years (Packaged Facts 2017). Additionally, as the population of individuals adopting vegan or vegetarian diets increases (Janssen et al. 2016; Radnitz et al. 2015), an increasing number of consumers are looking to feed a plant-based diet to their dogs as well (Wakefield et al. 2006; Knight and Leitsberger 2016). Vegan and vegetarian diets for dogs similarly contain a large proportion of high starch carbohydrate sources.

The health consequences of starch sources in companion animals are an area of great debate, though there is currently a lack of research to back up claims. The type and amount of starch source may play a key role in the maintenance of health and prevention of chronic disease
risk. Starch is made up of two glucose polymers: amylose and amylopectin (Nelson and Cox 2008). The linear structure of amylose makes it less susceptible to digestion, as compared to the branch-chained structure of amylopectin. The ratio of these polymers varies across starch sources and influences the rate of digestibility of starch sources (Biliaderis 1991). As a result, the postprandial glycemic response of starch sources will vary as well. The glycemic index (GI), a measure of post-prandial blood glucose response, was originally developed to help diabetic individuals with blood glucose control (Jenkins et al. 1981). However, the habitual consumption of low GI foods in humans has been associated with improved glucose control, insulin sensitivity and weight management, and overall reduced risks of both obesity and diabetes in healthy individuals (Bhupathiraju et al. 2014; Livesey et al. 2008; Lan-Pidhainy and Wolever 2011; Venn and Green 2007).

The increased interest that consumers have had in low GI foods for themselves has led to many pet food companies making marketing claims about GI as well. These claims are based on the inclusion of starch sources known to be low GI in humans, such as peas and lentils. Although these ingredients are frequently touted as being beneficial for dogs as well, these claims are not based on standardized in vivo GI testing in dogs. The GI of a food is determined by a number of factors, including the processing and storage conditions that the food has undergone (Brand and Nicholson 1985; Brouns et al. 2005). It is therefore inaccurate to assume that single starch sources have the same GI as a complete and balanced extruded pet food, which contains a variety of ingredients providing different amounts and types of starch. Moreover, there is limited research available in dogs on GI testing and the GI of commonly used starch sources. Previous research in dogs has demonstrated that pulses, such as peas and lentils, may produce delayed and
lengthened glycemic and insulinemic responses when fed alone and when included in an extruded diet (Adolphe et al. 2012; Adolphe et al. 2015; Briens 2018; Carciofi et al. 2008). However, these studies investigated single carbohydrate sources and commercial diets typically contain a variety of starch ingredients. Nguyen et al. (1998) found that the starch content of commercial dog foods had a significant impact on postprandial glucose, however the starch sources within the diets were not reported. To date, there is no published research investigating dry dog foods containing different starch sources and their effects on postprandial glycemic and insulinemic response in dogs. Additionally, there have been no studies investigating the GI of commercial extruded dog foods using in vivo testing.

The goal of this research is to examine the effect of foods that contain different starch sources in commercial dog food on postprandial glycemic and insulinemic response. Additionally, the GI of these dog foods will be calculated, and the use of GI methodology in dogs will be further investigated. It was hypothesized that the grain-free diet containing pulses would produce the lowest GI, and lowest glycemic and insulinemic responses in the dogs, as compared to the other commercial extruded diets tested.

3.3 Materials & Methods

All experimental procedures for this study were approved by the University of Guelph Animal Care Committee (AUP#3650), and were in accordance with national and institutional guidelines for the care and use of animals.
Animals

Eleven adult, client-owned Siberian husky dogs (n=4, male, neutered; n=5, female, spayed; n=2, female, intact) with a mean age of 5.63 ± 0.72 years (range 1.00-10.67 years) were used for this research. Body condition scores (BCS) for the dogs ranged between 3 and 6 on a 9-point-scale (Laflamme 1997), with a mean ± SEM body weight (BW) of 23.32 ± 1.15 kg (range 19.00-30.68 kg). All dogs were deemed healthy based on medical history, physical examination, complete blood count (CBC), and serum biochemistry profile. Dogs were not enrolled if they had received medications six months prior to enrolment, had abnormalities on their physical examination, complete blood count or serum biochemistry, or were younger than one year of age. All dogs remained with their owner throughout the duration of the study. The dogs were housed together in group housing, and were separated and individually handled on study days. All dogs were transitioned onto the same diet (GO! FIT + FREE™ Adult Dog Food, Petcurean Pet Nutrition, Chilliwack, BC, CA) two months prior to the start of the postprandial response tests. Dogs continued to eat this background diet throughout the entire study period. Dogs were fed the background diet in amounts deemed suitable to maintain an optimal BCS, based on previous energy intake from their diet history. Body weight and BCS were recorded at each study visit, and the amount fed was adjusted to maintain BW.

Dietary Treatments and Analyses

Four commercial dog foods were tested: Dog Chow® (Nestlé Purina Petcare®, St. Louis, MO, USA), SUMMIT™ Three Meat Adult Recipe (Petcurean Pet Nutrition, Chilliwack, BC, CA), GO! SENSITIVITY + SHINE™ Limited Ingredient Duck Recipe (Petcurean Pet Nutrition, Chilliwack, BC, CA), and Vegetarian Dry Dog Formula (Dick Van Patten’s Natural Balance Pet
The four test diets were classified based on the main starch sources that were listed on the ingredient panels: traditional grains (corn, wheat), whole grains (oats, rye), grain-free (peas, lentils), or vegan (no animal ingredients) (Table 3.1). A 50% (wt/vol) glucose solution was used as the control. Using a randomized, cross-over design, each dog tested each commercial diet once, and the glucose control which was tested twice. The washout period between testing was seven days. Test diets and the glucose solution were fed in amounts that provided 25 g of available carbohydrate (Nguyen et al. 1998), as determined through total starch and free sugar content of the foods (McCance and Lawrence 1929). The total (AOAC Method 996.11) and resistant starch (AOAC Method 2002.02) content of each food was determined enzymatically using commercially available assay kits (Megazyme International, Wicklow, Ireland). Total and resistant starches were calculated as a percentage of the total dry matter. Free sugar content was determined by extracting and analysing monosaccharides and disaccharides, as described by Brummer et al. (2015). Proximate analyses were performed by Central Testing Laboratories Ltd. (Winnipeg, MB, CA). Proximate analyses of the diets were performed as follows, according to AOAC (Association of Official Analytical Chemists) and AOCS (American Oil Chemist Society) methods: ash (AOAC 923.03), crude protein (AOAC 990.03), fat (AOCS Am 5-04), crude fiber (AOCS Ba6a-05), and moisture (AOAC 930.15).
Table 3.1. Primary starch sources in four commercial extruded dog foods categorized into traditional grain, whole grain, grain-free or vegan, selected to be fed in a meal response test to 11 client-owned Siberian huskies.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Category</th>
<th>Primary Starch Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog Chow®</td>
<td>Traditional Grain</td>
<td>Whole grain corn, corn gluten meal, soybean meal, whole grain wheat</td>
</tr>
<tr>
<td>SUMMIT™ Three Meat Adult Recipe</td>
<td>Whole Grain</td>
<td>Oatmeal, whole brown rice, rye, barley</td>
</tr>
<tr>
<td>GO! SENSITIVITY + SHINE™ Limited Ingredient Duck Recipe</td>
<td>Grain-Free</td>
<td>Peas, tapioca, lentils, chickpeas, pea flour</td>
</tr>
<tr>
<td>Vegetarian Dry Dog Formula</td>
<td>Vegan</td>
<td>Brown rice, oatmeal, cracked pearled barley, peas</td>
</tr>
</tbody>
</table>
**Meal Response Test**

Dogs underwent an overnight (14h) fast prior to each postprandial response test. On test days, dogs were weighed, BCS was recorded, and Emla cream (2.5% Lidocaine) was applied to the dogs’ legs. A 20Ga IV catheter (Insyte-W 20GA x 1.1, Becton Dickinson, Franklin Lakes, NJ, USA) was placed into a cephalic or saphenous vein. Once placed, catheters were immediately flushed with 2mL of 0.9 % sodium chloride, followed by 0.1mL of 4.0% sodium citrate, to prevent clotting. Prior to collection, catheters were flushed with 0.5 mL sodium chloride, and 0.5 mL of blood was withdrawn and discarded to avoid any dilution. Three baseline blood samples were taken from each dog prior to consumption of the test meals. Postprandial blood samples were taken at 15, 30, 45, 60, 90, 120,150, 180, 210, 240, 270, 300, 360, 420, and 480 min after the start of the meal. Time was started immediately when the dog started eating the meal or drinking the glucose solution. Each dog consumed all dietary treatments in less than 5 minutes. The volume of blood collected at each time point was 2.5 mL, and was collected into serum separation tubes (3.5mL gold top, Vacutainer™, Becton Dickinson, Franklin Lakes, NJ, USA). After collection, catheters were again flushed with 2mL sodium chloride and 0.1 mL sodium citrate to maintain patency.

**Whole Blood Glucose Analysis**

Collected whole blood was immediately tested for glucose using a handheld blood glucose monitor (AlphaTRAK 2®, Abbot Laboratories, North Chicago, IL, USA), validated for use in dogs (Kang et al. 2016). Each blood sample was tested twice, unless results varied by more than 0.3 mmol/L, in which additional testing was done until two results that were 0.3 mmol/L apart or less were obtained. Glycemic index was calculated as the post-prandial
incremental area under the curve (AUC) ≤ 150 min for glucose of each test diet, divided by the
AUC of the glucose control for the same dog (Wolever et al. 1991). The AUC of each treatment
was calculated as described by Brouns et al. (2005).

**Serum Insulin Analysis**

Following glucose analysis, whole blood was centrifuged at room temperature at 1000 g
for 10 minutes. Serum was aliquoted into two 1.5 mL microcentrifuge tubes, and stored at -20°C
until analysis. Serum insulin analysis was performed in duplicate using a canine-specific
Multiplex assay (Millipore Sigma, Burlington, MA, USA). Assays were performed according to
manufacturer’s instructions and run on a Bio-Plex 2000 system (Biorad, model# Luminex
100/200, S/N: LX10010315403). The quality control samples and standard curves were
evaluated based on manufacturer recommendations. Coefficients of variation (CV) for the results
were assessed for each set of duplicates. If CV per duplicate was < 20%, the results were deemed
acceptable and the mean of the duplicates was used for further analysis. However, if the CV was
≥ 20%, individual results were assessed. If the individual results of the duplicates were not in
agreement, results from that sample were removed.

**Statistical Analysis**

Statistical analyses were performed using the GLIMMIX procedure of SAS software
were tested for normality using Q-Q plots, box plots, and the Kolmogorov-Smirnov test. Data
were log-transformed when necessary. A one-way analysis of variance (ANOVA) model was
used to compare AUC, peak concentration, and time to peak for glucose and insulin, as well as
GI, among dietary treatments. This model was also used to assess the dogs’ body weights over the course of the study. A repeated measures ANOVA model was used to compare glycemic and insulinemic response over time, using dog as the random effect, and treatment and time as the fixed effects. Tukey's post-hoc test was performed for all multiple comparisons. A Pearson correlation was performed to investigate the correlation of total starch content and GI, as well as resistant starch content and GI. A p-value < 0.05 was considered significant. Results are expressed as mean ± SEM.

3.4 Results

*Dietary Treatments and Analyses*

The percentages of total starch, resistant starch, free sugars and available carbohydrates for each diet are as listed in Table 3.2. To provide 25 g of available carbohydrates, 62 g of the traditional grain diet, 77 g of the whole grain diet, 65 g of the grain-free diet, and 66 g of the vegan diet were fed. The grain-free diet had the lowest percentage of total starch, and the highest percentage of resistant starch of the commercial diets analysed. Proximate analyses on a dry matter basis for the diets are listed in Table 3.3. As-fed proximate analyses for each meal response test are listed in Table 3.4. All dogs tolerated the test diets well, no dog refused to eat the diets, and none showed signs of illness or maldigestion. Body weight and BCS were constant for all dogs throughout the study period (p=0.6699).
**Table 3.2.** In vitro starch and free sugar content (as fed basis) of four commercial extruded dog foods and their individual 25g available carbohydrate portion sizes (as fed basis) fed in a meal response test to 11 client-owned Siberian huskies

<table>
<thead>
<tr>
<th></th>
<th>Traditional Grain Diet</th>
<th>Whole Grain Diet</th>
<th>Grain-Free Diet</th>
<th>Vegan Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Starch (% As Fed)</td>
<td>39.26 ± 1.46</td>
<td>38.25 ± 1.78</td>
<td>31.69 ± 0.41</td>
<td>44.62 ± 3.96</td>
</tr>
<tr>
<td>Resistant Starch (% As Fed)</td>
<td>0.39 ± 0.02</td>
<td>0.15 ± 0.01</td>
<td>0.52 ± 0.02</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>Glucose (% As Fed)</td>
<td>0.094 ± 0.0031</td>
<td>0.022 ± 0.0008</td>
<td>0.030 ± 0.0012</td>
<td>0.027 ± 0.0017</td>
</tr>
<tr>
<td>Sucrose (% As Fed)</td>
<td>1.32 ± 0.026</td>
<td>0.52 ± 0.026</td>
<td>1.29 ± 0.021</td>
<td>0.81 ± 0.033</td>
</tr>
<tr>
<td>Av CHO (% As Fed)</td>
<td>40.02</td>
<td>38.53</td>
<td>32.36</td>
<td>45.05</td>
</tr>
<tr>
<td>Portion Size for 25g Av CHO (g diet As Fed)</td>
<td>62</td>
<td>77</td>
<td>65</td>
<td>55</td>
</tr>
</tbody>
</table>

Values equal to means ± SD; Av CHO, available carbohydrate; As Fed, as fed basis
Table 3.3. Proximate analysis of four commercial extruded dog foods containing different starch sources fed in a meal response test to 11 client-owned Siberian huskies (% DMB).

<table>
<thead>
<tr>
<th></th>
<th>Traditional Grain Diet</th>
<th>Whole Grain Diet</th>
<th>Grain-Free Diet</th>
<th>Vegan Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (% As Fed)</td>
<td>5.85</td>
<td>6.32</td>
<td>6.56</td>
<td>7.15</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>7.24</td>
<td>10.10</td>
<td>7.66</td>
<td>5.00</td>
</tr>
<tr>
<td>Crude Fiber (%)</td>
<td>1.05</td>
<td>1.24</td>
<td>3.14</td>
<td>2.10</td>
</tr>
<tr>
<td>Crude Protein (%)</td>
<td>25.55</td>
<td>26.10</td>
<td>28.59</td>
<td>23.46</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>10.25</td>
<td>10.21</td>
<td>11.58</td>
<td>8.20</td>
</tr>
<tr>
<td>GE (kcal/kg)¹</td>
<td>4712</td>
<td>4593</td>
<td>4728</td>
<td>4619</td>
</tr>
<tr>
<td>ME (kcal/kg)²</td>
<td>3959</td>
<td>3841</td>
<td>3816</td>
<td>3846</td>
</tr>
</tbody>
</table>

DMB, dry matter basis; ME, metabolizable energy; GE, gross energy; Values reported on DMB, except for Moisture.

¹GE (kcal/kg) = (5.7 x g protein) + (9.4 x g fat) + [4.1 x (g NFE + g crude fibre)] (National Research Council 2006)

²ME (kcal/kg) = 575 + [0.816 x GE (kcal/kg)] + (12.08 x percentage fat) –(52.76 x percentage crude fiber) –(20.61 x percentage protein) –(6.07 x percentage moisture) (Hall et al. 2013)
Table 3.4. Proximate analysis of four commercial extruded dog foods containing different starch sources fed in a meal response test to 11 client-owned Siberian huskies, expressed as the as-fed quantity in grams that each dog received to provide 25 g available carbohydrate.

<table>
<thead>
<tr>
<th></th>
<th>Traditional Grain Diet</th>
<th>Whole Grain Diet</th>
<th>Grain-Free Diet</th>
<th>Vegan Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash (g)</td>
<td>4.23</td>
<td>7.29</td>
<td>4.65</td>
<td>2.55</td>
</tr>
<tr>
<td>Crude Fiber (g)</td>
<td>0.62</td>
<td>0.89</td>
<td>1.90</td>
<td>1.29</td>
</tr>
<tr>
<td>Crude Protein (g)</td>
<td>14.92</td>
<td>18.83</td>
<td>17.36</td>
<td>14.38</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>5.98</td>
<td>7.37</td>
<td>7.03</td>
<td>5.03</td>
</tr>
<tr>
<td>GE (kcal)</td>
<td>275</td>
<td>331</td>
<td>287</td>
<td>283</td>
</tr>
<tr>
<td>ME (kcal)</td>
<td>231</td>
<td>277</td>
<td>231</td>
<td>235</td>
</tr>
</tbody>
</table>

ME, metabolizable energy; GE, gross energy; Values reported on an As Fed basis,

1GE (kcal/kg) = (5.7 x g protein) + (9.4 x g fat) + [4.1 x (g NFE + g crude fibre)] (National Research Council 2006)

2ME (kcal/kg) = 575 + [0.816 × GE (kcal/kg)] + (12.08 x percentage fat) − (52.76 x percentage crude fiber) − (20.61 x percentage protein) − (6.07 x percentage moisture) (Hall et al. 2013)
The post-prandial glycemic responses to extruded diets and glucose control are presented in Fig. 3.1 and Table 3.5. Overall, there was a significant effect of time on postprandial glycemic response (p < 0.0001), however the effects of treatment and the treatment by interaction were not significant (p > 0.05). The order of GI, from highest to lowest, was observed as: traditional grain diet > vegan diet > whole grain diet > grain-free diet. Although numerical differences were observed, there were no significant differences in GI between dietary treatments (p = 0.1537). However, there were significant differences noted in AUC ≤ 150 min, used to calculate GI, between the glucose solution and the whole grain and grain-free diets. There were no differences noted between the commercial extruded diets themselves. Overall differences in AUC (≤ 480 min), were not different among all dietary treatments (p = 0.9668). Similarly, there were no differences observed in immediate AUC (≤ 30 min). The glucose solution had the largest AUC ≤ 30 min, although not significant compared to any of the extruded commercial diets. The glucose solution and traditional grain diet had quicker times to peak compared to other diets (p = 0.0207). However, no differences were noted between dietary treatments following a Tukey-Kramer post-hoc test (p > 0.05), although a significant overall difference was observed. The glucose solution also presented the highest peak in postprandial blood glucose, although these differences were again not significant (p = 0.5260). In addition, there was no significant correlation between mean total starch content (41.74 ± 1.02) and GI (r=0.313, p=0.1364) or mean resistant starch content (0.3202 ± 0.031) and GI (r=-0.156, p=0.4983) when compared using Pearson correlation.
Figure 3.1. Mean increases in measured concentration of whole blood glucose from baseline (mmol/L) following acute feedings of 25 g available carbohydrates of a glucose control (50% wt/vol) and four commercial extruded dog foods in fasted client-owned Siberian huskies (n=11). Values expressed as mean ± SEM.
Table 3.5. Postprandial glycemic and insulinemic responses to acute feedings of 25 g of available carbohydrates of a glucose solution (50% wt/vol) and four commercial extruded dog foods in fasted client owned Siberian huskies (n=11).

<table>
<thead>
<tr>
<th></th>
<th>Glucose Solution</th>
<th>Traditional Grain Diet</th>
<th>Whole Grain Diet</th>
<th>Grain-Free Diet</th>
<th>Vegan Diet</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Concentration</td>
<td>8.43 ± 0.21</td>
<td>7.90 ± 0.23</td>
<td>7.95 ± 0.31</td>
<td>7.93 ± 0.37</td>
<td>8.05 ± 0.21</td>
<td>0.5260</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to Peak (min)</td>
<td>145.23 ± 17.86</td>
<td>147.27 ± 40.04</td>
<td>226.36 ± 38.29</td>
<td>250.91 ± 31.66</td>
<td>231.82 ± 28.63</td>
<td>0.0207</td>
</tr>
<tr>
<td>AUC ≤ 150 min (mmol/L min)</td>
<td>233.94 ± 21.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>177.23 ± 28.60&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>142.50 ± 20.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>104.72 ± 14.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>164.56 ± 38.94&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.0004</td>
</tr>
<tr>
<td>AUC ≤ 480 min (mmol/L min)</td>
<td>454.40 ± 39.17</td>
<td>496.91 ± 74.89</td>
<td>468.76 ± 65.75</td>
<td>452.01 ± 65.41</td>
<td>499.98 ± 79.70</td>
<td>0.9668</td>
</tr>
<tr>
<td>AUC ≤ 30 min (mmol/L min)</td>
<td>19.81 ± 2.29</td>
<td>10.00 ± 2.89</td>
<td>11.92 ± 2.32</td>
<td>14.46 ± 3.93</td>
<td>13.75 ± 3.85</td>
<td>0.1132</td>
</tr>
<tr>
<td>GI</td>
<td>-</td>
<td>83 ± 17</td>
<td>56 ± 8</td>
<td>41 ± 6</td>
<td>65 ± 15</td>
<td>0.1537</td>
</tr>
<tr>
<td><strong>Insulin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Concentration</td>
<td>796.59 ± 89.26</td>
<td>649.57 ± 87.71</td>
<td>699.28 ± 94.64</td>
<td>600.87 ± 80.88</td>
<td>744.35 ± 104.87</td>
<td>0.2119</td>
</tr>
<tr>
<td>(pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to Peak (min)</td>
<td>57.31 ± 11.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>194.92 ± 52.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.32 ± 23.55&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>120.05 ± 32.44&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>89.83 ± 24.24&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.0052</td>
</tr>
<tr>
<td>AUC ≤ 150 min (pg/mL min)</td>
<td>28095.28 ± 5723.93</td>
<td>12787.63 ± 3505.10</td>
<td>14437.78 ± 3954.53</td>
<td>16694.05 ± 4580.44</td>
<td>20537.93 ± 5627.09</td>
<td>0.0785</td>
</tr>
<tr>
<td>AUC ≤ 480 min (pg/mL min)</td>
<td>47017.08 ± 11393.05</td>
<td>30457.00 ± 9433.07</td>
<td>34974.28 ± 10833.78</td>
<td>52295.34 ± 16194.70</td>
<td>44856.20 ± 13903.21</td>
<td>0.5340</td>
</tr>
<tr>
<td>AUC ≤ 30 min (pg/mL)</td>
<td>5249.97 ± 1128.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1251.20 ± 394.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2842.30 ± 850.82&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1764.55 ± 505.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2141.70 ± 641.84&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM; n=11; Values in a row with superscripts without a common letter differ; p < 0.05, One-way ANOVA with Tukey-Kramer post-hoc test. AUC = area under the curve of postprandial glycemic response curve.
*Insulinemic Response*

There was a significant effect of time \((p = <.0001)\), treatment \((p = 0.0315)\), and treatment and time \((p = 0.0002)\) on postprandial insulinemic response between treatments. The post-prandial insulinemic responses to all dietary treatments, including the glucose solution, are presented in Fig. 3.2 and Table 3.5. There were no significant differences noted between dietary treatments in AUC \(\leq 150\) min or AUC \(\leq 480\) min \((p = 0.0785; p = 0.5340)\). However a trend was observed for AUC \(\leq 150\) where the glucose solution presented the highest AUC and the traditional grain diet presented the lowest. Similarly, for immediate AUC \((\leq 30\) min), the glucose solution was significantly greater than the grain-free diet and the traditional grain diet \((p = 0.0009)\). There were no differences between peak postprandial serum insulin concentration among any of the dietary treatments \((p = 0.2119)\), although the glucose solution did present the highest peak concentration, followed by the vegan diet. The grain-free diet presented the lowest peak concentration of \(600.87 \pm 80.88\) pg/mL. Time to peak was significantly faster for the glucose solution as compared to the traditional grain diet, which had the slowest time to peak of \(194.92 \pm 52.62\) min \((p = 0.0052)\), followed by the grain-free diet.
Figure 3.2. Mean increases in measured concentration of serum insulin from baseline (pg/mL) following acute feedings of 25 g available carbohydrates of a glucose control (50% wt/vol) and four commercial extruded dog foods in fasted client-owned Siberian huskies (n=11). Values expressed as mean ± SEM.
3.5 Discussion

The objectives of this research were to investigate the effects of various starch sources in commercial extruded dog foods on acute glycemic and insulinemic response and subsequent calculation of the GI of each diet. The commercial diets used were selected based on the broad category of starch sources listed on the ingredient panel and were categorized into the following: traditional grains (e.g. wheat, corn); novel whole grains (e.g. barley, oatmeal, rye); non-grain carbohydrates (e.g. peas, lentils, tapioca, potato); and vegan (e.g. no animal sourced ingredients).

In humans, the consistent consumption of low GI foods is believed to reduce the risks of both obesity and diabetes in healthy individuals, by improving overall glucose control, insulin sensitivity, and increasing the feelings of postprandial satiation (Bhupathiraju et al. 2014; Livesey et al. 2008; Lan-Pidhainy and Wolever 2011; Venn and Green 2007). These proposed health benefits have made consumers and pet food companies increasingly interested in low GI foods for companion animals to help prevent weight gain. As a result, pet food companies have started making marketing claims about the GI of their foods. However, these claims are based on the inclusion of ingredients known to be low GI for humans, such as pulses, and not through the use of standardized in vivo testing in dogs. Moreover, grain-free pet foods have remained very popular with pet owners in recent years due to their perceived health benefits, despite a lack of research to support these claims (Packaged Facts 2017). Currently, there has been limited research done to investigate the GI of various single starch sources, and extruded diets made with single starch sources in dogs. However, to our knowledge, there have been no studies investigating the GI of commercial extruded foods, which will contain a variety of starch sources as opposed to a single one.
Despite the varying starch sources listed on the ingredient panel of each diet, the mean percentage of resistant starch remained very low across all four diets, ranging from 0.15% to 0.52% on an as fed basis. This low amount of resistant starch is likely due to the high temperature and pressure used for extrusion processing and that led to the increased gelatinization of starch within the diets. However, the grain-free diet did have the lowest proportion of total starch, and the highest proportion of resistant starch. Research done on pulses has regularly reported higher resistant starch levels within this starch source (Murphy et al. 2008). Therefore, it is possible that these percentages are reflective of the inclusion of pulses within this diet. In comparison, the mean percentage of total starch between the extruded diets ranged from 31.69% to 44.62% on an as fed basis. The vegan diet contained the largest proportion of total starch. This may have been due to the increased proportion of overall plant matter used in this diet, as compared to the others that also contained animal derived ingredients. However, there was no significant correlation between the proportions of resistant starch and total starch in these diets and their mean calculated GI.

Previous work by Nguyen et al. (1998) investigating the glycemic and insulineic responses to various commercial dog foods with varying macronutrient profiles, proposed that the starch content within the diets was the main determinant for postprandial glycemic response in the dogs. In contrast, insulineic response was influenced by the fat and protein content, as well as starch content of the diets. These results suggest that in order to truly assess the effect of starch source in the diet on postprandial responses, the level of macronutrients between diets should be kept consistent. As the GI of these diets was calculated in the dogs using established human GI methodology, only the amount of available carbohydrates remained the same between
treatments. In addition, as this research aimed to investigate commercially available extruded diets, the amount of fat and protein provided by the test meals could not be kept consistent. As a result, the amount of fat and protein that were fed ranged from 5.03 g to 7.37 g, and 14.38 g to 18.83 g, respectively. Similarly, GI testing done in humans has reported that the protein and fat content within the dietary treatments provided may influence the postprandial glycemic response and the overall calculated GI for the food (Jenkins et al. 1981; Wolever et al. 1991). Therefore, it is possible that postprandial glycemic and insulinemic responses observed in these dogs, and the final mean calculated GI of each commercial pet food, was influenced by the fat and protein content of the meal provided. However, this study aimed to utilize in vivo GI methodology in dogs that mimicked human GI methodology, and not solely focus on postprandial responses to the starch sources in extruded pet foods.

Unfortunately, the starch sources within the commercial diets tested by Nguyen et al. (1998) were not reported. However, Carciofi et al. (2008) investigated the postprandial glycemic and insulinemic response of dogs to extruded diets containing one of the six following starch sources: sorghum, lentils, peas, corn, brewer’s rice, or cassava flour. The fat and protein levels were however not kept consistent across all the diets. The diets were meal fed in amounts to sustain maintenance energy requirements in the dogs. The results of this research demonstrated that the diets containing sorghum, lentils and peas, produced delayed and lengthened glycemic and insulinemic responses in the dogs, as compared to those made of rice, corn and cassava flour. The overall AUC was not lower for the sorghum, lentil and pea diet as compared to the other diets. Therefore, our results showing the delayed and lengthened responses in postprandial insulin and glucose in these dogs following the consumption of the grain-free diet containing
pulses is in agreement with previous research. Similarly, our research also did not find a lower overall AUC for the grain-free diet containing pulses as compared to the other commercial diets tested. Additionally, research done by Adolphe et al. (2012) and Briens (2018) investigating GI as well as acute glycemic and insulinemic effects of single starch sources in dogs reported lower GI, and overall delayed and lengthened glycemic responses to pulses (lentils, peas, faba beans) as compared to the other starch sources tested (barley, corn, rice, tapioca and wheat). Adolphe et al. (2012) reported a GI of 29 ± 5 for uncooked peas, while Briens (2018) reported a GI of 49 ± 15, 47 ± 10, and 46 ± 17 for lentils, peas and faba beans, respectively. Both research groups tested GI by feeding 10 g of available carbohydrates to beagles in a laboratory setting. When these research groups later tested extruded diets containing single starch sources (Adolphe et al. 2015; Briens 2018), they also found that the diets containing pulses produced delayed and lengthened responses, and overall had a lower GI compared to the diets containing corn or rice. The macronutrient profiles of these extruded diets were formulated to be identical. The extruded diet containing peas had a reported GI of 56 ± 12 by Adolphe et al. (2015). Similarly, Briens (2018) reported that extruded diets containing either peas, lentils or faba beans had GI values of 55 ± 20, 37 ± 11, and 48 ± 11, respectively. The GI of the grain-free diet tested in our research therefore falls within this range, with a calculated value of 41 ± 6. Overall in the research done by both Adolphe et al. (2015) and Briens (2018), with the exception of lentils, there was an increase in the calculated GI of each starch source when it was formulated into an extruded diet, versus when it was fed on its own. This is likely reflective on the effect of processing. Moreover, these research groups found that collectively, the diets containing pulses, as compared to those containing corn or rice, had lower peak concentrations in postprandial glucose and insulin, and took a longer time to peak. In our research, although the overall AUC for the grain-free diet was
not statistically different, it did have a lower peak in glucose and insulin concentration, as well as a longer time to peak for both of these responses, than the majority of the diets. Additionally, although the GI was not found to be statistically different, the calculated GI of the grain-free diet did show a noticeable numerical difference as compared to the traditional grain diet. The GI of the grain-free diet was calculated as 41 ± 6, categorizing it as a low GI food based on human GI methodology and categorization. In comparison, the higher GI of 83 ± 17 of the traditional grain diet, would classify it as a high GI food. In human GI testing, pulses have been observed to similarly be low GI foods with values of 10 to 54, based on the type of pulse, as well as the storage and processing conditions it has undergone. In comparison, corn typically has a higher GI value of 37 to 69, again based on type and the conditions it has been exposed to. Given the limited research currently available on canine GI, it is unknown at this time what the physiological benefits to differences in GI may be for dogs. However, extruded foods that produce minimal fluctuations in blood glucose and insulin concentrations, and demonstrate overall delayed and lengthened responses, may be beneficial in the dietary maintenance of dogs with diabetes mellitus, or glucose intolerance resulting from obesity (Graham et al. 1994).

Dietary treatments providing 25 g of available carbohydrates were chosen for GI testing in these dogs, based on the 50 g most commonly provided in human GI testing (Jenkins et al. 1981; Brouns et al. 2005), and the 10 g fed to beagles in existing canine GI testing (Adolphe et al. 2012; Adolphe et al. 2015; Briens 2018). When 10 g was initially fed to these Siberian Huskies in a pilot study done by this research group, the expected increase in postprandial glucose concentrations to glucose solution control was not observed; therefore 10 g was deemed an insufficient quantity of available carbohydrate for further GI testing in large breed dogs.
Additionally, Nguyen et al. (1998) suggested that 2 g per metabolic kilogram (2 g/kg BW$^{0.75}$) of available carbohydrates should be fed in canine GI testing. A sample size of 11 was originally deemed sufficient to demonstrate significant differences in GI based on sample size of 10 recommended in human GI testing (Brouns et al. 2005), as well as previous canine GI research done by Adolphe et al. (2012). Test conditions remained consistent between each dog and each dietary treatment; however, given the variability in glycemic response observed in this study, a sample size of 37 dogs would have been necessary to observe a statistically significant difference. Additionally, it has been proposed that breeds similar to the Siberian Husky, such as the Samoyed and the Greenland Sled dog, may have reduced copy numbers of the gene coding for pancreatic amylase, AMY2B, shown to correlate with the activity of serum amylase in dogs (Arendt et al. 2014). However, as noted by the authors, only a small percentage of the differences in serum amylase can be explained by this gene coding. Serum amylase levels are also influenced by diet, circadian rhythm, and age (Piccione et al. 2008), which had not been considered. Additionally, it is unknown whether the dogs were healthy, as the majority of blood samples were received from a Clinical Pathology service, and originally taken for diagnostic purposes (Piccione et al. 2008). Moreover, the Beagle, a common breed used for research, and the only breed used for published GI testing, demonstrated the most variation in its AMY2B copy numbers. As a result, it is difficult to interpret these results, and how they may affect the ability for these dogs to digest and absorb dietary starch and its products of digestion, respectively. Although Siberian Huskies competing in high intensity Iditarod races may have differences in their overall glucose transport activity (Davis et al. 2014), and oxidation of various macronutrients (Miller et al. 2015; Miller et al. 2017), the Siberian Huskies used in this research were not trained or competing to such a caliber. Additionally, these dogs had not been actively
racing or training during this research, or for at least two months prior, however they may still have been more insulin sensitive due to the effect of training (Dela et al. 1992). Overall, any potential differences in the postprandial responses observed as well as calculated GI caused by breed, cannot be speculated at this time. However the Siberian Huskies used in this research are believed to have been more anxious than the cohort of Beagle dogs used in the previous research by both Adolphe et al. (2015) and Briens (2018). As a result, the increased stress in these dogs may have affected their glucose homeostasis and induced hyperglycemia (McCowen et al. 2001).

Overall, although the grain-free diet did produce a more delayed and lengthened postprandial glycemic and insulinemic response as compared to the other diets, there were no significant differences in overall AUC, peak postprandial concentration or time to peak between the diets for either glycemic or insulinemic response. Moreover, there were no significant differences observed in the calculated GI between the extruded commercial diets, although the grain-free diet did have the lowest GI of 41 ± 6 as compared to the traditional grain diet that had the highest GI of 83 ± 17. At this time, the physiological relevance of low GI foods for dogs, and the health benefits they may produce if solely fed, is unknown. These results suggest that different starch sources in commercial extruded diets may not have significant effects on GI or postprandial glycemic and insulinemic response in dogs.
3.6 Acknowledgements

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3.8 References


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CHAPTER 4: POSTPRANDIAL EFFECTS OF FEEDING COMMERCIAL EXTRUDED DOG FOODS WITH VARIOUS STARCH SOURCES ON SATIETY-RELATED GUT HORMONES IN DOGS

4.1 Abstract

Obesity is the most common nutritional disorder affecting dogs and is considered a serious health condition that affects both the quality and quantity of life. The development of a food that could consistently stimulate feelings of satiation could be paramount in combating this epidemic. This research aimed to investigate the effects of commercial extruded dog foods containing different categories of starch sources on the postprandial response of gut hormones related to satiety. The four test diets included a traditional grain diet (corn, wheat), a whole grain diet (oats, rye), a grain-free diet (peas, lentils) and a vegan diet (no animal ingredients). Each diet was acutely fed, as opposed to meal fed, after an overnight fast (14 h) in a randomized crossover design to 11 adult client-owned Siberian Husky dogs. Each commercial diet was tested once in each dog, along with a 50% w/vol glucose solution control, which was tested twice. The amount of digestible carbohydrate was kept consistent between dietary treatments and was fed in amounts to provide 25 g of available carbohydrate. Pre- and post-prandial blood samples were collected at: 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, 420 and 480 minutes. The hormones were analyzed in serum using a canine-specific multiplex assay, and included ghrelin, leptin, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), pancreatic polypeptide (PP), and peptide tyrosine-tyrosine (PYY). An effect of treatment and treatment x time interaction was only observed for GLP-1, specifically for the glucose solution as compared to the extruded commercial diets. There was an effect of time for GIP,
PYY, PP and leptin, with postprandial concentrations decreasing with time. This research suggests that there may be no differences in the effects of satiety produced by these diets. However, at this time very limited research exists on the correlation of these hormones with voluntary food intake in dogs and more research is warranted.

4.2 Introduction

Obesity is the most common nutritional disorder affecting companion animals, with the prevalence of dogs considered overweight or obese estimated to be between 17-44 %, depending on the country and the criteria used to classify body condition (Jericoco and Scheffer 2002; Edney 1974; Robertson 2003; Lund et al. 1999; Vandendriessche et al. 2017; Montoya-Alonso et al. 2017; Mao et al. 2013; Lund et al. 2006; McGreevy et al. 2005). Obesity is defined as the excess accumulation of adipose tissue, to the point of adverse health consequences and low-grade inflammation (German 2006). Veterinarians are concerned with the large population of obese dogs, as obesity affects both the quality of life and overall life expectancy (Kealy et al. 2002; Crane 1991). Moreover, obesity increases the risk of orthopedic diseases, respiratory diseases, cardiac disease, and cancer (German 2006).

In human nutrition, the habitual consumption of low glycemic index foods, such as pulses, has been linked to decreased risk of various diseases, including obesity (Ludwig 2002; Barclay et al. 2008). Improved satiation after consuming low GI foods may contribute to a decreased risk of obesity (Ludwig 2002; Barclay et al. 2008). Satiation can be defined as the feeling of fullness that promotes meal termination, while satiety is the postprandial events that affect the interval to the next meal (Blundell and Halford 1994). Satiety can therefore be
measured by the many hormones that have a role in the regulation of appetite, including ghrelin, leptin, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), pancreatic polypeptide (PP), and peptide tyrosine-tyrosine (PYY) (Austin and Marks 2008). The majority of these hormones, with the exception of ghrelin, are released postprandially to reduce appetite and increase satiety. Ghrelin is the only known circulating orexigenic hormone, and therefore acts to stimulate appetite (Cummings et al. 2001).

The majority of dogs consume commercial dry food (Laflamme et al. 2008; Connolly et al. 2014; Michel et al. 2008; Packaged Facts 2017; Agriculture and Agri-Food Canada 2013; Agriculture and Agri-Food Canada 2016). Amongst these diets, the grain-free category has remained increasingly popular with pet owners (Packaged Facts 2017) who believe these foods to be more healthy, despite a lack of scientific evidence. Grain-free diets frequently contain pulses, such as peas, lentils and chickpeas. Pet diets are often marketed by citing the health benefits of pulses in humans. Currently, there has been no research to investigate postprandial hormones related to satiety after the consumption of commercial extruded food in dogs. Moreover, there has been no research done to investigate the effect of starch sources on these hormones in dogs.

This study aimed to investigate how commercial extruded dog foods with different starch sources may affect postprandial hormone concentrations related to appetite in dogs, while keeping the quantity of available carbohydrates fed consistent between treatments. It is hypothesized that the grain-free diet containing peas and lentils will have the greatest effect on satiety, as determined by these hormones. In addition, it is expected that there will be an effect of
time on the postprandial responses of these hormones, with leptin, GLP-1, GIP, PP and PYY initially increasing from baseline, and decreasing over time. The opposite effect is expected for ghrelin, with an initial decrease from baseline that increases over time.

4.3 Materials & Methods

Animals

The experimental protocol for this research was approved by the University of Guelph Animal Care Committee (AUP#3650), and was in accordance with national and institutional guidelines for the care and use of animals. Eleven adult Siberian Husky dogs were used for this study (n=2, female, intact; n=5, female, spayed; n=4, male, neutered). All dogs were deemed healthy prior to the meal response tests through their medical and dietary history, physical examination, and normal complete blood count (CBC) and serum biochemistry profile. The mean age of the dogs was 5.63 ± 0.72 years (range 1.00 - 10.67 years). The dogs’ body condition score (BCS) ranged from 3 to 6 (on a 9-point-scale (Laflamme 1997)), with a mean body weight (BW) of 23.32 ± 1.15 kg (range 19.00 - 30.68 kg). Exclusion criteria included dogs that were younger than one year of age, had abnormalities in their physical examination or blood work, or if they had received medication six months prior to the start of the study. All dogs were client-owned and remained with their owner throughout the duration of the study. Although dogs were normally group housed, they were separated and individually handled on the days of the meal response tests. All the dogs were transitioned onto the same background diet (GO! FIT + FREE™ Adult Dog Food, Petcurean Pet Nutrition, Chilliwack, BC, CA) at least two months prior to the start of the study. The background diet was fed in amounts to maintain the dog’s
current BW, as determined through their diet history. The dogs stayed on this diet throughout the duration of the study.

**Dietary Treatments and Analyses**

Four commercial dry dog foods that included a variety of starch ingredients were selected (Chapter 3; Table 3.1). The diets chosen included the following: Dog Chow® (Nestlé Purina Petcare®, St. Louis, MO, USA), SUMMIT™ Three Meat Adult Recipe (Petcurean Pet Nutrition Inc., Chilliwack, BC, CA), GO! SENSITIVITY + SHINE™ Limited Ingredient Duck Recipe (Petcurean Pet Nutrition Inc., Chilliwack, BC, CA), and Vegetarian Dry Dog Formula (Dick Van Patten’s Natural Balance Pet Foods®, Burbank, CA, USA). In addition, each dog consumed a 50% (wt/vol) glucose solution as the control. Each dog consumed each diet once, and the glucose solution twice, in a randomized crossover design. The amount of available carbohydrate fed was kept consistent providing 25 g of available carbohydrates. The washout period between meal response tests was seven days for each dog. Total starch, resistant starch, free sugar content, and proximate analysis of each food were determined as previously described in Chapter 3.

**Blood Collection**

Each dog was fasted overnight (14h) prior to the meal response tests. On the mornings of the study, dogs’ BWs were recorded and Emla cream (2.5% Lidocaine) was applied to the catheter insertion site to prevent discomfort. The protocol surrounding catheter insertion and subsequent flushing are as previously described in Chapter 3. Three baseline blood samples were taken for each dog. Each dog consumed each dietary treatment in less than five minutes. Blood was taken postprandially at the following time points: 15, 30, 45, 60, 90, 120, 150, 180, 210,
240, 270, 300, 360, 420, and 480 minutes. The time was started as soon as the dog started consuming the dietary treatment. At each time point, 2.5mL of blood was collected into serum separation tubes (3.5mL gold top, Vacutainer™, Becton Dickinson, Franklin Lakes, NJ, USA). Each tube had DPPIV inhibitor (DPP-IV inhibitor, Millipore Sigma, Billerica, MA, USA), protease inhibitor (Protease Inhibitor Cocktail, Sigma-Aldrich, St. Louis, MO, USA), and serine protease inhibitor (Pefabloc® SC, Sigma-Aldrich, St. Louis, MO, USA). The tubes were kept in the fridge or in a cooler box with ice after the addition of the inhibitors, and before and after collection. After each sample was taken, catheters were flushed with 2 mL sodium chloride and 0.1 mL sodium citrate to maintain patency.

**Analysis of Hormones**

Blood tubes were centrifuged at room temperature for 10 minutes at 1000 g. Serum was aliquoted and separated into two 1.5mL microcentrifuge tubes. The microcentrifuge tubes were stored at -20°C until time of analysis. Hormones were analysed using a canine-specific commercial multiplex kit (Millipore Sigma, Billerica, MA, USA). Analyses were performed in duplicate according to manufacturer’s instructions on a Bio-Plex 2000 system (Biorad, model# Luminex 100/200, S/N: LX10010315403). Duplicates with a coefficient of variation (CV) of 20 % or above were removed and not included in further statistical analysis.

**Statistical Analysis**

Data was analyzed using the GLIMMIX procedure of SAS (SAS® Studio, Version 9.4, SAS Institute Inc., Cary, NC, USA). Residuals were tested for normality using Q-Q plots, box plots, and the Kolmogorov-Smirnov test; and data was subsequently log-transformed. A repeated
measures ANOVA was used to examine the effects of treatment, time and the interaction of treatment x time, on postprandial hormone values, with dog as the random effect, and time and treatment as the fixed effects. Additionally, baseline values were added to the model as a confounding variable. A Tukey-Kramer’s post-hoc test was used for multiple comparisons. A p-value of < 0.05 was considered statistically significant. Results were back-transformed and reported as mean ± SEM.

4.4 Results

Dietary Treatments and Analyses

The quantity of free sugars, as well as total and resistant starch within each commercial diet are listed in Table 3.2 (Chapter 3). Proximate analysis on a dry matter basis for each dietary treatment is listed in Table 3.3 (Chapter 3). In addition, the as-fed proximate analyses for each meal response test diet is listed in Table 3.4 (Chapter 3). All dogs accepted and tolerated the dietary treatments provided. Throughout the study, BW and BCS were kept constant for all dogs.

Postprandial Hormone Concentrations

For ghrelin, there was no significant effect of treatment, time or treatment x time interaction (p > 0.05) (Figure 4.1). For GIP, PYY, PP, and leptin, there was no effect of treatment or treatment x time interaction (p > 0.05). However, there was a significant effect of time for these hormones (p < 0.05). Overall, concentrations decreased over time. Concentrations of GIP were significantly higher at 30 - 150 minutes compared to the concentrations at ≥ 180 minutes (Figure 4.2). Moreover, the concentrations of GIP were significantly lower at ≥ 420 minutes compared to those at 30 - 360 minutes. The concentrations of PYY at 30 - 60 minutes
were significant higher than at 360 - 480 minutes, and concentrations at 90 and 120 minutes were higher than at 420 minutes (Figure 4.3). For PP, concentrations at \( \leq 150 \) minutes were significantly higher than those at \( \geq 240 \) minutes (Figure 4.4). Postprandial leptin concentrations were significantly higher at 15 and 30 minutes when compared to 360 - 480 minutes, and at 46 and 60 minutes when compared to 420 - 480 minutes (Figure 4.5). For GLP-1, there was no effect of time (p > 0.05), however there was a significant effect of treatment and treatment x time interaction for the dogs fed the glucose solution (p < 0.05) (Figure 4.6).
Figure 4.1. Mean change in serum ghrelin concentrations from fasting and through the acute meal response (pg/mL) feedings of 25 g available carbohydrates of a glucose control (50% wt/vol) and four commercial extruded dog foods in fasted client-owned Siberian huskies (n=11). Values expressed as mean ± SEM.
Figure 4.2. Mean change in serum GIP concentrations from fasting and through the acute meal response (pg/mL) feedings of 25 g available carbohydrates of a glucose control (50% wt/vol) and four commercial extruded dog foods in fasted client-owned Siberian huskies (n=11). Values expressed as mean ± SEM.
Figure 4.3. Mean change in serum PYY concentrations from fasting and through the acute meal response (pg/mL) feedings of 25 g available carbohydrates of a glucose control (50% wt/vol) and four commercial extruded dog foods in fasted client-owned Siberian huskies (n=11). Values expressed as mean ± SEM.
Figure 4.4. Mean change in serum PP concentrations from fasting and through the acute meal response (pg/mL) feedings of 25 g available carbohydrates of a glucose control (50% wt/vol) and four commercial extruded dog foods in fasted client-owned Siberian huskies (n=11). Values expressed as mean ± SEM.
Figure 4.5. Mean change in serum leptin concentrations from fasting and through the acute meal response (pg/mL) feedings of 25 g available carbohydrates of a glucose control (50% wt/vol) and four commercial extruded dog foods in fasted client-owned Siberian huskies (n=11). Values expressed as mean ± SEM.
Figure 4.6. Mean change in serum GLP-1 concentrations from fasting and through the acute meal response (pg/mL) feedings of 25 g available carbohydrates of a glucose control (50% wt/vol) and four commercial extruded dog foods in fasted client-owned Siberian huskies (n=11). Values expressed as mean ± SEM. * = p < 0.05.
4.5 Discussion

Overall, the effect of time was significant for GIP, PYY, PP and leptin, with postprandial concentrations decreasing over time as expected. However, there was no effect of time on the postprandial response of ghrelin. Additionally, there was no effect of treatment or treatment x time interaction for the above mentioned hormones, including ghrelin. The only hormone where an effect of treatment and treatment x time interaction was observed on postprandial concentrations was GLP-1. The concentration of GLP-1 for the glucose solution was significantly higher at 30 - 60 minutes as compared to the other dietary treatments and sampling times. This was an unexpected observation as the glucose solution was not predicted to elicit a satiating effect in the dogs. Research by Lubbs et al. (2010) found that postprandial concentrations of GLP-1 in dogs were most affected by carbohydrate and fat, as opposed to protein. It is therefore possible that this initial spike was due to the more digestible nature of the monosaccharides in the glucose solution compared to the more complex carbohydrate sources found in the commercial diets.

To the authors’ knowledge, there is no published research on the effect of starch sources on postprandial hormonal regulators of appetite in dogs. However, similar to our results, Lubbs et al. (2010) found that postprandial ghrelin concentrations were not significantly reduced with time or treatment as expected when investigating the effects of dietary macronutrients (digestible carbohydrates, protein, and fat) on circulating ghrelin in dogs. It has therefore been suggested that ghrelin may be more affected by the volume or overall energy content of the food, as opposed to a specific macronutrient. Similarly, research done in dogs has suggested that the postprandial correlations of PYY may be correlated to energy intake (Lin and Chey 2003). As
the dogs in our study were fed between 55 - 77 g of food, as opposed to a full meal, it is possible that the volume or energy consumed was not enough to elicit a response in ghrelin. As mentioned earlier, GLP-1 in dogs is believed to be more affected by carbohydrate and protein, as opposed to fat. However, similar to our findings, there was no effect of time on GLP-1 (Lubbs et al. 2010). A high fiber diet was not shown to increase postprandial concentrations of GLP-1, PYY or ghrelin in dogs, as compared to a low fiber diet when investigated by Bosch et al. (2009). Additionally, the concentrations of these hormones did not correlate with the voluntary intake of food consumed by these dogs. To the authors’ knowledge, there appears to be no research on dietary modifications and their effects on postprandial PP concentrations in dogs. However, research does suggest that PP concentrations increase after a meal in dogs (Taylor et al. 1979).

It is possible that the quantity of food fed to these dogs was not sufficient to elicit enough of a postprandial response in these hormones. Factors such as sex and BW may have also contributed to the postprandial concentrations observed (Greenman et al. 2004). Namely, leptin concentrations in dogs have been found to positively correlate with their BCS (Ishioka et al. 2002; Ishioka et al. 2007; Mazaki-Tovi et al. 2010; Jeusette et al. 2005). Similarly, ghrelin concentrations appeared to be down regulated in negative association with adipose tissue and fat storage in dogs (Jeusette et al. 2005). Moreover, as these dogs were client-owned dogs, it is possible that the dogs may have associated the people involved in this research with feeding, subsequently altering their hormone concentrations in anticipation of food. A study by Drazen et al. (2006) observed that rats anticipating a meal exhibited significant increases in their preprandial ghrelin concentrations, that peaked 30 min prior to the meal. Rats that were not
anticipating a meal did not demonstrate the same preprandial rise in their ghrelin concentrations. As a result, it has been hypothesized that there may be a learned component related to meal availability and ghrelin. Additionally, our results demonstrated a biphasic effect in the postprandial concentrations of the hormones, suggesting a role of gastric emptying on postprandial response. Very little research has been done to investigate the postprandial concentrations of these hormones and their correlation with satiety and appetite in dogs, and therefore it is possible that data on these hormones cannot be extrapolated from humans and applied to dogs.

Overall, despite differences in the amount of resistant starch and macronutrient content, along with the differences in starch sources between the commercial diets chosen, there did not appear to be an effect of treatment on the satiety-related hormones observed in the present study. At this time, there is very little research concerning these hormones in dogs, and how they may be affected by dietary modification, including differences in macronutrient profile or ingredients. Moreover, very little research has been done to investigate whether a correlation exists in the postprandial concentrations of satiety hormones and feeding behaviors in dogs. Future research should be done to investigate the postprandial concentrations of these satiety-related hormones in dogs, in conjunction with subjective measurements of satiety such as voluntary food intake, and other behavioral markers. Commercial diets that could consistently induce satiety and the feeling of fullness in dogs could be paramount in addressing the epidemic of obesity in companion animals, and assist in maintaining a good quality and quantity of life for our pets.
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4.8 References


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CHAPTER 5: GENERAL DISCUSSION

5.1 Discussion

At present, commercial dry dog foods formulated to be grain-free are popular with consumers and have remained this way for several years (Packaged Facts 2017). Although consumers believe that these foods may provide more health benefits for their dogs, research is currently lacking. Grain-free diets containing pulses, known to be low GI in humans, are being advertised as low GI for dogs and for the associated health benefits that low GI foods may provide in humans. At this time there has been no research investigating the GI of commercial pet foods containing multiple starch sources, or their effects on acute postprandial glucose and insulin concentrations, or satiety. In addition, very limited work has been done to investigate the use of GI methodology in dogs, and how the GI values of single starch sources may compare to the values published for human consumption.

The results of this work have supported the need for further research on the effects of different starch sources in commercial extruded dog foods on glycemic index (GI), glycemic response, insulinemic response, and satiety-related hormones in dogs. In addition, this thesis further investigated the use of GI methodology in dogs through the testing of different single starch sources. The conclusions drawn from this work have been highlighted in the discussion of Chapters 2 – 4.

When first investigating the GI of various single starch sources (cooked green lentils, cooked white rice, and white bread) as compared to a glucose control in Siberian Huskies, this thesis failed to observe differences. In addition, the observed results were inconsistent with those
previously published in humans and beagle dogs (Foster-Powell et al. 2002; Adolphe et al. 2012; Briens 2018). The GI values for both rice and lentils were higher than those previously published in dogs (Adolphe et al. 2012; Briens 2018). These increased values could be a result of the cooking process imposed on these starch sources, resulting in a higher proportion of rapidly digestible starch. However, our results were also higher than those observed of cooked rice and cooked lentils in human GI testing (Foster-Powell et al. 2002). This thesis also observed a low GI for white bread that does not agree with human GI values (Foster-Powell et al. 2002). At this time, it is unclear what may have caused these differences in GI values, especially the low GI observed for the white bread. It is unclear whether these results were a reflection of differences in carbohydrate metabolism in dogs as compared to humans, or a result of the large variability seen in postprandial glycemic responses between dogs to the various starch treatments. However, based on these results, the glycemic response to pulses in dogs requires further investigation. Additionally, based on the unexpected GI of the white bread, a glucose solution may be considered a more reliable control for future canine GI testing. The quantities of 10 g of available carbohydrates fed to the dogs in our research may not have been enough to elicit a significant difference in postprandial blood glucose concentrations, and should have been increased to account for the larger size of the Siberian Huskies as compared to beagles previously used in canine GI testing (Adolphe et al. 2012; Briens 2018). Future studies investigating GI methodology in dogs will need to adjust the amount of available carbohydrates fed based on the size and breed of the dogs used. However, more research may be necessary to determine how to calculate this ideal amount, and if significant differences in postprandial glycemic responses and calculated GI values exist between breeds. These results have advanced
our knowledge on the steps that need to be taken so that canine GI testing can be further improved upon, and before GI testing can become a validated method for use in dogs.

When the effects of various starch sources in commercial extruded dog foods on acute glycemic and insulinemic response and subsequent calculation of the GI were tested in these dogs, the differences were not statistically different. The calculated GI was the lowest for the grain-free diet and highest for the traditional grain diet. The low GI of the grain-free diet may be a reflection of the inclusion of pulses in this diet, as this diet was also found to contain the highest proportion of resistant starch and lowest proportion of total starch, when compared to the other commercial diets tested. However, overall AUC, peak postprandial concentration or time to peak between the diets were not significant for glucose and insulin. Additionally, when these commercial diets were later tested for their effects on postprandial hormones related to satiety, there were no differences observed for treatment or treatment x time interaction between any of the hormones tested. As a result, it does not appear that one single commercial diet caused more of a satiating effect as compared to the others. It is possible that the lack of observed differences in postprandial glucose, insulin, and satiety-related hormones between diets could due to the high temperature and pressure conditions of extrusion. As these diets were fed in acute meal response tests where only the amount of available carbohydrates was kept consistent between treatments, these results may not be reflective of the feeding practices imposed on pet dogs that are consistently meal-fed the same diet. It has been suggested that the postprandial concentrations of several of the hormones, including ghrelin and PYY, may be affected by volume and energy content in dogs (Lubbs et al. 2010; Lin and Chey 2003). As such, the test meals fed to these dogs may not have been large enough to elicit the expected postprandial responses. Additionally, although the effects of starch sources within these diets were of primary interest in this research,
the amount of fat and protein within the diets may have also had an effect on the observed results. To more accurately assess the effects of commercial diets on glucose, insulin and the satiety-related hormones, commercial diets should be tested in a long term feeding trial and fed in isoenergetic amounts to maintain ideal body condition score in dogs. Additionally, there appears to be very little research published investigating the postprandial concentrations of these satiety-related hormones with food intake or feeding behaviour in dogs. Therefore, more canine research is necessary to further explore the postprandial concentrations of these hormones, how they may be affected by various dietary modifications, and their relation to satiety and appetite.

This research was developed to address gaps in the literature regarding the use of GI methodology in dogs, in addition to the effects of starch sources in commercial dog foods on GI, glycemic response, insulinemic response, and satiety in dogs. This thesis found that despite the praised benefits of lentils for humans, the GI of lentils as compared to rice and white bread was not significantly different in the dogs. Additionally, despite the popularity of grain-free dog diets with consumers, this thesis did not observe any differences in postprandial glycemic response, insulinemic response, or any of the satiety-related hormones measured in the dogs, as compared to the other diets tested. Given the large variability noted between the dogs in both glycemic and insulinemic responses, further research in this area should focus on further modifying and improving canine GI testing so that it may become a reliable methodology for use in dogs. Future studies should consider increasing the amount of dogs used in their research. Given the limited amount of knowledge available on canine GI testing at this time, it is unknown what the physiological and health benefits of feeding low GI foods may be for dogs. Additionally, research is warranted to investigate how postprandial concentrations of satiety-related hormones
correlate with the observed feeding behavior and voluntary food intake of dogs, and how dietary modifications such as volume, energy, and macronutrient content may affect these results. By doing said research, the pet food industry will have knowledge that could potentially be used to manufacture dog foods that produce a greater feeling of satiation, and help in combating the obesity epidemic in our pets. At this time, it does not appear the dog foods formulated with pulses to be grain-free can be marketed as being low GI, or as being more beneficial when compared to other diets formulated with more traditional ingredients.
5.2 References


