

EFFECT OF PARTICLE SIZE AND EXTRUSION PROCESSING PARAMETERS ON
IN VITRO STARCH FRACTIONS, IN VIVO STARCH DIGESTIBILITY AND
GLYCEMIC INDEX OF FIELD PEA IN DOGS

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ABSTRACT

A meta-analysis was performed to determine the effect of hydrothermal processing of peas on starch digestibility in monogastric species. From a total of 415 studies on hydrothermal processing of peas, nine studies were identified for inclusion in the meta-analysis. Effect sizes were standardized by converting them to Cohen's d (CD). The combined nine studies showed a significant increase in pea starch digestibility by CD = 6.94 (95% CI; 4.50-9.37; $P < 0.001$) after hydrothermal treatment. A regression of processing temperature on the effect size showed a nearly significant quadratic response ($CD = -0.009(\text{temp})^2 + 2.345(\text{temp}) - 146.103$, $r^2 = 0.303$; $P = 0.096$). This suggests that the rate of pea starch digestion can be manipulated by controlling processing temperature. The hypothesis of this research was that processing parameters, namely particle size and extrusion, would alter pea starch *in vitro* degradability, and *in vivo* digestibility and glycemic response in laboratory beagles. A preliminary experiment found that, although not significant ($P = 0.07$), pea starch had a lower total tract apparent digestibility coefficient (TTADC) than rice starch (81% vs. 100% respectively) ($n = 6$). A second experiment found no significant effect of pea particle sizes 195, 309, and 427 μm on glycemic index (GI) in laboratory beagles ($n = 6$). A third experiment was performed to determine the effect of extrusion processing on pea starch. The experiment used a completely randomized 2 x 2 x 2 x 2 factorial design with 2 levels of temperature (110 vs. 150°C), moisture (20 vs. 28%), particle size (288 vs. 407 μm) and cooling method (freezing vs. drying). Extrudates were analyzed for their rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) contents. Particle size was the only significant effect; large particle size increased RS and decreased RDS ($P < 0.05$).

There was also significant negative correlation between particle size and RDS and SDS fractions ($P < 0.05$) and a trend toward particle size being positively correlated with RS content ($P = 0.059$). Subsequently, four of the 16 extruded treatments were selected for the measurement of GI in beagles ($n = 6$): 3) 150°C, 288 μm , 20% H₂O, dried; 7) 110°C, 288 μm , 20% H₂O, dried; 10) 150°C, 407 μm , 28% H₂O, frozen; 14) 110°C, 407 μm , 28% H₂O, frozen. There was no relationship between GI and particle size, but GI was negatively and RDS was positively correlated with temperature ($P < 0.05$). These results suggest that *in vitro* starch fractions are not good predictors of GI in dogs. However, the rate of pea starch digestion may be manipulated by controlling processing temperature. Further studies are needed to determine the effect of multiple temperatures on the GI of starch.

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ABBREVIATIONS

ADC	Apparent digestibility coefficient
AEE	Acid ether extract
AUC	Area under curve
cAMP	Cyclic adenosine monophosphate
CD	Cohen's d
CI	Confidence interval
CP	Crude protein
DM	Dry matter
FG	Free glucose
GE	Gross energy
GI	Glycemic index
GLM	General linear model
GLP-1	Glucagon like peptide-1
GLP-2	Glucagon like peptide-2
GLUT-2	Glucose transporter-2
GLUT-5	Glucose transporter-5
HbA1	Glycosylated hemoglobin
HDL	High density lipoprotein
IAUC	Incremental area under curve
LDL	Low density lipoprotein
PAI-1	Plasminogen activator inhibitor 1
RDS	Rapidly digestible starch

RS	Resistant starch
SCFA	Short chain fatty acid
SDS	Slowly digestible starch
SEM	Standard error of the mean
SGLT-1	Sodium glucose co-transporter-1
T1R2	Taste receptor type 1 member 2
T1R3	Taste receptor type 1 member 3
TAUC	Total area under curve
TS	Total starch
TTADC	Total tract apparent digestibility coefficient

1.0 INTRODUCTION

Nutrition plays a fundamental role in the health and wellbeing of both humans and animals. In America, there has been a general increase in consumption of energy dense, nutrient-poor convenience foods. Due to the increased consumption of nutrient poor diets combined with a general decrease in physical activity, obesity has become a North American plague (Lieberman, 2003; WHO, 2009). Obesity has not only prevailed in the human population but it is now the most commonly occurring nutritional problem associated with companion animals (German, 2006). Research has demonstrated links between the state of obesity and chronic disease risk factors for diseases such as: type II diabetes (Kahn and Flier, 2000; Gayet et al., 2004; Khaodhiar et al., 2009; WHO, 2009), coronary heart disease (Mayer-Davis et al., 2001; Flight and Clifton, 2006), cardiovascular disease (O'Keefe et al., 2008), hypertension (Lee et al., 2008), stroke (Flight and Clifton, 2006), and musculoskeletal problems (Laflamme, 2006), as well as some forms of cancer (Giovannucci, 2001).

The misbalance between energy intake and energy output is the most prevalent cause of obesity, thus diet therapy to combat obesity is of much interest and is by no means a new proposal (Kerl and Johnson et al., 2004). Carbohydrates are the most common source of dietary energy in most American diets. One mechanism linking diet to disease is the rate of glucose absorption from the diet (Wolever et al., 1991; Jenkins and Kendall, 2000; Wong and Jenkins, 2007). Research has shown that foods eliciting high glucose and insulin responses post-prandially may lead to increased hunger, promoting over eating and weight gain (Bornet, 1993; Jenkins et al., 2002; Appleton et al., 2004; Wong and Jenkins et al., 2007). Furthermore, it has been shown that the use of

carbohydrate sources producing slower absorption rates will result in lower glucose and insulin responses, promoting satiety (Wolever et al., 1991; Englyst et al., 1999; Costacou and Mayer-Davis, 2003).

Pulses characteristically contain high levels of resistant and slowly digestible starch, which are digested and absorbed at a slower rate than conventional cereal grains, producing lower glucose and insulin responses and leading to prolonged satiation. The use of peas as a carbohydrate source will increase satiety, improve weight control and decrease disease risk factors by having a naturally occurring lower glycemic response than conventional carbohydrates. Not only can pulses act as an alternate carbohydrate, they can also be used as a source of protein and fibre. Peas are an important source of protein and energy in human and animal diets around the world. However, there is increasing awareness that peas have properties that go beyond the provision of nutrients.

Both intrinsic and extrinsic factors play a role in carbohydrate digestion and can greatly influence subjects' glycemic responses (O'Dea et al., 1980; Costacou and Mayer-Davis, 2003). It is generally accepted that application of extrinsic processing on carbohydrate sources increases palatability, digestibility and glycemic response (Cheftel, 1986; Bengala-Freire, et al., 1991; Lankhorst et al., 2007; Copeland et al., 2009). However, little is known about optimal processing techniques to minimize the glucose response. The following meta-analysis and experiments were performed to determine the effect of extrusion processing of peas on starch digestibility in monogastric animals and glycemic index in dogs.

2.0 LITERATURE REVIEW

2.1 Carbohydrate metabolism

2.1.1 Digestion and absorption

Carbohydrates typically provide the majority of energy in both human and canine diets. Canadian Diabetes Association Clinical Practice Guidelines state that to reduce risk for chronic disease carbohydrate intake should be no less than 45% of energy intake and can become greater than 60% of total energy intake if from a low glycemic high fibre diet (Barnard et al., 2006; Otten et al., 2006; Gougeon et al., 2008). Carbohydrates are a vital macronutrient and regulation of their metabolism is an important component of disease control (O'Dea et al., 1981; Englyst et al., 1996b and 1999). Carbohydrate digestion and absorption is an intricate process of mechanical, enzymatic and chemical processes. Typically, carbohydrates are digested into monosaccharides and disaccharides via amylases in the small intestine in the canine, as canines lack salivary amylase (Best et al., 1959; Bach Knudsen et al., 2000; Reece, 2004; NRC, 2006). Pancreatic α -amylases begin carbohydrate catabolism in the small intestine cleaving glycosidic linkages of starch, forming α -limit dextrins and oligosaccharides (Gray, 1992; Bach Knudsen et al., 2000; Zhang and Hamaker, 2009). End products of α -amylase digestion are transported to the brush border of the small intestine where disaccharide catabolism takes place through the use of α -glucosidases, maltase-glucoamylase and sucrase-isomaltase (Gray, 1992; Bach Knudsen 2000). These enzymes cleave α -1,4 and α -1,6 glycosidic linkages to form monosaccharides, i.e. glucose, galactose and fructose (Gray, 1992; Zhang and Hamaker, 2009).

2.1.2 Glucose absorption and homeostasis

Monosaccharides, the breakdown products of carbohydrate digestion, are subsequently absorbed into intestinal epithelium post digestion. Monosaccharides, glucose and galactose, are absorbed in the small intestine through transport-mediated Na^+/K^+ ATP dependant transporters (Bach Knudsen et al., 2000). Fructose is transported via facilitative transport mechanisms (Bach Knudsen et al., 2000; Ferraris, 2001; Pencek et al., 2002). Sodium glucose co-transporter-1 (SGLT-1) is an active transport mechanism located on the brush border of the intestinal lumen and transports both glucose and galactose with Na^+ from the lumen into the intestinal cystol (Ferraris, 2001). Glucose transporter-5 (GLUT-5), a facilitative transport mechanism, carries fructose from the intestinal lumen into the cystol. Once all three monosaccharides enter the intestinal cystol, glucose transporter-2 (GLUT-2), a basolateral transporter, carries the end products of carbohydrate metabolism into the blood stream (Ferraris, 2001). A study by Pencek et al. (2002) has shown that passive transport of glucose into the enterocyte plays a minor role in post-prandial glucose absorption, agreeing with previous research findings (Uhing and Kimura, 1995). Once absorbed in the small intestine, sugars are directly transported to the liver via portal blood. In the liver, monosaccharides are made available for energy formation through glycolysis, the citric acid cycle and oxidative phosphorylation to form ATP.

Glucose homeostasis is a tightly regulated mechanism with pancreatic glucagon and insulin playing major roles. Once glucose is detected in the blood, the pancreas responds by activating specialized β -cells to secrete insulin (Best et al., 1959; Reece, 2004). Insulin is a complex peptide hormone with anabolic activity. Insulin works with muscle and fat tissue increasing the uptake of glucose from the blood. Insulin also

activates glucose storage, glycogenesis and protein accretion (Best et al., 1959; Reece, 2004). Hypoglycemia causes a reverse cascade of events, activating α -cells in the pancreas to secrete the hormone glucagon. Glucagon, along with growth hormone, cortisol and catecholamines, help to decrease uptake of blood glucose into tissues and mobilize glucose stores by glycogenolysis and start the synthesis of glucose via gluconeogenesis (Best et al., 1959; Reece, 2004). The control of blood glucose in healthy individuals is tightly regulated via the pancreas and intestinal absorption. The physiological blood glucose response to a food of both humans and animals directly and indirectly affects many other physiological mechanisms.

2.2 Measuring starch degradation

2.2.1 *In vitro*

Starch is the primary source of energy in canine diets and its degradation rate directly influences glucose release and absorption. Predicting starch degradation rates is important in order for nutritionists to formulate satisfactory diets in terms of energy balance as well as achieving and maintaining satiation. Several methods for determining starch degradation rates exist. An *in vitro* method, known as the Englyst Method (Englyst et al., 1992), quantitatively measures the glucose released from a test feed after digestion with amyloglucosidase and pancreatin invertase, mimicking *in vivo* digestion (Englyst et al., 1992). With this assay starch is divided into three types: rapidly digestible starch (RDS), the glucose released after 20 minutes of enzymatic digestion; slowly digestible starch (SDS), the glucose released after 100 minutes of enzymatic digestion; and resistant starch (RS) that portion of starch which remains after the total 120 minute digestion (Englyst et al., 1992). Rapidly digestible starch, as the name suggests, is that

fraction of starch that is readily digested by pancreatic amylase and other digestive enzymes to absorbable monosaccharides as well as further digestible oligosaccharides malto-dextrins and maltose. Indicative of the name, SDS, is that component of starch which is digested slowly, releasing a slow, constant cascade of glucose post-prandially into the bloodstream.

Resistant starch is the fraction of starch not absorbed in the small intestine and which enters the large intestine intact (Sun et al., 2006). Resistant starch can be further broken down into four categories, RS₁, RS₂, RS₃ and RS₄ (Sun et al., 2006; Cummings and Stephen, 2007). RS₁ refers to that part of the starch fraction which is physically inaccessible and is trapped within whole grains (Sun et al., 2006; Cummings and Stephen, 2007). RS₂ is that fraction that has naturally occurring resistant starch granules (Type B; tuber starch) (Englyst et al., 1992). RS₃ is the resistant starch fraction formed by retrogradation, a process post food processing where crystalline regions reform in the starch making it inaccessible to digestive enzymes (Sun et al., 2006; Cummings and Stephen, 2007). The fourth type of resistant starch (RS₄) is a starch made resistant to digestion through chemical modification (Sun et al., 2006). The incorporation of RS in diets is known to decrease post-prandial glucose levels, due to the lack of digestion and absorption in the small intestine (Costacou and Mayer-Davis, 2003). The consumption of diets high in resistant starch has been shown to help alleviate many of the health problems associated with obesity (Flight and Clifton, 2006).

2.2.2 Slaughter technique

Although *in vitro* assay methods are inexpensive and quick, they do not always parallel what is occurring physiologically. One *in vivo* method to determine starch

disappearance in the gut is to euthanize test animals at various time points post-prandially and calculate starch disappearance from different intestinal sections. Typical protocol suggests animals are to be euthanized at times points of 0, 0.25, 0.75, 1, 2, 3 or 4 hours post-prandially (Weurding et al., 2001; Weurding et al., 2003; Morales et al., 2002; Bach Knudsen et al., 2006). Digesta from euthanized animals is sampled from varying parts along the gastrointestinal tract, characteristically the posterior jejunum, anterior ileum, posterior ileum and excreta (Weurding et al., 2001; Weurding et al., 2003; Bach Knudsen et al., 2006). A major advantage of the slaughter technique is that prior to euthanasia, there are no invasive surgeries that may alter the gastrointestinal digestive or absorptive capacity (Bach Knudsen et al., 2006). However, this method is expensive and time consuming and precludes performing multiple experiments on one animal. Thus, other techniques have been instituted in order to eliminate these hurdles.

2.2.3 Glycemic index

Glycemic index (GI) is the third means of classifying the rate of absorption of glycemic carbohydrates. GI is measured by quantifying the post-prandial release of glucose into the blood and is defined as:

“The incremental area under the glucose response curve of a 50 g carbohydrate portion of a test food expressed as a percentage of the response to the same amount of carbohydrate from a standard food taken by the same subject” (FAO, 1998).

In other words, the GI is a quantitative property of feedstuffs, which is related to the rate of carbohydrate digestion in the small intestine (Wolever et al., 1998). Dr. David Jenkins and colleagues developed GI methodology in order to formulate low glycemic diets for diabetic patients, helping control blood glucose and insulin surges. Since dietary

carbohydrates play such an important role in human and animal energetics, their classification and modification have much been the focus of recent nutrition research. Carbohydrates were previously classified only on their chemical structure, ignoring the physiological effects of this structure. The use of GI has aided in determining the physiological responses to carbohydrate structure and the positive and negative implications of the physiochemical properties of carbohydrates.

2.2.3.1 Generalized protocol

There are many variables which affect the GI of a feedstuff including, but not limited to: portion size, reference food, frequency and length of blood sampling, blood sample location, time of day, and the calculation of the area under the glucose curve (Wolever et al., 1991; Wolever et al., 2003). Publications by Wolever et al. (1991, 2003) summarize the standard GI testing technique. To determine the GI of a specific food FAO protocol suggests the use of six subjects and using the resulting average GI (FAO, 2008). For the GI obtained to be a representative indication of the physicochemical property of a food, The FAO recommends that the standard food should be repeated three times in each subject and the test food repeated twice (FAO 1998). FAO also recommends minimizing daily variation by randomly assigning test feed and reference feed to subjects.

The reference food used in GI testing is typically 100% available glucose, usually given in the form of a glucose drink, diabetic screening product or white bread. The portion size of reference and test food consumed to determine GI is important as the blood glucose response differs depending on the amount of carbohydrate consumed (Wolever et al., 1991, 2003; Aziz, 2009). In standardized human GI testing, a 50 g

portion of available carbohydrate is used. Available carbohydrate, as defined by the FAO, is the total carbohydrate minus dietary fibre, and is that portion of carbohydrate which is fully available for absorption in the small intestine (Wolever et al., 1991, 2003; FAO, 1998). To decrease day-to-day variation, GI testing is always performed in the morning after an overnight fast of 12-16 hours (Wolever et al., 1991). Baseline, fasting blood glucose levels are taken immediately before subjects are given their test meals. Subjects are allotted a strict 10 - 15 minute time period in which they must consume the test meal or reference meal to minimize subject and repeat differences in digestion and absorption.

Standard blood glucose is typically measured post-prandially in capillary whole blood for two reasons. First obtaining capillary blood is much easier and less invasive than venous blood collection, and second, capillary whole blood allows for higher rises in blood glucose to be detected with less variability (Wolever et al., 1991; Aziz, 2009). In healthy individuals, blood glucose is measured over two hours in 15-minute intervals (Wolever et al., 1991, 2003). Diabetic subjects have characteristic impaired glucose tolerance, and thus take longer to clear glucose from their blood and requiring blood glucose to be measured for a third hour in 30 minute increments (Wolever et al., 1991, 2003; FAO, 1998). Blood glucose is plotted as glucose concentration vs. time and the incremental area under the curve (IAUC) is measured to calculate the *change* in blood glucose concentration.

2.2.3.2 Blood glucose curve

Blood glucose and blood insulin are commonly expressed as the area under the curve (AUC) (Wolever et al., 2003). Blood glucose is measured as mmol of glucose per

litre of blood over time. The first step in calculating the GI of a feedstuff is on the incremental area under the glucose curve for a foodstuff and standard food. The glucose AUC can be expressed as Total AUC (TAUC) or Incremental AUC (IAUC) (Wolever et al., 2003). Total area under the curve measures the average blood glucose concentration during the test period, whereas IAUC measures the change in blood glucose from the fasting concentration (Jenkins et al., 1981; FAO, 1998; Wolever et al., 2003). For GI, IAUC is almost always used and only utilizes the area above the fasting level, ensuring that AUC can never be less than zero (FAO, 1998; Wolever et al., 2003). The IAUC above blood glucose fasting levels is calculated using the simple trapezoid rule applied to all blood glucose time increments (FAO, 1998; Wolever et al., 2003).

2.2.3.3 Mechanism of action

It is hypothesized that the GI ranking of food relates to the rate of glucose absorption from the small intestine (O'Dea et al., 1981; Jenkins et al., 1981, 1982a; Mourot et al., 1988; Bornet, 1993; Wolever et al., 2003; Wong and Jenkins, 2007). Both intrinsic and extrinsic properties of a food source will dramatically affect the rate of digestion and absorption. Highly digestible carbohydrates increase gastric emptying rate, increasing potential glucose production in the small intestine and thereby causing a rapid rise in blood glucose concentration, producing a high GI (Bornet, 1993). A study by Mourot et al. (1988) showed a significant negative correlation between gastric emptying and blood glucose variation ($P < 0.0001$) in the starch sources potatoe, bread, rice and spaghetti. Slower digestion and absorption of a carbohydrate source results in a slower rise and fall in blood glucose concentration, producing a lower GI. With a lowered post-prandial rise in glucose, subsequent rises in gut hormones and insulin are decreased

(Jenkins et al., 2002). Low GI foods have prolonged absorption rates and can suppress free fatty acid and other counter regulatory processes of high blood glucose levels (Jenkins et al., 2002).

2.3 Intrinsic factors influencing carbohydrate digestion and absorption

2.3.1 Carbohydrate source

Native carbohydrate sources can be classified into three major groups, namely cereals, tubers and legumes. Carbohydrate sources are metabolized and function differently in the gastrointestinal tract according to intrinsic factors such as molecular structure (Crapo et al., 1980; Jenkins et al., 1981; Brand et al., 1985; Biliaderis 1991; Englyst et al., 1999; Tran et al., 2008). Carbohydrates are typically classified on the basis of size such as the degree of polymerization (dp) or the type of linkages, e.g. α or β (Cummings and Stephen, 2007). Characteristically, carbohydrates are organized into three groups: sugars (1-2 dp) consisting of monosaccharides, disaccharides and sugar alcohols including glucose, fructose, galactose, sucrose, lactose, maltose, trehalose, sorbitol and mannitol; oligosaccharides (3-9 dp) including malto-oligosaccharides, raffinose, stachyose; and polysaccharides (> 10 dp) including starch and non-starch polysaccharides such as amylose, amylopectin, cellulose, hemicellulose, pectin, arabinoxylans and β -glucans (Cummings and Stephen et al., 2007). Studies have demonstrated that various carbohydrate sources elicit markedly different post-prandial glucose responses due to carbohydrate composition (Crapo et al., 1980; Appleton et al., 2004; Thomas et al., 2007). Highly digestible cereal carbohydrates, such as rice and white bread, result in higher post-prandial glucose responses than do sources such as pulses (Crapo et al., 1980; Wolever et al., 1991; Appleton et al., 2004). Carbohydrate

sources such as pulses have been shown to have low GI and decreased insulin surges (Lieberman 2003; Thomas et al., 2007). Thus, these foods are desirable in the control of the glycemic response after a meal.

2.3.2 Starch composition

Starch, a non-structural polysaccharide, is the main storage carbohydrate in plants (40-90% of dry matter) and is thus the main carbohydrate in food and feed sources (Biliaderis, 1991; Bornet, 1993; Annison and Topping 1994; Åkerberg et al., 1998). The composition and structure of the starch in a carbohydrate source, being a large storage of glucose, has the greatest influence on digestion, absorption and GI (Rosin et al., 2002). The basic components of starch are found in two polymers of glucose, arranged in a semi-water-insoluble granule (Lindeboom et al., 2004; Bach Knudsen et al., 2006; Copeland et al., 2009). Amylose, a linear D-glucose polymer, is made of unbranched α -1, 4 linkages (Bornet, 1993; Lindeboom et al., 2004; Bach Knudsen et al., 2006; Copeland et al., 2009). Amylopectin consists of an α -1,4 chain but is highly branched with α -1,6 links (Åkerberg et al., 1998; Bach Knudsen et al., 2006; Copeland et al., 2009). Amylose has a molecular weight of approximately 10^5 - 10^6 , whereas amylopectin has a molecular weight of 10^8 due to its larger size (Copeland et al., 2009). Most starches contain, on average, a higher percentage of amylopectin, between 60 and 90%, compared to amylose (Copeland et al., 2009). Native starch molecules containing high levels of amylopectin are highly digestible due to increased access of digestive enzymes to multiple reducing ends (Copeland et al., 2009). Amylose, due to its lack of branching tends to form insoluble semi-crystalline aggregates during processing (Copeland et al.,

2009). Amylose and amylopectin are deposited in the endosperm and other plant reserve organs in the form of starch granules, which are classified based on structural arrangement (Biliaderis, 1991).

2.3.3 Starch granules

Starch granules are made up of 98-99% amylose and amylopectin on a dry matter basis (Copeland et al., 2009). Amylose and amylopectin form patterns visible through X-ray diffraction and electron microscopy (Englyst et al., 1992; Sun et al., 2006; Copeland et al., 2009). These granules range in size with an average diameter estimated between 1 and 100µm (Lindeboom et al., 2004; Copeland et al., 2009). Granules also come in an array of shapes, primarily due to arrangement of amylose and amylopectin in the crystalline regions (Lindeboom et al., 2004; Copeland et al., 2009). Tubers typically have an oval granule structure, whereas peas and beans characteristically have granules shaped like thick discs with an indentation present at one end or in the middle (Lindeboom et al., 2004).

Crystalline regions are distinguished as either A-, B- or C- type and are characteristic of different starch sources (Englyst, 1992; Sun et al., 2006). Amylopectin chains greater than 10 glucose units long are typically formed into double helices (Copeland et al., 2009). These double helices are then arranged into type A or B forms (Copeland et al., 2009). Cereal starch granules are most commonly of Type A crystalline structure and are known to be highly compact and more rapidly digestible than Type B (Englyst et al., 1992; Copeland et al., 2009). Type B starch granules are commonly found in high amylose plants, such as raw tubers, and the crystalline regions form dense hexagonal patterns known to have an open structure with a hydrated core

(Copeland et al., 2009). Both Type A and B crystalline structures are quite similar, as they contain a double helical structure (Copeland et al., 2009). Finally, Type C granules, which occur in leguminous plants, are a combination of both Type A and Type B and are generally more resistant to digestion (Englyst et al, 1992; Copeland et al., 2009).

2.3.4 Resistant starch

Resistant starch (RS) and soluble fibre behave in similar fashion in that they resist digestion in the upper intestinal tract but are fermented in the colon, producing short chain fatty acids which can provide energy as well as other physiological benefits (Bornet, 1993). Studies have shown that high RS diet not only slow gastric emptying and decrease glycemic responses, but may also affect the regulation of gut hormones (Bornet, 1993). Fermentation by colonic bacteria produces short chain fatty acids (SCFA), which may have significant effects on lipidic and glucidic metabolism (Bornet, 1993; Topping and Clifton, 2001). Wolever et al. (1989) have previously shown that addition of SCFA has the ability to decrease peripheral fatty acids which are known to alter the ability to utilize insulin and glucose. SCFA, such as butyrate is beneficial for colonocyte health (Annison and Topping, 1994). Delayed gastric emptying due to RS and SCFA products may induce a lower GI effect of the meal consumed (Liljeberg and Björck 1996; Robertson et al., 2005). Massimino et al. (1998) and Cuche et al. (2000) were able to show that SCFA have the ability to increase the incretin hormones glucagon like peptide-1 (GLP-1) and polypeptide YY. These hormones are known to reduce gastric motility, delaying transit time and the prolonging glycemic response.

2.3.5 Fibre

Definitions of dietary fibre have evolved dramatically over the years from the skeletal remnants of plant cell walls to indigestible polysaccharides and lignin that are not digested by endogenous enzymes in the intestinal tract of man (DeVries et al., 1999). Characteristically, fibre includes cellulose, hemicellulose, oligosaccharides, pectins, gums, waxes and lignin (Asp et al., 1993; Asp, 1995; Tosh and Yada, 2010). Dietary fibre can further be classified as insoluble or soluble. Insoluble fibre typically contains cellulose, hemicellulose and lignin and is known to improve laxation, and supports the growth of intestinal microflora (Tosh and Yada, 2010). Soluble fibre includes oligosaccharides, pectins, β -glucans and gums (Tosh and Yada, 2010).

Some research has related lower glycemic responses seen in some starchy foods to an increase in the fibre content of the food source (Crapo et al., 1980; Jenkins et al., 1982a). Dietary fibre was first thought to act as a barrier for nutrient diffusion, resulting in slower absorption of nutrients (Jenkins et al., 1982a). This slower absorption of nutrients was thought to then result in a prolonged glucose response (Jenkins et al., 1982a). In order for fibre to reduce the digestibility of starch, it must have highly viscous properties and be distributed evenly throughout a feedstuff (Bornet, 1993).

2.3.6 Other

Lipids, proteins and antinutritional factors all have been shown to impact either the rate or the efficiency of carbohydrate metabolism. It has been hypothesized that these factors slow gastric emptying or digestion, delaying starch degradation and absorption and reducing GI (Thompson et al., 1984). Lipids are naturally occurring in plant sources and form complexes with amylose (Lin et al., 1997). These complexes increase the

hydrophobicity of starch granules, reducing water and enzyme access to the starch granule and inhibiting digestion, absorption, and in turn, glycemic response. Proteins also form complexes with starch and may reduce digestion rates and GI (Jenkins et al., 1987a). Antinutritional factors such as lectins, tannins and phytic acid have been associated with inhibition of enzymatic degradation in the small intestine, ultimately slowing down glucose absorption (Thompson et al., 1984; Jenkins et al., 1987a). Studies have shown a correlation between lowered glycemic response and the presence of phytic acid, an antinutritional factor, which is explained by a decrease in the rate of digestion (Yoon et al., 1983).

2.4 Extrinsic factors influencing carbohydrate digestion and absorption

2.4.1 Feed processing

Extrinsic mechanisms applied to starch sources may also have an effect on the rate of luminal digestion and absorption, in turn affecting glycemic and insulinemic indices (Jenkins et al., 1982b; Brand et al., 1985; Bornet, 1993; Rosin et al., 2002). Feed processing takes many forms, including but not limited to cracking, grinding, rolling, flaking, pelleting, steaming, expanding and extruding. Increasing susceptibility to enzymatic break down along with degradability will increase glucose release from a food or feed source and thus will increase the corresponding GI (Bornet, 1993). Processing causes a disorganized state of the starch granule and thus increases α -amylase susceptibility and bioavailability (Bornet, 1993). Some types of heat denaturation can reduce the water absorption rate (Choi and Han, 2002). Grinding feedstuffs is a common practice prior to diet formulation, as whole grains are often not included due to poor palatability. As particle size decreases, surface area and pore volume increase, allowing

water retention and absorption and increasing the rate of uptake, essentially increasing palatability (Auffret et al., 1994; Tosh and Yada, 2010). Increasing absorptive capacity increases digestive susceptibility, as do further processing mechanisms (e.g. extrusion and gelatinization).

2.4.2 Hydrothermal processing

2.4.2.1 Starch gelatinization

Hydrothermal processing involves exposing feedstuffs to high temperature, moisture and mechanical shear for a short period of time. These processes are used to cook starches to form highly digestible and palatable products (Cheftel, 1986; Lankhorst et al., 2007). The process of extrusion alters the physiochemical properties of foodstuffs, altering the nutritional value and bioavailability of proteins, carbohydrates, lipids and vitamins for metabolism (Brand et al., 1985; Cheftel, 1986; Lankhorst et al., 2007; Tran et al., 2008). Extrusion is widely used in the preparation of human food and animal feeds and is the primary processing technique used in pet food manufacturing.

The main effect of extrusion on starch is coined gelatinization. Starch gelatinization is a process where intermolecular bonds are broken down in the presence of water and heat. As temperature increases, the disruption of hydrogen bonds occurs, allowing water to be absorbed by the granules, which is termed starch swelling (Bornet, 1993, Tran et al., 2008). This structural change allows hydrogen-bonding sites, typically bonded to oxygen and hydroxyl hydrogen, to bind extra water. The second phase, amylose leaching, occurs as starch increases in solubility and a gradual increase in viscosity is observed (Bornet, 1993). The final step, gelatinization, causes a paste to form, and is characteristic of increasing randomness of a molecule and decreased size and

number of crystalline regions (Tran et al., 2008). A decrease in granular structure improves digestibility by increasing access of digestive α -amylases, resulting in higher caloric density (Cheftel, 1986). Gelatinization is a function of the gelatinization temperature, which is intrinsic to the starch source and ranges from 65°C to above 100°C (Bornet, 1993).

The ability of a starch granule to become gelatinized is characteristic of the size, distribution and structure of the native crystalline regions and the ability for the starch to become hydrated (Lindeboom et al., 2004). In general, it is proposed that the smaller the starch granule, the lower the gelatinization temperature (Lindeboom et al., 2004). The Type B starch structure found in tubers has a lower gelatinization temperature caused by a lower degree of organization and stability than seen in Type A starches typical of cereals (Lindeboom et al., 2004). The increased number of amorphous regions in Type B starch granules allows for increased hydration (Lindeboom et al., 2004). The amylose-lipid complexes found in native starch granules are also resistant to hydration and thus gelatinization (Lindeboom et al., 2004). Ralet et al. (1993) found that extrusion of pea hulls resulted in solubilization of cell wall polymers significantly transforming insoluble to soluble fibre. As extrusion parameters increased (temperature and shear) the water solubility of pea increased predisposing it to starch gelatinization (Ralet et al., 1993).

2.4.2.2 Starch retrogradation

Although hydrothermal processing can increase digestibility, rapid cooling can allow crystalline complexes to reform between amylose and amylopectin molecules (Åkerberg et al., 1998; Spears et al., 2004). This process of recrystallization is termed starch retrogradation and involves the repacking of molecules and includes the loss of

water (Bornet, 1993; Åkerberg et al., 1998; Rosin et al., 2002). Amylose molecules easily facilitate retrogradation due to their linear chain structure, while the branched structure of amylopectin resists retrogradation (Rosin et al., 2002). Storage can also have a significant effect on starch composition and function (Bornet, 1993; Rosin et al., 2002). Rosin et al. (2002) found that a significant amount of resistant starch can be formed via 30-day storage at -20°C in a multitude of starch sources, including rice, corn, spaghetti, potato and legumes.

2.4.2.3 Formation of resistant starch

Resistant starch, that portion of starch escaping digestion in the small intestine, can naturally occur within the native starch granule. Chemical and physical processing can also cause the formation of RS by amylose retrogradation, amylose-lipid complex formation and chemical modification (Bornet 1993; Sun et al., 2006). Two types of chemical modifications can take place, substitution or cross-linking. Substitution is the process of etherification or esterification of the hydroxyl groups on glucose units which decreases retrogradation (Cummings and Stephen, 2007). Cross-linking is a second type of chemical modification where linkages are formed between amylose and amylopectin molecule to reinforce the naturally occurring hydrogen bonds within the starch granule (Cummings and Stephen, 2007). The chemical modification of starches is a common occurrence in food processing to alter the taste, texture and shelf life of food (Cummings and Stephen, 2007).

2.5 Benefits of low glycemic diets

2.5.1 Blood glucose management

The starch in high GI diets is rapidly digested and absorbed resulting in hyperglycemia, which is followed by a hypoglycemic event. Constant fluctuations in blood glucose generate high stress on regulatory mechanisms of glucose homeostasis (Ludwig, 2002). Studies have shown that high post-prandial glucose levels are associated with decreased levels of serum antioxidants, increasing oxidative damage risk (Ceriello et al., 1998; Rao and Agarwal, 1999). It has long been advised that diabetic patients stringently control blood glucose levels to reduce future complications (Crapo et al., 1980; Jenkins et al., 1982a). Studies have shown that subjects with decreased glucose tolerance have exaggerated post-prandial glucose responses (Crapo et al., 1980; Collier and O'Dea, 1982).

Diabetes is a common disease affecting over 9 million Canadians and can be characterized into three main types, Type 1, Type 2 or gestational diabetes (Canadian Diabetes Association, 2011). The most common form of diabetes, Type 2, otherwise known as non-insulin dependant diabetes mellitus (NIDDM) is characterized by an adulteration in cell transport and metabolism of glucose. It is the result of glucose intolerance due to insulin resistance in some cases insulin resistance is due to defective insulin receptors (Canadian Diabetes Association, 2011). Low glycemic diets were first proposed as a mechanism to help diabetics control their blood glucose levels (Jenkins et al., 1981; Collier and O'Dea, 1982; Jenkins et al., 1985). Many studies have found that with the intake of low glycemic diets, both glycosylated serum proteins and glycosylated hemoglobin (HbA1) are decreased (Jenkins et al., 1987b, 1988; Wong and Jenkins, 2007). Glycosylated serum proteins are a measure of plasma glucose concentration over a short time, specifically when there is a dietary change (Reece, 2004). HbA1 is another

indicator of plasma glucose concentrations, usually over a 12-week time period, note red blood cells have a 120-day life cycle and if exposed to glucose, remain glycosylated until apoptosis (Reece, 2004). Diabetics typically have raised HbA1 levels as compared to subjects who clear glucose normally (Jenkins et al., 1988). Studies have also shown a correlation between increasing β -cell function and low glycemic diets (Jenkins et al., 1987b; Wong and Jenkins, 2007). The inclusion of low glycemic foods may increase insulin sensitivity by avoiding major fluctuations in blood glucose levels (Thomas et al., 2007).

2.5.2 Weight management

Dietary evolution plays a major role in today's obesity and diet related health problems. Obesity is known to contribute to hyperinsulinemia, an over production of insulin by β -cells of the pancreas, as a response to decreased insulin sensitivity (FAO, 1998). Low GI diets are beneficial to obesity related disease by helping to control glucose release, insulin response and satiety (Cheftel, 1986; Jenkins and Kendall, 2000). Countless studies have demonstrated that controlling the GI of a diet can positively influence the health status of humans (O'Dea et al., 1980; Wolever, 2006). Consuming slowly digestible carbohydrates has been shown to reduce post-prandial glucose levels, decreasing the rise in gut hormones such as insulin surges (Jenkins et al., 2002). Another phenomenon is the second meal effect. It has been shown that a low GI meal in the morning can improve glucose tolerance in the following meal (Jenkins et al., 1981; Björck and Elmståhl, 2003).

Positive correlations have been observed between the consumption of low GI diets and body weight regulation through increased satiety and insulin sensitivity (Jenkins et al., 2002; Ebbeling et al., 2003; Ludwig 2002; Warren et al., 2003; McMillan-Price and Brand-Miller 2006; Thomas et al., 2007; Du et al., 2008). Low GI diets are beneficial to obesity related disease by controlling satiety (Cheftel, 1986; Jenkins and Kendall, 2000). Human research has shown that consumption of food with a high post-prandial glucose responses is coupled with greater subsequent intake (Ludwig, 2003; Warren et al., 2003). Animal research, by Appleton et al. (2004), supports this theory and it is documented that cats fed *ad libitum* had significantly higher energy consumption with a rice based diet, which elicited a higher glucose response, than with a sorghum/corn based diet, 140kcal/feeding versus 71 kcal/feeding, respectively. This implies that the reduced glucose response of a feed will increase the satiety of an animal, which is intrinsic to the starch source itself.

2.5.3 Other

Prolonged periods of high insulin have been correlated with an increase in blood pressure, triglyceride level and a decrease in high-density lipoprotein (HDL) cholesterol, all of which are predisposing factor to cardiovascular disease (O'Keefe et al., 2008). Decreasing low-density lipoprotein (LDL) cholesterol has also been correlated with decreasing the risk for cardiovascular disease mortality and morbidity (Jenkins et al., 1985, 1987b). Jenkins et al. (1985) have shown that a one-month low GI diet therapy in hyperlipidemic patients significantly decreased serum cholesterol and triglycerides, thus decreasing their cardiovascular disease risk. Plasminogen activator inhibitor-1 (PAI-1) is used as in indicator for impaired fibrinolysis (Reece, 2004). Fibrinolysis is the ability to

break down a fibrin clot caused by coagulation, and an increased level of PAI-1 is a substantial risk factor for coronary heart disease (Wolever et al., 1992). A study by Wolever et al. (1992) showed that a low GI diet in diabetics significantly reduced PAI-1, by 58%. Researchers have hypothesized that this is due to a low GI diet having an improved metabolic profile with lowered insulin concentrations (Leeds, 2002).

A new aspect of GI research is looking into its relevance to cancer prevention. Insulin and insulin-like growth factors have been correlated with cancers such as those of the colon, breast and prostate (Jenkins et al., 2002). Researchers are hypothesizing that high GI diets and sedentary lifestyles may be associated with an increased risk of cancer (Giovannucci, 2001). Insulin, being an anabolic hormone, causes protein accretion, and since cancer is the excessive proliferation of disease cells, it is hypothesized that there is a correlation between high insulin levels, insulin resistance and cancer (Giovannucci, 2001; Jenkins et al., 2002). Low GI diets are proposed to reduce post-prandial insulinemic responses, thus lowering insulin's risk in cancer cell production. However, there have been conflicting reports as Flood et al. (2006) found that GI or glycemic load showed no association with prostate, lung and or ovarian adenomas or cancers. Thus, more research is required to elucidate the role of GI in cancer.

2.6 Beneficial properties of peas

Biblical reference in the book of Daniel (1:8-15) portrays the benefits of consuming a diet of pulses and water for 10 days (Kritchevsky, 1988). Along with the advantageous attributes of pea, they are also widely available within Canada and Saskatchewan. Canada is the leader in production and exportation of peas, with

Saskatchewan producing 65% of Canadian production (Saskatchewan Pulse Growers, 2011). In 2010, Canada produced 2.9 Mt of peas with Saskatchewan producing 1.86 Mt (Canadian Grain Commission, 2010; Saskatchewan Ministry of Agriculture, 2011).

A year-long study by Jenkins et al. (2006) noted that diets consisting of plant sterols, soy protein and viscous fibres, including the consumption of peas, beans or lentils, significantly reduced serum cholesterol. Fibrous carbohydrate sources are able to bind bile acids and increase their excretion, which is what causes a reduction in plasma cholesterol (Schneeman 1999; Tosh and Yada, 2010). Pulses, such as peas, are commonly incorporated into food to increase dietary fibre (Tosh and Yada, 2010). Field pea are known to contain both insoluble and soluble fibre (Jenkins et al., 1982b). They also are known to contain small amounts of antinutritional factors such as enzyme inhibitors, lectins, phytates and tannins, which may also be an influencing factor in their reported low GI (Jenkins et al., 1982b). Rosin et al. (2002) found that the starch present in legumes was only 89-93% digestible due to the presence of RS, and that cereals and tubers showed little to no RS formation post processing. Reduced digestibility of legumes is proposed to be from the structure of the cell wall, which entraps starch granules hindering hydrolysis and gelatinization (Rosin et al., 2002). A meta-analysis by Sievenpiper et al. (2009) demonstrated that pulses alone and in high fibre diets improved markers of long term glycemic control including lowering fasting blood glucose levels and glycosylated blood proteins.

2.7 Dogs, peas and application of glycemic index

With the industrialization of the modern world, not only are people suffering from obesity-related syndromes, canines undoubtedly suffer from similar diseases due to owners' sedentary lifestyles. Using the same ideology for humans, it is hypothesized that canines can be treated with the same diet therapy remedied for humans to help alleviate their obesity-related ailments such as glucose intolerance. For the convenience of owners, pet food is typically processed into a kibble form using highly digestible carbohydrates such as rice. Using pulses, such as peas and lentils has been shown in human studies to help with glucose homeostasis as mentioned previously. This begs the question: why aren't peas commonly used in canine diets? The problem is research is inconclusive as to what happens to peas once they are processed. Since grains are typically ground and further processed to be in a diet, the present study looks at how grind size and extrusion parameters alter glycemic responses to the consumption of pea.

Based on the literature review and meta-analysis observations which follows, the overall objective of this study was to determine if processing of field pea could change their glycemic response in dogs and if the glycemic response could be correlated to the three starch fractions RDS, SDS, and RS. Adjusting extrusion conditions was hypothesized to affect the naturally occurring physiochemical properties of peas, namely altering the high proportion of slowly digestible starch and low GI measured through *in vivo* and *in vitro* starch digestibility and glycemic testing.

3.0 THE EFFECT OF HYDROTHERMAL TREATMENT OF PEAS ON STARCH DIGESTIBILITY: A META-ANALYSIS

3.1 Introduction

It is well established that starch consists of fractions that are absorbed and digested at different rates (Englyst et al., 1996b). Three major methods are currently used to assess the rate of starch digestion in monogastric animals. The first of these involves slaughtering animals at various time points after a meal and sampling digesta from different sites in the gastrointestinal tract (Low et al., 1982; Fuller et al., 1994; Schafer et al., 2007). This method, while comprehensive, is expensive and time consuming. A less invasive *in vivo* method to estimate starch digestion rates is the glycemic index (GI) (Wolever et al., 1991). Glycemic index measures the rate and height of the glucose response curve after a meal relative to a control food. While less invasive, GI remains expensive and laborious. To overcome these limitations, Englyst et al. (1992) developed an *in vitro* method to determine starch degradability rates. This method is based on the release of glucose from a test feed after digestion with amyloglucosidase and pancreatic invertase. The amount of glucose released from the test feed after 20 minutes is termed RDS, after 100 minutes SDS, and the remaining undigested starch is termed resistant RS. The Englyst Method has the advantage of being inexpensive, fast and easy to perform, but requires validation to correlate *in vitro* results with actual *in vivo* responses.

Rapidly digestible starch in humans promotes large spikes in blood glucose and insulin release after a meal (O'Keefe et al., 2008). Low glycemic diets were first proposed as a mechanism to help diabetics control their blood glucose levels, but now have many other proposed health benefits (Jenkins et al., 1981, 1985). Diabetics

typically have elevated HbA1 levels as compared to subjects who clear glucose normally (Jenkins et al., 1987b). Research has shown that with the intake of low GI diets, both glycosylated serum proteins and HbA1 are decreased, which may increase β -cell function (Jenkins et al., 1987b, 1988). High GI diets are associated with weight gain and impaired hormonal control of glucose (Ludwig et al., 1999). Further, epidemiological studies indicate that slowly digestible starch from dietary whole grains and legumes is protective against chronic diseases, including cancer, cardiovascular disease, diabetes and obesity (Flight and Clifton, 2006; McKeown et al., 2009).

Pea starch is slowly digestible and is therefore desirable in human diets. However, peas are generally subjected to some form of processing before consumption and this can significantly affect the starch degradation rate (Tovar et al., 1992; Berhall and Scholfield, 2005; Marques et al., 2007; García-Zaragoza and Sánchez, 2010). Hydrothermal processes, including cooking, microwaving and extrusion can all significantly affect the glycemic properties of starch (García-Zaragoza and Sánchez, 2010). During processing, the slow starch degradation rate of peas may be destroyed, thus, abrogating the benefits of pea starch in human diets. Despite this, there is only one reported study on the effect of hydrothermal processing on pea starch digestion kinetics measured using any of the three methods discussed above (Bornet et al., 1989). The digestibility of foods is correlated with starch digestion kinetics (Weurding et al., 2001). Based on this observation and given the lack of studies measuring the effect of hydrothermal processing on starch digestibility kinetics, the effect of processing on the digestibility of peas may serve as a useful estimate of these effects. Thus, a meta-analysis was performed to

systematically review the effects of hydrothermal processing on the digestibility of peas in monogastric animals and humans.

3.2 Materials and methods

3.2.1 Search strategy and inclusion criteria

The meta-analysis was performed using Mix Version 1.7 (Bax, 2008) and was conducted as described by the Cochrane Handbook for Systematic Reviews of Interventions (Higgins and Green, 2008). Reporting of results was done according to the quality of Reporting of Meta-analyses (QUOROM) guidelines (Moher et al., 1999). Study selection was conducted searching MEDLINE(1950-2010); ISI WEB OF KNOWLEDGE(1899-2010); CABI(1910-2010); WEB OF SCIENCE(1989-2010); EMBASE(1947- 2010); and the Cochrane Library (including the Cochrane Central Register of Controlled Trials (Clinical Trials; CENTRAL) database)(1800-2010) using the following search terms and Boolean operators: Topic=(pea OR peas) AND Topic=(cook OR cooking OR gelatinization OR extrusion OR steam OR heat treatment OR fractionation) AND Topic=(digestibility OR glycemic OR glycaemic). The search included all monogastric species. No limit was placed on language. Manual searches supplemented the database search strategy. Our pre-specified inclusion criteria were: 1) random allocation of participants; 2) use a variety of *Pisum sativum*; 3) studied *in vivo* starch digestion; 4) use of humans or other monogastric vertebrates; and 5) presence of a non-hydrothermally-processed control group.

3.2.2 Data extraction

The authors independently extracted relevant data on study characteristics and outcomes using a standardized performa. These data included information on study design (parallel, crossover, factorial, etc.), randomization, blinding, sample size, participant characteristics (age, sex, species, BMI, diabetes status and presence of

preexisting conditions), pea variety, form and dose, inclusion of appropriate control diets, and macronutrient profile of background diet.

3.2.3 Statistical analysis

Starch digestibility values from the studies selected for inclusion in the meta-analysis are presented as a percent (%) relative to the control. For trials with a factorial design only main results on 2-way analyses were reported, that is, all participants evaluated for starch digestibility of processed peas were compared with all participants whose digestibility was recorded on non-processed peas (Al-Marzooqi and Wiseman, 2009). Whereas some studies included only one cultivar or variety of peas, others contained multiple varieties (Flemming and Vose, 1979; Conan and Carre, 1989; Bengala-Freire et al., 1991). Furthermore, some studies contained one evaluation of digestibility, whereas other studies contained two (Sun et al., 2006). Due to the apparent effect of all of the above variables, data were not pooled for these individual studies, but were used as individual comparisons if an appropriate control was available. Summary statistics were calculated using a random-effects model, which took into account true heterogeneity and sampling error (Hedges and Vevea, 1998). Data was pooled and weighted using the DerSimonian and Laird method (1986) and effect size was measured CD (Cohen, 1988) with 95% CI with an alpha level of $P < 0.05$. CD measures effect size based on the mean difference divided by the pooled standard deviation. Heterogeneity was assessed by Q index and quantified by t^2 . Weighted regression analysis of CD on processing temperature was performed using PASW Statistics Standard Version 18.0 (Version 18.0.0, SPSS Inc., Chicago, IL.). Linear and quadratic regressions were calculated and the model with the lowest P -value was reported.

3.3 Results

3.3.1 Study description

There was only one trial measuring how hydrothermal processing affected glycemic responses in humans (Bornet et al., 1989). This trial was not included in the meta-analysis because it did not measure starch digestibility. Figure 3.1 summarizes the trial selection process for this meta-analysis. Fifty randomized, controlled trials were identified, of which 41 were excluded. Reasons trials were excluded included: one trial did not include a heat treatment (Carré et al., 1998), one trial because the experimental species was not monogastric (Goelema et al., 1999), two trials because they did not contain an appropriate control (Burel et al., 2000, Stein and Bohlke, 2007), four trials because they were not on a *Pisum sativum* variety (Niba, 2003; Stone et al., 2003; Lichovnikova et al., 2004; Rehman and Shah 2005), six trials because they were not on an appropriate pea treatment (were included as a blend) (Carré et al., 1987; Gomes et al., 1993; Fasina et al., 1997; Thacker and Qiao, 2002; Golian et al., 2007; Htoo et al., 2008), nine trials because they measured *in vitro* digestibility (Hove et al. 1978; Estévez et al., 1991; Saharan and Khetarpaul, 1994; Habiba, 2002; Masoero et al., 2005; Chung et al., 2009; Eyearu et al., 2009; Ravindran et al., 2010; Yao et al., 2010) and 17 trials because they did not measure starch digestibility (Goodlad and Mathers, 1992; Van Der Poel et al., 1997; Van Der Poel et al., 1998; O'Doherty and Keady, 2000; Alonso et al., 2001; Leontowicz et al., 2001; O'Doherty and Keady, 2001; Owusu-Asiedu et al., 2002; Allan and Booth, 2004; Ramachandran and Ray, 2004; Thacker et al., 2005; Kiarie and

Nyachoti, 2007; Nagra and Bhatta, 2007; Ramachandran and Ray, 2008; Davies and Gouveia, 2010; Laudadio and Tufarelli, 2010; Stein et al., 2010).

Only nine trials (Flemming and Vose, 1979; Longstaff and McNab, 1987; Conan and Carré, 1989; Bengala-Freire et al., 1991; Moher et al., 1999; Gutiérrez et al., 2002; Thiessen et al., 2003; Sun et al., 2006; Al-Marzooqi and Wiseman, 2009) met the inclusion criteria (Table 3.1). The selected trials were reported between 1979 and 2009, and their sample sizes varied between 4-16. The total number of participants was 282. Species included were rainbow trout, chickens, rabbits, pigs and rats. Six trials measured apparent total tract digestibility, two trials measured apparent ileal digestibility, and one trial measured both apparent ileal digestibility and total tract digestibility. Pea inclusion rates ranged from 200 g/kg up to 1000 g/kg. Hydrothermal processing techniques of the nine incorporated studies included autoclaving, toasting, extrusion, and boiling. The digestibility of the control treatments ranged from 24.7 to 99.9% and the digestibility of the experimental hydrothermally processed treatments ranged from 82.0 to 100.7%.

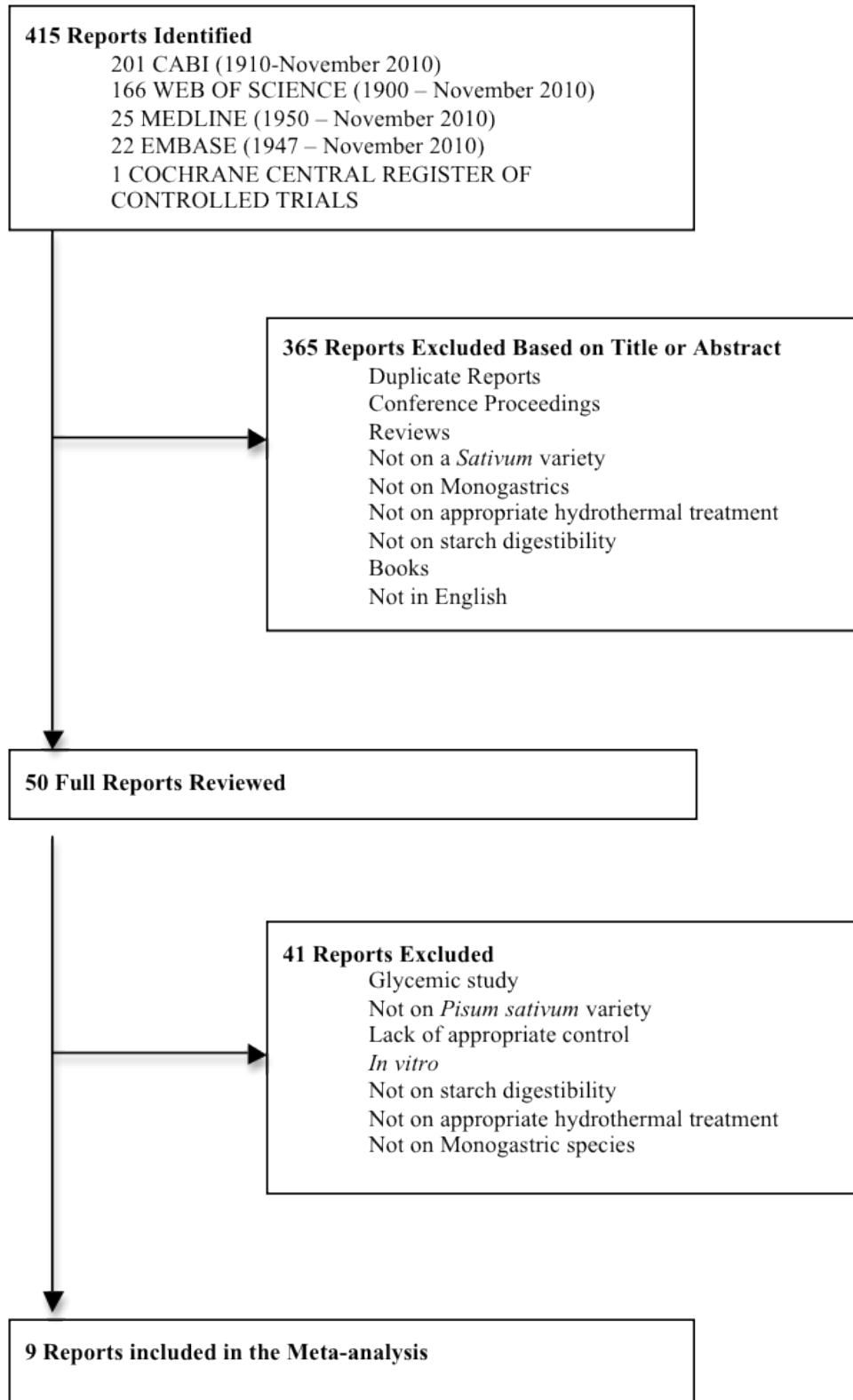


Figure 3.1 Flow chart of selection process.

Table 3.1 Data extracted from studies included in the meta-analysis.

1st Author	Year	Species	N Control	N Treated	Processing Technique	Temp (°C)	Inclusion Rate (g/kg)	Measure	Control Digest. (%)	Expt Digest. (%)
Al-Marzooqi	2009	Poultry	8	16	Extruded	70	500	AID	71.3	82.1
Al-Marzooqi	2009	Poultry	8	16	Extruded	140	500	AID	71.3	78.5
Bengala- Freire	1991	Swine	6	6	Extruded	150	450	AID	94.4	99.1
Bengala- Freire	1991	Swine	6	6	Extruded	150	450	AID	97.1	98.9
Canibe	1997	Swine	4	4	Toasted	139	661	AD	92.9	94.2
Conan	1989	Poultry	6	6	Autoclaved	130	400	AD	92.1	96.7
Conan	1989	Poultry	6	6	Autoclaved	130	400	AD	92.2	97.1
Conan	1989	Poultry	6	6	Autoclaved	130	400	AD	90.6	96.6
Conan	1989	Poultry	6	6	Autoclaved	130	400	AD	89.3	96.6
Conan	1989	Poultry	6	6	Fractioned/ Autoclaved	130	400	AD	82.9	94.0
Conan	1989	Poultry	6	6	Fractioned/ Autoclaved	130	400	AD	82.5	94.8
Conan	1989	Poultry	6	6	Fractioned/ Autoclaved	130	400	AD	78.3	93.4
Conan	1989	Poultry	6	6	Fractioned/ Autoclaved	130	400	AD	74.3	93.3
Flemming	1979	Rats	6	6	Cooked and Fractioned	121	550	AD	99.9	99.7
Flemming	1979	Rats	6	6	Cooked and Fractioned	121	550	AD	96.9	96.9
Gutiérrez	2002	Rabbits	9	9	Cooked	98	374	AD	99.5	98.9
Longstaff	1987	Poultry	8	8	Heated	121	1000	AD	75.6	91.4
Longstaff	1987	Poultry	8	8	Autoclaved	121	1000	AD	75.6	91.4
Sun	2006	Swine	6	6	Extruded	145	810	AID	79.0	91.5
Sun	2006	Swine	6	6	Extruded	145	810	ATTD	99.0	100.0
Thiessen	2003	Fish	4	4	Extruded and Dehulled	145	200	AD	24.7	100.7

AD = Apparent Digestibility Coefficient; AID = Apparent Ileal Digestibility coefficient; ATTD = Apparent Total Tract Digestibility

3.4 Combined effect of hydrothermal processing on pea starch digestibility

All nine studies were normally distributed according to *Z* scores and normal quantile plot (Figure 3.2). Of the nine studies used in the meta-analysis, seven reported an increase in starch digestibility post-hydrothermal processing. Figure 3.3 shows a forest plot and illustrates the pooled effect of hydrothermal processing on pea starch digestibility. The overall results of the meta-analysis showed that hydrothermal processing caused a significant increase in pea starch digestibility (CD = 6.94; 95% CI: 4.50-9.37; $P < 0.001$).

Subsequent analysis identified three of the included studies as outliers (Al-Marzooqi and Wiseman, 2009, Sun et al., 2006, Longstaff and McNab, 1987). Furthermore, the weight of these studies within the meta-analysis was less than 1% thus they were excluded and the data was re-analyzed (Figure 3.4). The results of this analysis were similar to the previous analysis (CD = 5.47; 95% CI: 3.67-7.27; $P < 0.01$). A weighted regression of temperature on CD values was performed using the second data set (Figure 3.5). A quadratic model showed a near significant relationship (CD = $-0.009(\text{temp})^2 + 2.345(\text{temp}) - 146.103$, $r^2 = 0.303$; $P = 0.096$). The regression model indicates that starch digestibility increases as processing temperature increases to approximately 130°C and decreases as temperature continues to rise.

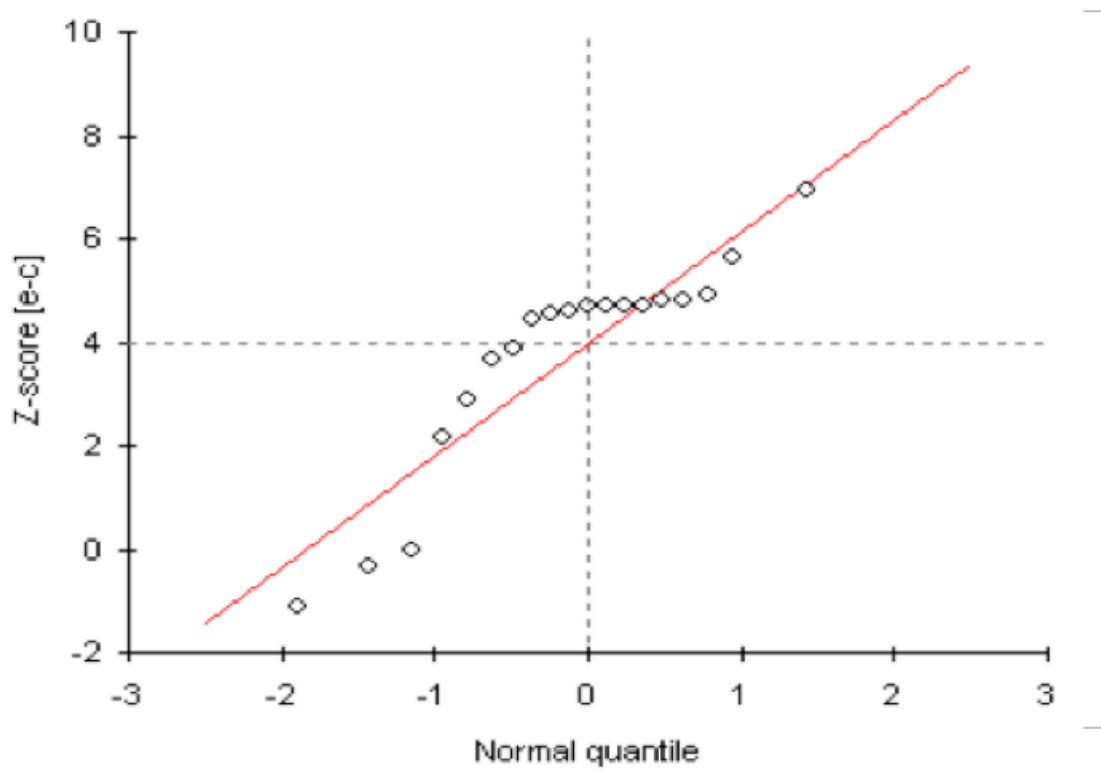


Figure 3.2 Normality quantile vs. Z-score plot.

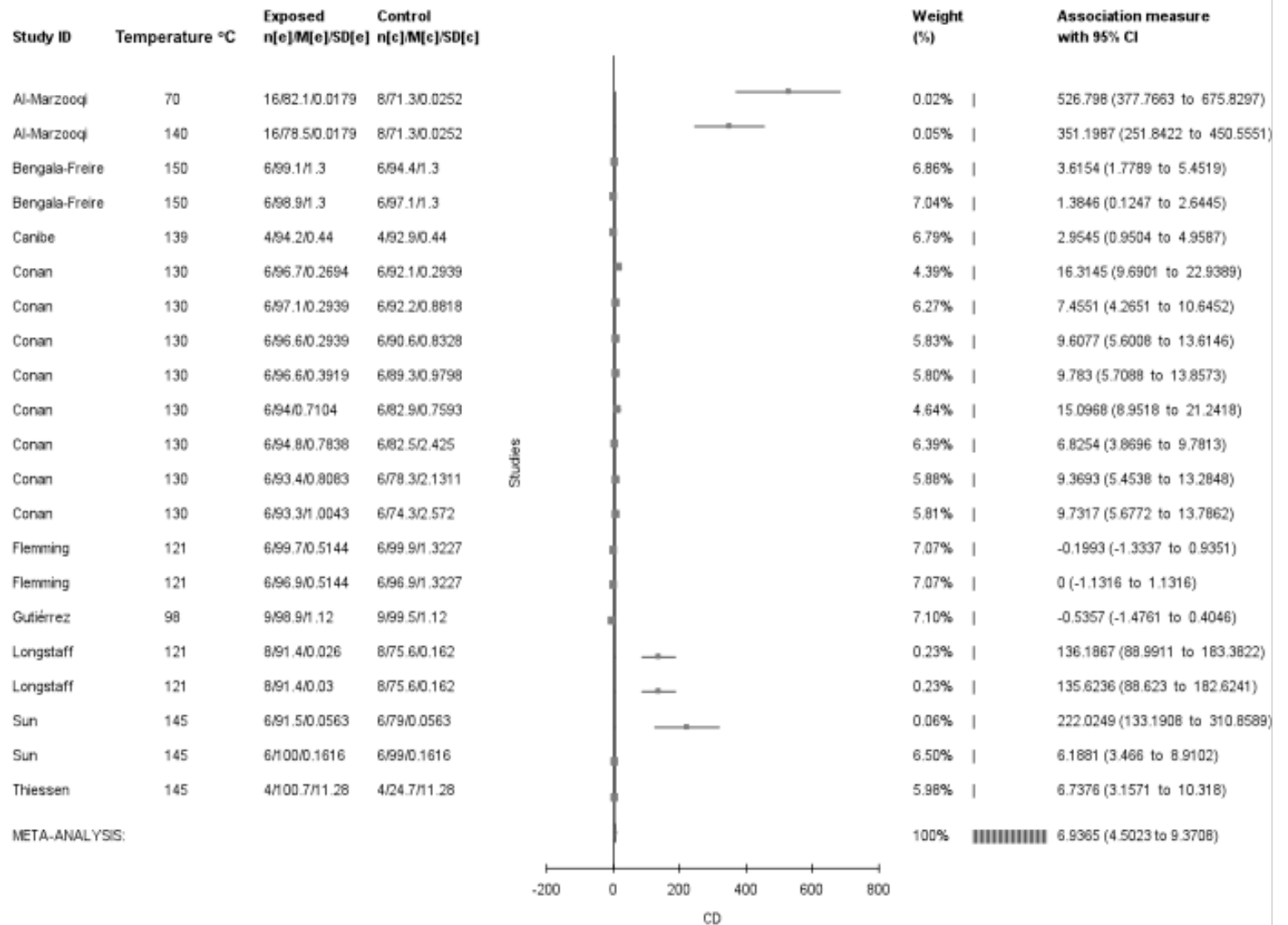


Figure 3.3 Annotated forest plot showing change in digestibility in all included studies as a result of hydrothermal processing using CD as the association measure.

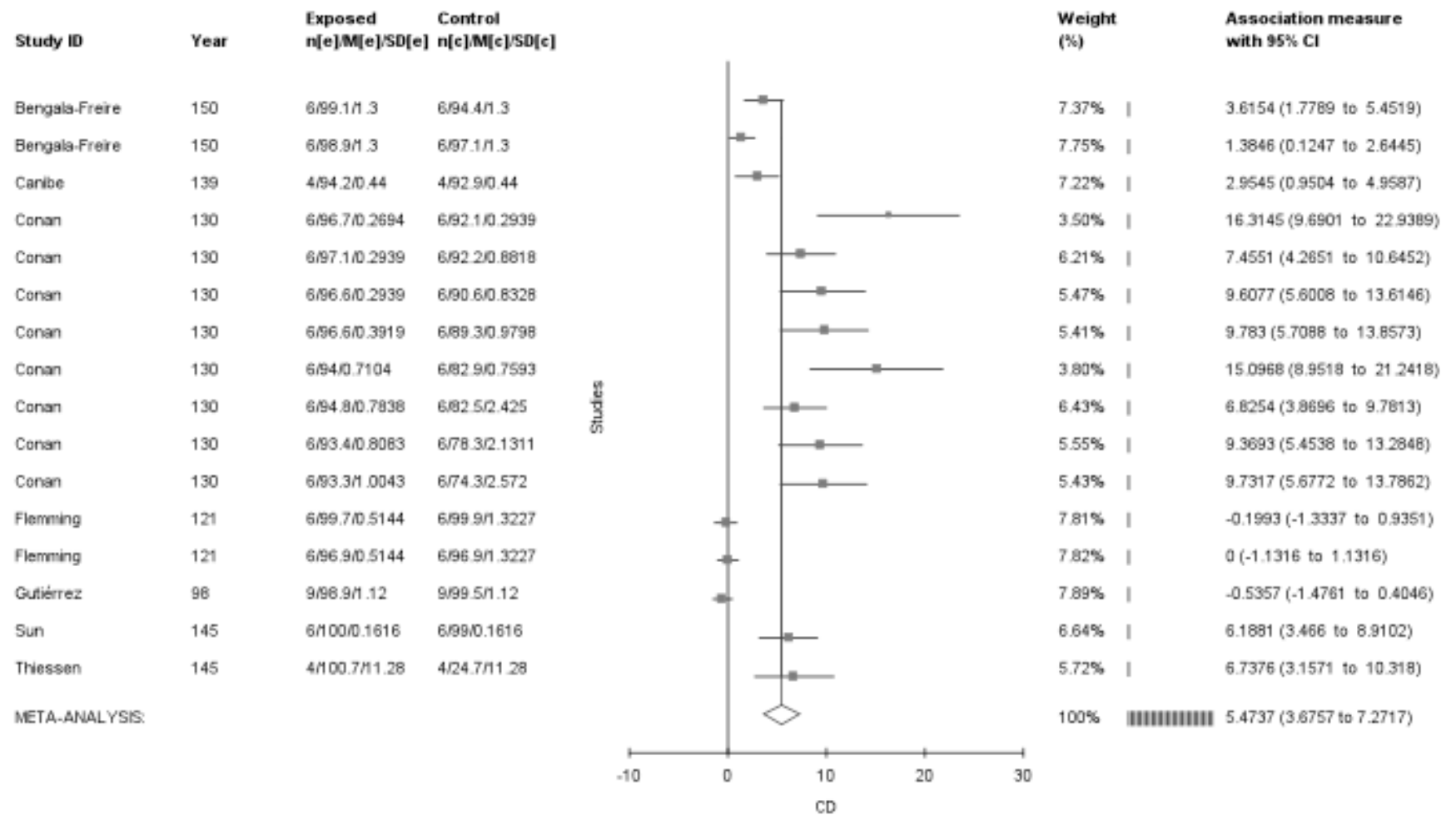


Figure 3.4 Annotated forest plot excluding outliers showing change in digestibility as a result of hydrothermal processing of peas using CD association measure.

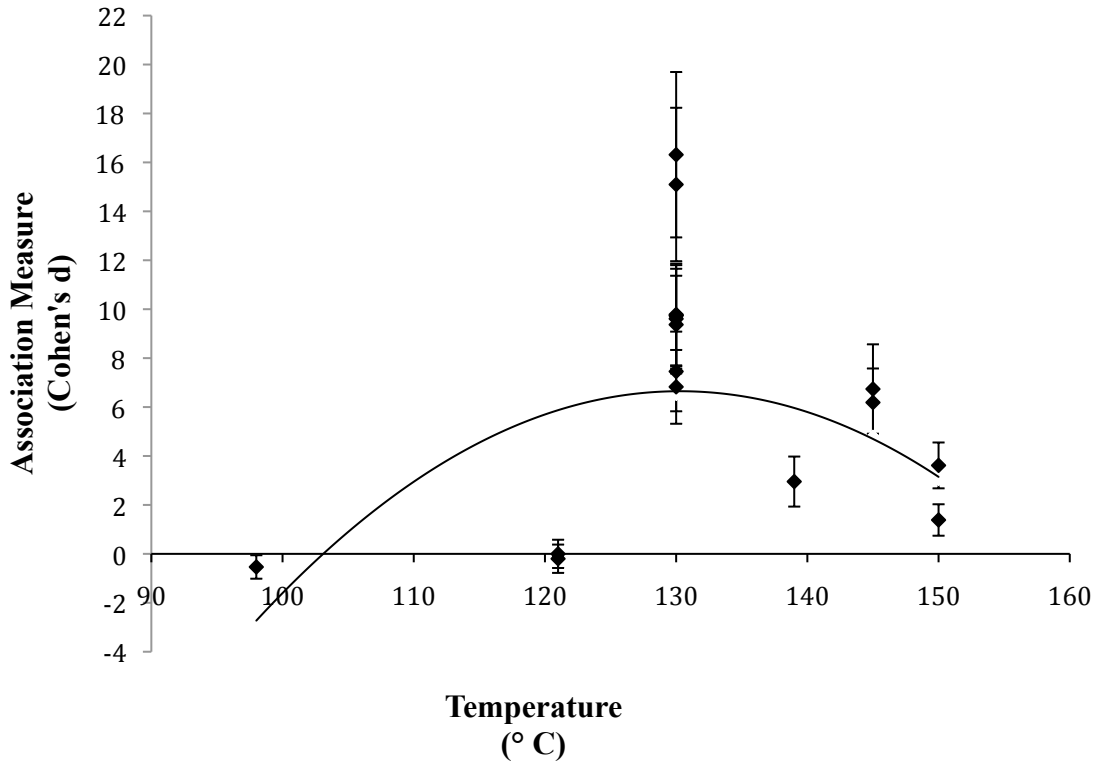


Figure 3. 5 Quadratic regression model of digestibility's association measure (CD \pm SE) as influenced by temperature ($^{\circ}$ C) during processing. (CD = $-0.009(\text{temp})^2 + 2.345(\text{temp}) - 146.103$, $r^2 = 0.303$; $P=0.096$).

3.5 Discussion

Peas have a unique starch composition influencing their digestion, absorption and GI, making them a desirable carbohydrate source (Rosin et al., 2002). Starch granules have a hierarchical structure made of multiple layers of concentric growth rings with amylose and amylopectin forming alternating amorphous and crystalline regions respectively (Lindeboom et al., 2004, Copeland et al., 2009). Cereal starches are most commonly Type A crystalline structure and are known to be highly compact, but also more digestible than Type B (Chung et al., 2008). Type B starches are commonly found in high amylose varieties of grains and raw tubers and the crystalline regions form dense hexagonal patterns known to have an open structure and hydrated core (Rosin et al.,

2002, Lindeboom et al., 2004, Chung et al., 2008). Type C granule structure, typical of leguminous plants such as peas, is a combination of both Type A and Type B and is resistant to digestion (Chung et al., 2008). Peas have been shown to contain relatively high proportions of SDS (53.7 to 59.0%) and RS (8.1 to 12.6%) and low proportions of RDS (18.2 to 23.8%) (Chung et al., 2008). Diets high in RS and SDS are proposed to not only slow gastric emptying but may decrease glycemic responses by improving hormone sensitivity (Bornet, 1993; Liljeburg and Björck, 1996; Robertson et al., 2005).

The GI of peas in humans has been reported in a number of studies. However, most of these studies measured the GI of cooked peas. In three separate studies, the GI of boiled peas averaged 68 ± 7 (Foster-Powell et al., 2002; Jenkins et al., 1981; Otto and Niklas, 1988; Kurup and Krishnamurthy, 1992). In contrast, Bornet et al. (1989) reported that the area under the plasma glucose response curve in healthy human subjects was 49 ± 19 for raw pea starch compared to 143 ± 29 for gelatinized pea starch. Furthermore, the cooking/processing methods used have differing effect on pea starch digestibility kinetics. Eyaru et al. (2009) compared the effects of soaking, boiling and pressure-cooking on pea starch fractions. They reported that the percentage of rapidly digestible starch increased from 17.6% in raw peas to 64.2% in boiled peas to 81.8% in pressure-cooked peas. Interestingly, soaking peas in water for 16 hours was found to reduce RDS to 4.4% (Eyaru et al., 2009).

Several studies have suggested that the GI of foods relates to the rate of glucose absorption from the small intestine (Jenkins et al., 2002, Wong and Jenkins, 2007). Highly digestible carbohydrates increase the gastric emptying rate, subsequently increasing potential glucose production in the small intestine and eliciting a rapid rise in

blood glucose concentration (Bornet, 1993). The digestion and gastric emptying rate of starch determines the uptake of starch in the small intestine and thus greatly influences glycemic responses (O’Dea et al., 1981, Jenkins et al., 1982a, Mourot et al., 1988, Rosin et al., 2002; Sola-Oriol et al., 2010). A study by Mourot et al. (1988) reported a significant correlation between gastric emptying and blood glucose variation in potatoes, bread, rice and spaghetti. A more recent study by Sola-Oriol et al. (2010) corroborates the relationship between GI and digestion rate. This study found that GI is positively correlated with the ileal digestibility of organic matter (Sola-Oriol et al., 2010). They also found that the rate of passage was also significantly positively correlated with GI (Sola-Oriol et al., 2010). Other studies have also shown that *in vitro* digestion is positively correlated with GI and can be used as a predictive tool (Goñi et al., 1997, Englyst et al., 1999). O’Dea et al. (1981) found that the rate of starch hydrolysis *in vitro* with pancreatic amylase correlated strongly with peak glucose response. Thus, although not completely interchangeable, the correlations between digestibility, both *in vitro* and *in vivo* starch hydrolysis, and GI indicate that digestibility may be used as a useful predictor of glycemic responses.

Seven out of the nine studies included in this meta-analysis found that hydrothermal processing increased the digestibility of peas. Such processing modifies the physicochemical properties of a foodstuff altering the nutritional value and bioavailability of proteins, carbohydrates, lipids and vitamins (Cheftel, 1986; Lankhorst et al., 2007; Tran et al., 2008). The main effect of hydrothermal processing on starch is gelatinization. Gelatinization results in the increase of the randomness of a molecule, decreasing molecular size and the number of crystalline regions increasing digestive

susceptibility (Rosin et al., 2002). Hua and Bureau (2009) reported that the concentration of gelatinized starch relative to total starch was the most important factor in determining the digestibility of starch in salmonid fish. The efficiency of starch gelatinization is dependent on, not only the processing temperature, but moisture, particle size and processing time as well. The studies included in the meta-analysis used extrusion, pelleting, autoclaving, boiling and toasting. Despite these differences, the effect of temperature was relatively similar between methods. However, the weighted regression of temperature on CD had an r^2 of only 0.303. Thus, the temperature at which peas are processed accounts for only about one-third of the variation in starch digestibility. Another source of variability in this analysis is the use of data from different species. Animals as different as rainbow trout, chickens and pigs were all included in the analysis. While the absolute digestibility of starch varies greatly between these species, the improvement in digestibility due to hydrothermal processing measured in the meta-analysis was similar between species. This suggests that the increase in digestibility by hydrothermal processing is independent of species and is due primarily to the effect of gelatinization of starch and concomitant improvement in intestinal amylase activity.

The digestibility of starch decreased when the processing temperature was above approximately 130°C. This effect may have been due to interactions between starch and other chemical components, including lipids, proteins and antinutritional factors. The interaction of these pea constituents with starch is known to reduce digestibility. This suggests it may also decrease GI. Lipids form complexes with amylose, which increase the hydrophobicity of starch granules, and reducing water and enzyme access, inhibiting digestion and absorption (Putseys et al., 2010). Protein-starch complexes such as

Maillard products are also formed during hydrothermal processing. These complexes are resistant to digestion and therefore decrease starch digestibility and potentially GI.

Jenkins et al. (1987a) found that when bread was made without gluten protein, *in vitro* digestibility as well as GI significantly increased. Peas are also known to contain small amounts of antinutritional factors such as enzyme inhibitors, lectins, phytates and polyphenols which have been associated with inhibition of enzymatic degradation in the small intestine, ultimately slowing down glucose absorption (Khattab and Arntfield, 2009). The production of starch-lipid and starch-protein complexes and interactions with antinutritional factors might explain the quadratic relationship between digestibility and processing temperature. This indicates that optimum processing conditions that maintain the slowly digestible and resistant starch fractions present in peas are achievable.

In addition to the effect of hydrothermal processing on the GI of foods, it may also have significant effects on gut health and microbiology. The unabsorbed nutrients in the gut are the major factor controlling the composition of the intestinal microbiota and can change microbial numbers, species and species diversity (Dahiya et al., 2002, Drew et al., 2002, Pieper et al., 2008). While rapidly digestible starch is absorbed primarily in the jejunum, SDS and RS starch reach the distal ileum and colon where they are fermented by the large anaerobic bacterial communities present at these sites. A major product of microbial fermentation of starch is SCFA such as acetate, propionate and butyrate (Topping and Clifton, 2001). Butyrate is the preferred substrate for the epithelial cells lining the gut, and it is thought to be important for maintaining a healthy intestinal cell wall. The reduction in pH associated with increased SCFA production reduces the solubility of bile acids limiting the microbial metabolism to secondary bile acids

implicated in colon cancer (McGarr et al., 2005). Further SCFA may have significant effects on lipidic and glucidic metabolism (Khattab and Arntfield, 2009). Wolever et al. (1989) has previously shown that addition of SCFA can decrease peripheral fatty acids, which are known to alter the cells ability utilize insulin and thus glucose. Massimino et al. (1998) reported that SCFA have the ability to increase the incretin hormones GLP-1 and polypeptide YY. Specific members of the normal gut microbiota are thought to be beneficial with respect to SCFA production including lactic acid bacteria, in particular *Lactobacillus* spp., bifidobacteria and some members of *Clostridium* clusters XIVa and IV (Hope et al., 2005; Flint et al., 2007). The positive impacts of some *Lactobacillus* sp. on digestive function, immunity and health have been reported in a number of studies (Chowdhury et al., 2007; Danielsen et al., 2007; Willing and van Kessel, 2007). Resistant starch may also drastically alter the abundance of putrefactive bacteria, which ferment protein liberating ammonia, toxic amines and H₂S which are associated with colonic neoplasms (Hughes et al., 2000; Hope et al., 2009). The rate of glucose uptake from the gut is therefore important not only for glycemic responses but also for gut health and associated bacterial microflora.

3.6 Conclusion

While the GI of starch is correlated to the starch digestibility of foods, it is not an equivalent measure. Thus, the present meta-analysis is a suggestive but not a definitive measure of the effects of hydrothermal processing on GI. However, it suggests that there may be optimum processing parameters for maintaining or improving the glycemic properties of peas. The paucity of research on the effects of heat/cooking on peas specifically and pulses in general indicates a gap in our present understanding of GI in the

human diet. Future research in this area should address our lack of knowledge of the effect of hydrothermal processing on glycemic responses in humans. Such studies should: 1) be performed on monogastric animals and preferably humans; 2) include an unprocessed control treatment; 3) include experimental treatments with one and preferably a range of processing parameters including temperature, moisture, particle size, etc.; and 4) utilize this information to validate rapid assessment technology for predicting GI, such as near infrared reflectance. The goals of future research should also be to improve *in vivo* and *in vitro* assays defining glycemic responses to peas. The glycemic properties of peas are important and there is a large gap in knowledge where improvements can be made to processing techniques to improve the use of peas in human and animal diets.

3.7 Objectives and hypothesis

Based on observations of the meta-analysis, heat processing appears to have an effect on the digestibility of pea starch measured in a variety of animal species. Understanding how processing affects pea digestibility, starch degradability and glucose absorption are important tools for feed formulation. With companion animal obesity a common problem in the developed world, diet therapies used in the human world seem valid options to entertain. Therefore, the objectives of this study were to:

1. Characterize the apparent total tract digestibility of peas in laboratory beagles in comparison to other common pet food ingredients.
2. Determine how particle size of peas influences glycemic response using GI testing.

3. Determine how extrusion processing parameters modify *in vitro* pea starch degradability using Englyst starch fractionation methodology.
4. Determine how extrusion processing parameters applied to peas change their *in vivo* glucose response using GI testing in laboratory beagles.
5. Determine the correlation between *in vitro* starch degradability and *in vivo* GI of extruded peas.

Adjusting particle size and extrusion conditions is hypothesized to affect the naturally occurring physiochemical properties of peas, namely maintaining the high proportion of slowly digestible starch and low GI measured through *in vivo* and *in vitro* pea starch degradability, digestibility and glycemic testing.

4.0 THE EFFECT OF EXTRUSION ON PEA STARCH DEGRADABILITY RATE IN DOGS

4.1 Abstract

Peas have a low GI due to their low content of RDS and high content of SDS and resistant starch RS fractions. Low GI foods are thought to protect against chronic diseases, thus, the use of peas as a starch source in dog foods may improve the health of dogs. However, peas intended for canine diets require extrusion processing to increase palatability and digestibility. This may affect the GI of pea starch. Three experiments were designed to identify possible processing techniques to maintain the low GI property of peas. The first trial tested cold-pelleted peas versus rice starch total tract apparent digestibility in laboratory beagles ($n = 3$) in a completely randomized design. Although not significantly different ($P > 0.05$), peas had a lower TTADC than rice, 81% versus 100% respectively. The second trial tested the affect of various pea particle sizes 195, 309 and 427 μm , on GI in laboratory beagles ($n=6$) in a replicated randomized controlled trial. Using repeated measures of SPSS, there was no significant difference noted between glycemic responses of the three particle sizes ($P > 0.05$). The third trial utilized a completely randomized $2 \times 2 \times 2 \times 2$ factorial design testing 2 levels of temperature (110°C vs. 150°C), moisture (20% vs. 28%), particle size (288 μm vs. 407 μm) and cooling method (freezing vs. drying) on Englyst starch fractions RDS, SDS and RS. Using backwards-stepwise ANOVA of SPSS temperature, moisture and cooling method had no significant effect on Englyst starch fractions RDS SDS and RS ($P > 0.05$). However, as particle size increased RDS decreased and RS increased ($P = 0.039$ and 0.024 respectively). Subsequently, four of the 16 extruded pea treatments were selected

for measurement of GI in laboratory beagles ($n = 6$): 1) 150°C, 288 μm , 20% H_2O , dried; 2) 110°C, 288 μm , 20% H_2O , dried; 3) 150°C, 407 μm , 28% H_2O , frozen; 4) 110°C, 407 μm , 28% H_2O , frozen in a randomized controlled trial. All test diets were fed in amounts that provided 10 g of available carbohydrate. A 20% glucose solution was used as a control. Using GLM-ANOVA of SPSS, no significant difference was observed for GI between the four extruded pea treatments ($P > 0.05$). Using correlation analysis of SPSS, no relationship between GI and starch fractions, particle size, moisture content or cooling rate was detected ($P > 0.05$). However, GI was negatively correlated with temperature ($P < 0.05$). These results suggest that *in vitro* starch fractions are not good predictors of GI in dogs. However, starch fractions and GI may be manipulated by controlling processing temperature. Further studies are needed to determine the effect of multiple temperatures on the GI of various starch fractions.

4.2 Introduction

Obesity has become a widespread problem in the developed world, predisposing subjects to chronic disease risk such as, but not limited to, diabetes (Kahn and Flier, 2000; Gayet et al., 2004; Khaodhiar et al., 2009; WHO, 2009), cardiovascular disease (O'Keefe et al., 2008) and coronary heart disease (Mayer0Davis et al., 2001; Flight and Clifton, 2006), as well as some forms of cancer (Giovannucci, 2001). This trend towards obesity and associated disease risk factors has not only stricken the human population but is now the number one problem in companion animals (Crane, 1991; German, 2006). Obesity is a multifactoral problem occurring in most cases due to a combination of

decreased physical exercise and over consumption of calories. Diet therapy is a proposed and proven method to improve the health status of both humans and animals.

Typically, canine diets contain highly digestible carbohydrate sources such as rice and corn, which is characteristically ground and extruded with other ingredients to achieve a homologous diet. Intrinsic factors, carbohydrate source, granule size, crystallinity and amylose: amylopectin ratio, are known to affect starch digestion and glucose absorption kinetics (Copeland et al., 2009). Pulses, compared to typical cereal grains in canine diets, are known to have a higher amylose content, and Type C granule structure, thus increasing their RS content, and allowing them to have a low GI which has been shown to improve long term glycemic control (Sievenpiper et al., 2009). It has been shown that hydrothermal processing of carbohydrate-containing food sources cause gelatinization that elicits higher glucose responses in comparison to their unprocessed counterparts (Brand et al., 1985). Diets causing high glucose responses are known to stimulate increased hormone responses, such as insulin, increasing ones risk for chronic diseases such as diabetes. On the other hand, diets having low glycemic responses are shown to be protective against chronic diseases by decreasing cardiovascular and diabetic risk factors including PAI-1 (Wolever et al., 1992) and HbA1c respectively (Jenkins et al., 1987b; Jenkins et al., 1988; Wong and Jenkins, 2007). Diets with low glycemic responses are also hypothesized to increase satiety by having a slower, more stable release of glucose and corresponding insulin release (Cheftel, 1986; Jenkins and Kendall, 2000; Jenkins, 2002).

Processing applications typically used in pet food manufacturing causes diets to become high glycemic diets. Extrinsic heat, moisture, pressure treatments are shown to

increase glycemic responses to food stuffs (Jenkins et al., 1982b; Brand et al., 1985; Bornet, 1993; Rosin et al., 2002). Therefore adjusting particle size and extrusion conditions is hypothesized to affect starch degradability kinetics in terms of *in vitro* and *in vivo* digestibility and GI.

4.3 Materials and Methods

4.3.1 Animals

Six purebred beagles, three castrated males and three spayed females, obtained from Covance (Kalamazoo, Michigan) or University of Guelph (Guelph, Ontario) and one year of age or older, were included in this experiment. Experimental animals were kept individually in indoor-outdoor runs provided by the Animal Care Unit of the Western College of Veterinary Medicine, University of Saskatchewan, for the duration of the trial. Dogs were given *ad libitum* access to clean fresh water and kept on a 14-hour photoperiod with an ambient room temperature of 21°C. Dogs were fed according to a predetermined amount to maintain an ideal body weight (within 5%) using NRC 2006 (ME/Day (KJ/kg) = 140 x BW kg^{0.75}). Dogs were kept in accordance with guidelines approved by the University of Saskatchewan Animal Research Ethics Board under the guidelines established by the Canadian Council on Animal Care (2005).

4.3.2 Experiment 1 - Ingredient digestibility

4.3.2.1 Diet formulation and data collection

An indirect method was used to measure apparent digestibility coefficients (ADC, %). A non-absorbable marker, celite, was added at a 1% inclusion rate to the experimental diets. A reference diet (Table 4.2) was formulated according to Fahey et al. (1992) and met or exceeded nutrient requirements of dogs according to NRC (2006). The

seven experimental diets were formulated using 70% of the reference diet with 30% of the experimental ingredient (as is basis). Feedstuffs were ground with a 3 mm screen prior to mixing using a hammer mill. The diets were cold extruded using a 5 mm die on a Hobart Pelleter (Model 4822; Ohio, USA), dried in a forced air oven (55°C, 12 h), chopped and screened to obtain the appropriate pellet size. Ingredients tested included barley, chicken meal, corn, field pea, wheat gluten, rice and spray dried egg.

On the first day of the experiment, dogs were fed a 50:50 mixture of the new experimental diet and the previous diet. On days two to seven dogs were fed only the experimental diet. On the morning of the eighth day of the experiment, fecal samples were collected intermittently throughout the day and pooled per dog until approximately 2 kg of feces was obtained. Feces were dried in a forced air oven at 55°C for ~ 12 hours. Post drying, feces were ground using a Retsch Mill (Brinkmann Corp) using a 1 mm screen and stored in 30-dram snap cap vials at an ambient temperature of $21 \pm 5^\circ\text{C}$ until analyzed. Feeding took place until three dogs had been fed each of the seven experimental diets and all six dogs had been fed the reference diet.

Table 4.1 Ingredient composition of the reference diet and seven experimental diets.

Diet	Reference¹	CM²	WG³	Barley⁴	Rice⁵	Peas⁶	Corn⁷	SDE⁸
Ingredients	Amount (g/kg)							
Chicken Meal	497.00	347.00	347.00	347.00	347.00	347.00	347.00	347.00
Corn	235.00	164.50	164.50	164.50	164.50	164.50	164.50	164.50
Chicken Fat	89.00	62.30	62.30	62.30	62.30	62.30	62.30	62.30
Wheat Flour	64.00	44.80	44.80	44.80	44.80	44.80	44.80	44.80
Cornstarch	60.80	42.56	42.56	42.56	42.56	42.56	42.56	42.56
SDE	13.50	9.45	9.45	9.45	9.45	9.45	9.45	9.45
Premix ⁹	10.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Celite	10.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
KCl	7.00	4.90	4.90	4.90	4.90	4.90	4.90	4.90
CaP	6.70	4.69	4.69	4.69	4.69	4.69	4.69	4.69
NaCl	5.00	3.50	3.50	3.50	3.50	3.50	3.50	3.50
DL-Met	2.00	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Test Ingredients								
SDE	-	-	-	-	-	-	-	300.00
Corn	-	-	-	-	-	-	300.00	-
Peas	-	-	-	-	-	300.00	-	-
Rice	-	-	-	-	300.00	-	-	-
Barley	-	-	-	300.00	-	-	-	-
WG	-	-	300.00	-	-	-	-	-
CM	-	300.00	-	-	-	-	-	-

¹Reference diet formulated using Fahey et al. (1992)

²Chicken Meal – Supplied by Horizon Pet Food

³Wheat Gluten – Dawn Foods Ltd.

⁴Barley – Hulless variety, supplied by Horizon Pet Food

⁵Rice – Gold Mountain long grain white

⁶Peas – Mozart (yellow cotyledon variety)

⁷Corn – Supplied by New-Life Feeds

⁸Spray Dried Egg-Supplied by Horizon (Innovatech Egg Products)

⁹Premix Supplied by Univar Canada Ltd.

Table 4.2 Vitamin and mineral premix nutrient profile¹.

Vitamins/Minerals	Inclusion	Unit
Selenium	0.2	mg/kg
Magnesium	800.0	mg/kg
Zinc	15.0	mg/kg
Copper	20.0	mg/kg
Manganese	15.0	mg/kg
Iron	125.0	mg/kg
Iodine	2.5	mg/kg
Vitamin A	37000.0	IU/kg
Vitamin D	3450.0	IU/kg
Vitamin E	325.0	IU/kg
B-12	22.0	mcg/kg
Biotin	500.0	mcg/kg
Thiamin	13.4	mg/kg
Riboflavin	15.3	mg/kg
Niacin	52.0	mg/kg
Pantothenic Acid	27.0	mg/kg
Pyridoxine	8.2	mg/kg
Folic Acid	1.5	mg/kg
Vitamin C	425.0	mg/kg

¹Ingredients: Wheat, Magnesium Oxide, Zinc, methionine, Vitamin C, Alltech Bio-Mos, Vitamin E, Zinc Sulphate, Ferrous Sulphate, Iron Proteinate, Vitamin D3, Alltech Deodorase, Mineral Oil, Copper Proteinate, Copper Sulphate, Niacin, Selenium Enriched Yeast, Calcium Iodate, Vitamin A, Manganese Proteinate, Calcium Pantothenate, Biotin, Vitamin B12, Riboflavin, Manganese Oxide, Thiamine, Sodium Selenite, Pyridoxine, Folic Acid

4.3.2.2 Analytical methods and calculations

Diets and fecal material were analyzed for moisture (AOAC, 1990, method no. 934.01), energy (oxygen bomb calorimetry; Parr Adiabatic Calorimeter, Model 1200), crude protein, acid ether extract (AOAC, 1995, method no. 954.02) and acid insoluble ash (AOAC 1995, method no. 954.02). The combustion method (AOAC, 1995) was used to determine nitrogen content, which was multiplied by 6.25 in order to estimate protein. Starch was analyzed using an AOAC approved assay (996.11) Megazyme Assay Kit K-TSTA (Megazyme, Bray Co., Wicklow, Ireland).

The total tract apparent digestibility coefficient (TTADC) (%) for the individual diets was calculated using the following equations adapted by Bureau and Cho (1999) from Cho et al. (1982) and Sugiura et al. (1998):

$$\text{TTADC} = 1 - (\text{F/D} \times \text{Di/Fi})$$

Where: D = % nutrient in the diet (dry matter (DM) basis)

F = % nutrient in the feces (DM basis)

Di = % indicator in the diet (DM basis)

Fi = % indicator in the feces (DM basis)

The ADC of the test ingredient was calculated using the following equation:

$$\text{ADCI} = \text{ADCT} + ((1-s) \text{DR/s DI}) (\text{ADCT} - \text{ADCR})$$

Where: ADCI = Apparent digestibility coefficient of test ingredient

ADCT = Apparent digestibility coefficient of test diet

ADCR = Apparent digestibility coefficient of the control diet

DR = % nutrient (or kJ/g gross energy) of the control diet mash
(DM basis)

DI = % nutrient (or kJ/g gross energy) of the test ingredient (DM
basis)

s = Proportion of test ingredient in test diet mash (DM basis)

4.3.2.3 Statistical analysis

Digestibility data was analyzed using the GLM procedure of SPSS (PASW Statistic v.18.0, SPSS Inc., Chicago, IL, USA). When significant ($P < 0.05$), means were separated using the Ryan Einot Gabriel Welsch F-Test.

4.3.3 Experiment 2 - Effect of particle size on glycemic index in dogs

4.3.3.1 Product formulation, data collection and calculations

Peas (CDC Mozart) were ground using a Christy Norris hammer mill with 5-, 2- and 1-mm screens. US standard Sieves 20, 30, 45, 50, 60, 100 and pan were used to measure the particle size of ground grain using an adapted method of the ASABE standards (2008). The glycemic indices (GI) of the pea samples were measured using a modification of the method of Wolever et al. (1991).

Individual beagles ($n = 6$) were considered as experimental units in testing each of the three pea samples and control (20% glucose solution) in duplicate. The control and test diets provided 10 g of available carbohydrate. The dogs were fasted overnight for 12 hours. They were then given the test diets. Dogs were given 10 minutes to consume the entire test diet, otherwise they were excluded from the trial. No subjects were excluded in this trial based on 10 minute consumption time. Venous blood glucose was measured using a HemoCue® Glucose 201 analyzer (HemoCue Inc., Lake Forest, CA). Prior to blood collection, area intended was clean-shaven and disinfected using hibitane and 70% isopropyl alcohol. Blood samples were taken from the femoral vein using 30.5 G needles and 1mL Luer-Lok Syringes at times of 0, 15, 30, 45, 60, 90, 120, 150, and 180 min following the test meal. Glycemic index was calculated as the incremental area under the blood glucose response curve ($\text{mmol} \times \text{min/L}$) for the test meal, expressed as a percentage of the corresponding mean incremental area under the blood glucose response curve for the two control (D-glucose) tests taking by that subject (FAO/WHO 1998).

The resulting glycemic values were averaged for each of the three test diets to determine the glycemic index (Wolever et al., 1991).

$$GI_{ijk} = A_{ijk}/A_{ijControl}$$

Where:

GI_{ijk} = Glycemic index for the i^{th} dog in the j^{th} period for the k^{th} ingredient

A_{ijk} = Area under the glucose response curve for the i^{th} dog in the j^{th} period for the k^{th} ingredient

$A_{ijControl}$ = Area under the glucose response curve for the i^{th} dog in the j^{th} period for the 20% glucose control

4.3.3.2 Statistical Analysis

In Experiment 2, the Mixed Model – Repeated Measures procedure of SPSS (PASW Statistic v.18.0, SPSS Inc., Chicago, IL, USA) was used to determine effect of particle size on glycemic response in dogs. When significant ($P < 0.05$), means were separated using the Ryan Einot Gabriel Welsch F-Test.

4.3.4 Experiment 3 - Effect of extrusion of peas on starch degradability kinetics

4.3.4.1 Product formulation and data collection

Peas (CDC Mozart) were extruded at the Saskatchewan Food Industry Development Centre at the University of Saskatchewan using a Clextrol Evolum EV32 twin-screw extruder (Firminy, France) with a 20:1 length:diameter ratio and a 3.88 mm die. Utilizing a 2 x 2 x 2 x 2 factorial arrangement of treatments extrusion parameters studied included: two levels of particle size (288 μm vs. 407 μm), extruder barrel

temperature (110°C vs. 150°C), moisture content of product in extruder (200 g/kg vs. 280 g/kg) and cooling rate (room temperature vs. freezing) for a total of 16 treatments.

Products that were frozen were subjected to -40°C temperatures until an internal product temperature of 0°C was achieved. Extrudates were dried using a flow through cooling conveyer at 250°C at a 5 minute flow through rate. Particle size of peas, prior to extrusion, was determined using an adapted method from ASABE standards (2008).

The GIs of selected samples (3 = (150°C, 288 µm, 20% H₂O, dried); 7 = (110°C, 288 µm, 20% H₂O, dried); 10 = (150°C, 407 µm, 28% H₂O, frozen); 14 = (110°C, 407 µm, 28% H₂O, frozen) were then analyzed in dogs as described above except that blood samples were taken from an intravenous catheter in either the cephalic or saphenous vein. The cephalic or saphenous vein catheter was inserted using a 22-gauge catheter. After placement, the catheter was immediately flushed with 2-5 mL of saline and 0.1 mL of citrate. Prior to drawing blood at the indicated test times, the catheter was be flushed with 2-5 mL of saline. At the test times, indicated above, ~75 µm of blood was drawn and discarded before 1 mL of test blood, using a 3 mL syringe. The 1ml of test blood was drawn and placed immediately into a labeled test tube and kept on ice. Following each aliquot of drawn blood, the catheter was flushed with saline and citrate.

4.3.4.1 Analytical methods

Extrudates were stored according to treatment in separate plastic tubs at an ambient room temperature of 21 ± 5°C. Samples were analyzed for total starch, free glucose, RDS, SDS, and RS according to methodology described by Englyst et al. (1992). This procedure used constituents of the Megazyme Resistant Starch Kit (K-RSTAR)

(Bray Co., Wicklow, Ireland). Rapidly digestible starch, SDS, and RS were converted into percent of total starch dry matter (DM) by dividing DM fraction by DM total starch content. Data was normalized using Arcsine transformation, as the starch fractions were proportions of total starch content resulting in a skewed distribution. Test diets for Experiment 2 were portioned to contain 10 g of available carbohydrate determined by: available carbohydrate = total starch + free glucose x 0.9.

Blood samples were centrifuged at 3000 x g at 4°C for 20 minutes twice, with the fibrin clot being removed between centrifuge sets. Serum was transferred to microcentrifuge tubes and stored at -50°C. Serum glucose content was determined using the glucose oxidase / peroxidase method (Megazyme K-GLUC). The absorbance of the samples was read at 440 nm at 37°C against the reagent blank to obtain Δ^A_{sample} and $\Delta^A_{\text{D-glucose standard}}$. The area under the glycemic response curve for each test diet was expressed as a percentage of the mean glycemic response to the control meal.

4.3.4.2 Statistical analysis

In Experiment 3, results of the *in vitro* assay determining the effects of extrusion on RDS, SDS and RS fractions of peas, were analyzed using a multivariate ANOVA backwards-stepwise method with SPSS (PASW Statistic v.18.0, SPSS Inc., Chicago, IL, USA). Those variables not normally distributed were transformed using ARCSINE prior to statistical testing. Data was pooled for the *in vivo* GI experiment and analyzed as a completely randomized design using a 1-way ANOVA with SPSS. When significant ($P < 0.05$), *post hoc* analysis of individual means were compared using Ryan Einot Gabriel Welsch F Test. Pearson's correlation calculations between different variables were

performed using SPSS correlation analysis. A *P*-value of < 0.05 was considered significant and a *P*-value > 0.05 and < 0.10 was considered a trend.

4.3.5 Results

4.3.5.1 Experiment 1 - Digestibility of common pet food ingredients

The digestibilities of the ingredients are shown in Table 4.3. Dry matter TTADC of peas was the lowest at 52% and was significantly different from all other ingredients namely barley, chicken, corn, egg and rice ($P < 0.05$). Dry matter TTADC of chicken was significantly different from egg and rice ($P < 0.05$). None of the ingredients crude protein TTADC or acid-ether extract TTADC was significantly different from one another ($P > 0.05$). However, pea gross energy TTADC was the lowest of all ingredients at 55% and was significantly different from all other ingredients ($P < 0.05$). The GE TTADC of barley was significantly different from that of egg and rice ($P < 0.05$). Although starch digestibility of peas and rice was not significantly different (81% and 100%, respectively) there was a trend to the TTADC of pea starch being significantly lower than that of rice ($P = 0.06$).

Table 4.3 Total tract apparent digestibility coefficients of nutrients¹.

Ingredients	DM ²	CP ³	GE ⁴	AEE ⁵	Starch
	(ADC DM %)				
Barley	78 ^{ab}	69	75 ^b	38	
Chicken	73 ^a	84	82 ^{ab}	78	
Corn	89 ^{ab}	62	85 ^{ab}	76	
Egg	92 ^b	91	94 ^a	97	
Peas	52 ^c	62	55 ^c	51	81
Rice	95 ^b	74	94 ^a	84	100
SEM	0.045	0.045	0.045	0.047	0.026
<i>P</i> -Value	0.02	0.126	0.001	0.189	0.06

¹ ^{a,b,c} Means in the same column not sharing superscripts are considered significantly different

when $P \leq 0.05$.

² Dry matter

³ Crude protein

⁴ Gross energy

⁵ Acid ether extract

4.3.5.2 Experiment 2 - Effect of pea particle size on glycemic response in dogs

The mean particle sizes of peas ground using 5-, 2- and 1-mm screens were 427 μm , 309 μm and 195 μm respectively. No significant differences in GI were observed to be due to particle size (Table 4.4). The glycemic response curves for the three particle sizes 427 μm , 309 μm , and 195 μm , are depicted in Figure 4.1. There was no significant difference in peak glycemic response or time to peak ($P > 0.05$).

Table 4.4 Mean GI for three particle sizes of Mozart variety field pea.

Pea Particle Size (μm)	427	309	195	P - value
Glycemic Index ¹	45.1 \pm 13.0	39.1 \pm 11.3	45.6 \pm 13.2	-
Time	-	-	-	0.352
Particle Size	-	-	-	0.871
Particle Size x Time	-	-	-	0.395
Peak (mmol/L)	5.1 \pm 0.1	5.0 \pm 0.1	5.0 \pm 0.1	0.540
Time to peak (min)	75.0 \pm 9.2	83.5 \pm 9.3	98.8 \pm 13.5	0.308

Data reported as mean \pm SEM and values in a row not sharing the same letter are significantly different ($P \leq 0.05$)

¹ Glycemic Index determined using FAO/WHO 1998 as a ratio between the incremental area under the glucose response curve of a 10g carbohydrate portion of a test feed expressed as a percent of the response of a standard food from the same subject

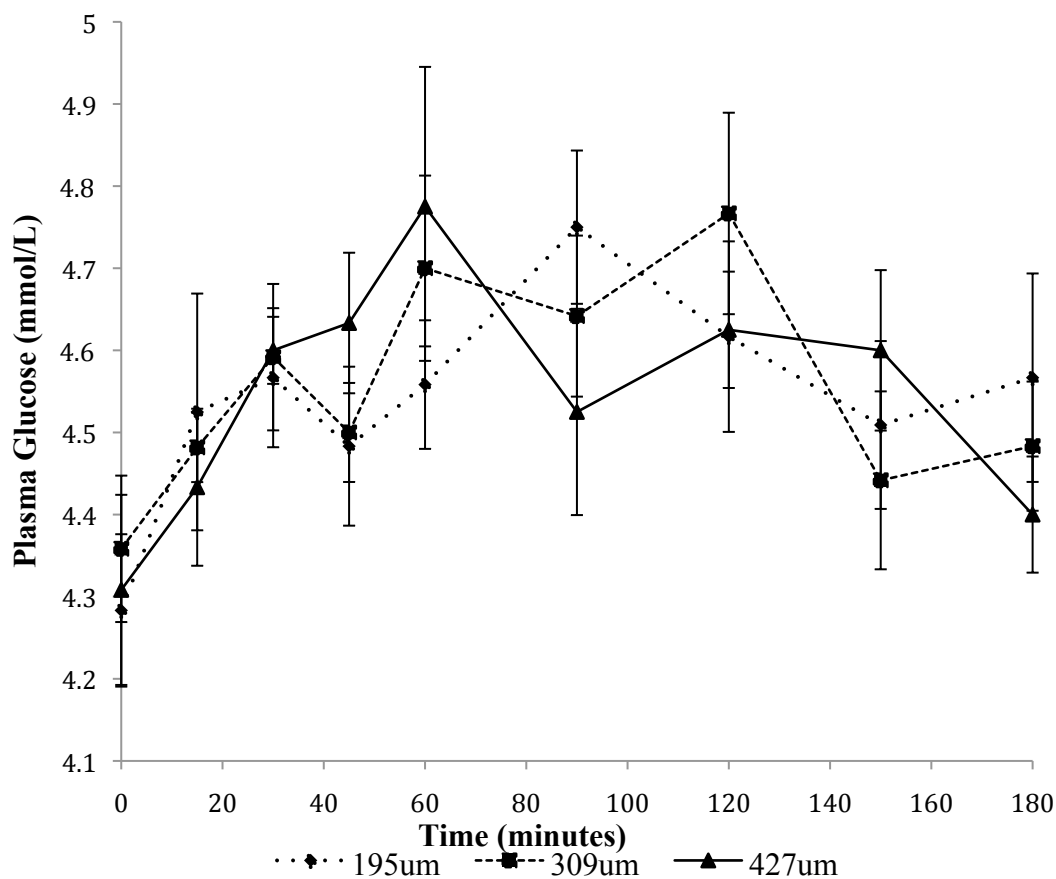


Figure 4.1 Glycemic response curves of the three particle sizes.

4.3.5.3 Experiment 3 - Effect of pea extrusion on *in vitro* digestibility and *in vivo* glyceic response in dogs

There was no significant effect of treatment temperature, moisture or cooling on the starch fractions (RDS, SDS and RS) ($P > 0.05$; Table 4.6). However, as particle size increased from 288 μm to 407 μm the RDS fraction significantly decreased and the RS significantly increased ($P = 0.039$ and 0.024 respectively) but particle size did not affect SDS content ($P > 0.05$; Table 4.7). There were no significant interactions between the main effects ($P > 0.05$).

Table 4.5 The effect of extrusion on average RDS, SDS and RS content.

ID	RDS	SDS	RS	Temp ¹	Moisture ²	Particle Size ³	Cooling ⁴
(% of Total Starch in DM)							
Unprocessed	17	21	62	n/a ^e	n/a	n/a	n/a
1	21	23	56	1	1	1	2
2	20	23	57	1	1	1	1
3	20	25	55	1	2	1	2
4	19	27	55	1	2	1	1
5	20	27	53	2	1	1	2
6	21	23	57	2	1	1	1
7	20	24	55	2	2	1	2
8	17	24	59	2	2	1	1
9	16	24	60	1	1	2	2
10	18	24	58	1	1	2	1
11	17	24	59	1	2	2	2
12	20	23	57	1	2	2	1
13	19	22	59	2	1	2	2
14	18	25	57	2	1	2	1
15	18	22	60	2	2	2	2
16	20	23	57	2	2	2	1

Data presented as % total starch (DM)

¹1 = 150°C, 2 = 110°C

²1 = 28%, 2 = 20%

³1 = 288 μm , 2 = 407 μm

⁴2 = Dryer, 1 = Freezer,

n/a=not applicable

Table 4.6 Main effects of temperature, moisture, cooling and particle size on RDS, SDS and RS¹.

Parameters	RDS ²	SDS ²	RS ²
% of Total Starch in DM			
Temperature(°C)			
150	18 ± 2	24 ± 2	57 ± 2
110	19 ± 1	24 ± 2	57 ± 2
<i>P</i> -value	0.68	0.38	0.87
Moisture(%)			
28	19 ± 2	24 ± 2	57 ± 2
20	19 ± 1	24 ± 1	57 ± 2
<i>P</i> -value	0.76	1.0	0.87
Cooling			
Freezing	19 ± 1	24 ± 1	57 ± 1
Drying	19 ± 2	24 ± 2	57 ± 3
<i>P</i> -value	0.84	0.66	1.0
Particle Size(µm)			
407	18 ± 1 ^b	23 ± 1	58 ± 1 ^b
288	20 ± 1 ^a	25 ± 2	56 ± 2 ^a
<i>P</i> -value	0.04	0.11	0.02

¹^{a,b} Means are presented ± SEM and means in the same column with different superscripts are significantly different ($P \leq 0.05$).

None of the interactions were significant.

² Presented as a fraction of total starch

Total starch, free glucose and available carbohydrate of experimental pea treatments chosen to determine glycemic response in dogs are summarized in Table 4.7. There was no significant difference observed in GI of the four extruded pea treatments (Table 4.8; $P > 0.05$). The four glycemic response curves for the extruded pea treatments 3, 7, 10 and 14 are shown in Figure 4.2. There were no significant differences in glycemic response observed at any of the 12 specific time points ($P > 0.05$). Furthermore, no significant differences were observed for maximum glucose response between each of the four treatments ($P > 0.05$).

Correlation analysis indicated that SDS, RDS and RS were not correlated with GI

(Table 4.9). However, RDS and SDS fractions were significantly negatively correlated to particle size ($P < 0.05$; Table 4.9). Temperature was negatively correlated with GI as well as positively correlated with RDS ($P < 0.05$). Moisture and cooling were colinear and thus were not included in the correlation analysis.

Table 4.7 Chemical compositions of the four extruded pea treatments.

Treatment ID¹	3	7	10	14
Dry matter (DM), g/kg	981.8	969.0	916.7	903.5
TS (DM), g/kg	421.5	460.6	478.5	489.3
FG (DM), g/kg	8.3	10.4	9.6	10.2
Available Carbohydrate(DM), g/kg	429.2	469.9	446.6	450.4

¹3 = (150°C / 288µm / 20% H₂O / dried); 7 = (110°C / 288µm / 20% H₂O / dried)
 10 = (150°C / 407µm / 28% H₂O / frozen); 14 = (110°C / 407µm / 28% H₂O / frozen)

Table 4.8 Average glycemic index of the four treatments¹.

Treatment ID²	3	7	10	14	P-value
Peak (mmol/L)	6.7 ± 0.2	6.5 ± 0.3	6.3 ± 0.2	6.1 ± 0.1	0.225
Time to Peak (min)	47.5 ± 9.8	45.0 ± 3.9	65.0 ± 14.8	62.5 ± 9.8	0.424
GI ³	47.7 ± 4.3	81.7 ± 20.0	26.6 ± 7.1	70.9 ± 19.4	0.076

¹Data is presented mean ± SEM and means in a row not sharing common letters are significantly different ($P \leq 0.05$)

²3 = (150°C / 288µm / 20% H₂O / dried);

7 = (110°C / 288µm / 20% H₂O / dried)

10 = (150°C / 407µm / 28% H₂O / frozen);

14 = (110°C / 407µm / 28% H₂O / frozen)

³Glycemic Index determined using FAO/WHO 1998 as a ratio between the incremental area under the glucose response curve of a 10g carbohydrate portion of a test feed expressed as a percent of the response of a standard food from the same subject

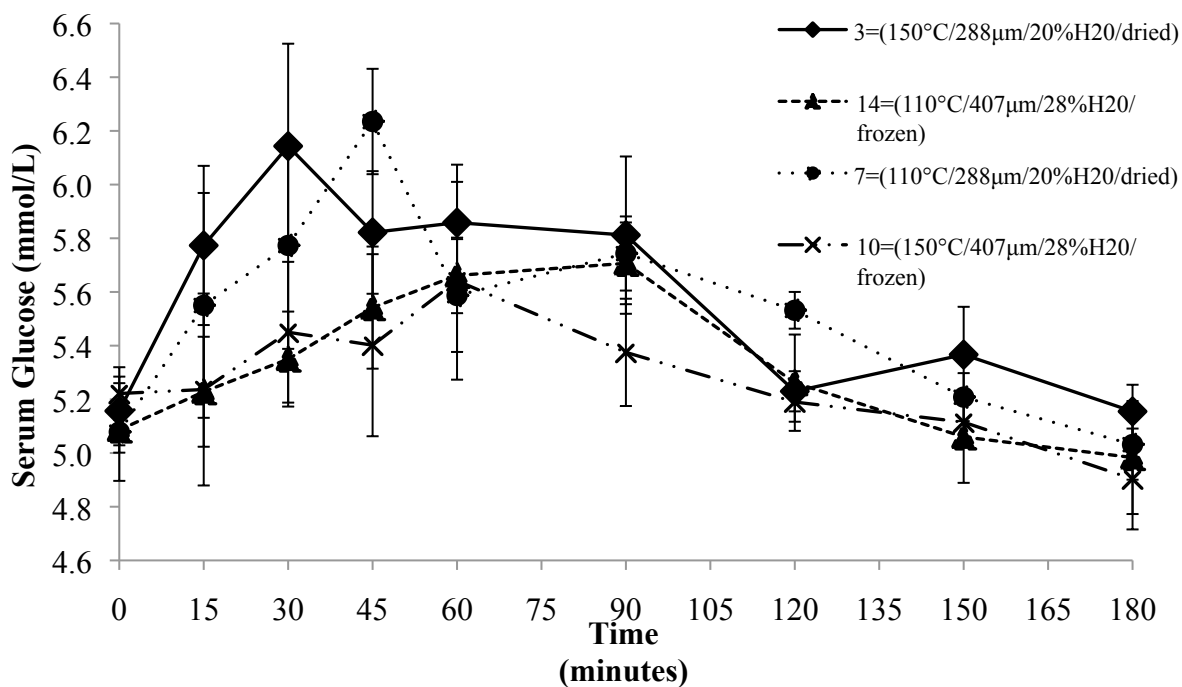


Figure 4.2 Glycemic response curves of the four extruded treatments.

Table 4.9 Pearson correlation (r) coefficients between temperature, particle size, starch fractions and glycemic index.

Parameters	RDS	SDS	RS	GI
Particle Size	-0.577**	-0.667**	0.391	-0.219
Temperature	0.577**	0.333	-0.130	-0.504*
RDS	-	-	-	-0.126
SDS	-	-	-	0.031
RS	-	-	-	-0.093

*Correlation is significant at a 0.05 level

**Correlation is significant at a 0.001 level

5.0 DISCUSSION

The TTADC of pea starch was 81% in the present study. In contrast, Carciofi et al. (2008) reported that the TTADC of pea starch was 98.7% in dogs. This difference may have been due to the processing method used to prepare the peas. The present study used cold extrusion, rather than hydrothermal extrusion processing to make the experimental diets. The temperature, pressure and shear forces the diets were subjected to in the experiment by Carciofi et al. (2008) might have gelatinized pea starch thus, increasing digestibility.

The total tract digestibility of raw pea starch is greater than 99% in pigs (Sun et al., 2006; Stein and Bohlke, 2007) and rats (Fleming and Vose, 1979). However, the digestibility of pea starch in the present study was only 81%. These differences may be due to the anatomy and physiology of the canine intestinal tract. Dogs have a short intestinal tract, small ceca and produce no salivary amylase (Ellison, 1968; Kararli, 1995). These factors might explain not only the low total tract digestibility of starch in dogs but also the lack of change in GI due to particle size. As particle size decreases, surface area and pore volume increase allowing the feed granule to increase water absorption and retention, ultimately increasing the susceptibility of starch to hydrolysis by intestinal amylases (Auffret et al., 1994; Tosh and Yada, 2010).

In the present study, barrel temperature, moisture and cooling rate did not affect the levels of RDS, SDS and RS in pea extrudates. In contrast, a number of studies reported that these parameters affected starch fractions. Unprocessed peas have been shown to contain relatively high proportions of SDS (53.7% to 59.0%) and RS (8.1% to 12.6%) and low proportions of RDS (18.2% to 23.8%) (Chung et al., 2008). Eyearu et al.

(2009) compared the effects of soaking, boiling and pressure-cooking on pea starch fractions. They reported that the percentage of rapidly digestible starch increased from 17.6% in raw peas to 64.2% in boiled peas to 81.8% in pressure-cooked peas. Interestingly, soaking peas in water for 16 hours was found to reduce RDS to 4.4%. In the present study, RDS was below 21% of the total starch content. Although gelatinization is a function of temperature it is also influenced by retention time and moisture content. The limited moisture employed in the present study could have decreased the amount of starch gelatinized. In contrast, Eyaru et al. (2009) employed higher moisture levels and this may have allowed for more complete starch gelatinization. However, a study by Sun et al. (2006) found that extrusion of peas at 145°C increased RDS as a fraction of TS, from 15% in unprocessed peas to 92%. The same study found that SDS decreased from 37% to 4% and that RS decreased from 48% to 4%. Toasting soaked peas at 350°C for 3-4 minutes in a study by Canibe and Bach Knudsen (1997) found similar results to Sun et al. (2006), where toasting peas increased RDS as a fraction of TS from 24.5% to 80.2% and from 35.9% to 64.0%. Canibe and Bach Knudsen (1997) also found that SDS and RS were both reduced with toasting. SDS was reduced from 47.9% to 13.3% and 45.1% to 27.5% and RS was reduced by 27.8 to 6.6% and 19.0 to 8.5% (Canibe and Bach Knudsen, 1997).

Many studies have indicated the importance of processing on the glyceemic response of foods (Jenkins et al., 1982b; Brand et al., 1985; Bornet et al., 1989). The present study found no significant difference between the four extruded pea treatments ($P > 0.05$). A comparable GI of boiled peas was found when averaged from three previous studies 68 ± 7 (Jenkins et al., 1981; Otto and Niklas, 1988; Kurup and Krishnamurthy,

1992; Foster-Powell et al., 2002). Bornet et al. (1989) reported a significant change of the area under the plasma glucose response curve in healthy human subjects from 49 ± 19 for raw pea starch to 143 ± 29 for gelatinized pea starch. Gelatinization is a function of temperature which is intrinsic to starch source ranging from 65°C to above 100°C (Bornet, 1993). Although hydrothermal processing can increase digestibility, excess processing and rapid cooling can allow crystalline complexes to reform between amylose and amylopectin molecules (Åkerberg et al., 1998; Spears et al., 2004). Retrogradation may have occurred lowering the GI of the extruded pea treatments. Although not significant, treatments 3 and 10 had the lowest GI of 47.7 ± 4.3 and 26.6 ± 7.1 , respectively. The only similarities these two treatments had were that they both were extruded at 150°C . It can be hypothesized that the low GI reflected a high retrogradation or recrystallization as compared to the other two treatments, 7 and 14, which were extruded at 110°C . Brand et al. (1985) performed a study comparing the glycemic responses to conventionally cooked food versus highly processed convenience food. Brand et al. (1985) discovered that with increased processing of corn, rice and potato, increased digestibility and GIs were observed, except potato chips did not have significantly higher GI than their conventionally cooked counterpart thought to be due to intrinsic factors such as amylose-lipid complexes. A study by Lankhorst et al. (2007) draws parallel conclusions to Brand et al. (1985) confirming that there was an increase in carbohydrate digestibility and glucose absorption post heat extrusion as well as post-temperature and moisture increase (Lankhorst et al., 2007; Carciofi et al., 2008). Freezing and toasting have also been shown to significantly decrease glycemic response (Burton and Lightowler, 2008). Since each of the four products tested was either oven

dried or frozen, these processing methods could have played a role in starch retrogradation and reformation of resistant starch. Ranawana et al. (2010) also reported an inverse correlation between particle size and RDS content. This agrees with the results of the present experiment. An increase in RDS with decreasing particle size would be predicted to increase GI, as glycemic response is hypothesized to be a function of available starch and digestibility (O'Dea et al., 1981, Jenkins et al., 1982b, Mourot et al., 1988, Rosin et al., 2002; Sola-Oriol et al., 2010).

Glycemic testing is time consuming and expensive, thus many studies have attempted to correlate *in vitro* glycemic testing with *in vivo* starch digestibility and degradability rates. O'Dea et al. (1981) found that rate of starch hydrolysis *in vitro* with pancreatic amylase correlated strongly with peak glucose response. Thus, while not completely interchangeable, the correlations between starch degradability, digestibility, *in vitro* starch fractions and GI indicate that *in vitro* assays should be a useful predictor of glycemic responses. However, these correlations are measured in human studies and there appear to be significant species differences affecting this model.

Glycemic responses are a function of the rate of starch digestion and absorption. Many studies have found that altering starch digestibility or gastric retention time alters glycemic responses (Jenkins et al., 1987a). Starch degradability and digestibility can be predicted by *in vitro* assays, such as the Englyst assay used in the present study. Wolever et al. (1991) found that the fractions RDS and SDS are related to the GI ($r^2 = 0.62$). Conversely, Priebe et al. (2008) and Kim et al. (2003) have correlated the RS fraction with glucose response. Wolever et al. (1991) correlated RDS with an increasing glucose response. Since digestibility is correlated with both GI and *in vitro* starch fractions, it

was hypothesized in this study that RDS could be positively correlated and RS inversely correlated, with GI. However, the present study found that RDS, SDS and RS were not correlated with GI ($P > 0.05$). Rather, RDS and RS were negatively and positively correlated, respectively with particle size ($P < 0.05$). Furthermore, GI was negatively correlated with extruder barrel temperature, but temperature was positively correlated with RDS ($P < 0.05$). In this study, RDS was the smallest fraction of the starch in field pea and this may explain why it was not correlated with GI. Other studies using glucose, starch or sucrose found low correlation with the glycemic index ($r^2 = 0.17$) (Englyst et al., 1999). Furthermore, gastric retention time is not accounted for by the Englyst Method, which may cause an over estimation of glucose absorption. A recent study by Van Kempen et al. (2010) found that when Englyst starch fractions are corrected for gastric emptying time, they form a linear regression with glucose appearance and are able to predict glucose appearance up to 8 hours, post-prandially. Also, a study by Regmi et al. (2010) found that rapid, moderately rapid and moderately slowly digestible starches had differing 12-hour cumulative glucose absorption in pigs in comparison to slowly digestible starch. This study also found that rapidly digestible starch had negative net glucose absorption after 8 hours indicating a high utilization of glucose by intestinal tissues (Regmi et al., 2010). This study may indicate that a 3-hour glycemic testing may not allow adequate time to measure the full glucose response of a high amylose starch such as peas. In support of this, Weurding et al., (2001) reported that *in vitro* digestion of starch for 2 hours was not sufficient to accurately model starch digestion in the distal ileum of broiler chickens. They reported that a 4-hour incubation was required to predict ileal starch digestibility. This may explain why the present study did not find any

correlation between RDS, SDS or RS and GI. Alterations to the incubation times for starch samples need to be made to predict GI in dogs.

6.0 GENERAL CONCLUSION

Canine obesity is a problem, in part due to types of ingredients and diets used for dogs. Assessment of ingredients using *in vivo* methods is expensive and useful *in vitro* methods that can predict glycemic responses are essential to improve our ability to do this type of research. The Englyst methodology (1992) was not correlated to GI in the present study, meaning it is not a good predictive model to use in dogs. Rice, a commonly used carbohydrate in pet food manufacturing, had a much higher TTADC than did peas. Peas will therefore provide less glucose to the canine due to a lower starch digestibility compared to rice. Peas, even with processing, elicit only moderate glycemic responses and therefore are a good alternate carbohydrate source for use in canine diets. The choice of starch sources, particle size and processing techniques in both human and animal foods has the potential to create foods/feeds with improved glycemic properties.

Much biological variation exists between and within dog species, and there is limited information on canine gastrointestinal physiology and function. Large and small breed dogs are known to have different gastrointestinal size (3-4% large 5-6% small), which can be hypothesized to alter starch digestion and glucose absorption capabilities (NRC, 2006). There is little information on the production and activity of intestinal amylase in dogs and this is essential to our understanding of starch digestion. Research investigating physiological differences between breeds would be beneficial to formulate feeds to optimize the formulation of dog foods.

The use of low GI diets to control obesity in dogs is the long-term goal of this research. However, the use of these diets may still not overcome the issue of overfeeding by owners and concomitant obesity. Despite this, low GI diets may still be beneficial to canine health. Controlling glucose homeostasis is an important aspect in controlling diabetes, coronary heart disease, cardiovascular disease, stroke, musculoskeletal problems and some forms of cancer in humans (Kahn and Flier, 2000; Mayer-Davis et al., 2001; Gayet et al., 2004; Flight and Clifton, 2006; Lee et al., 2008; O'Keefe et al., 2008; Khaodhiar et al., 2009; WHO, 2009). Although low glycemic diets have been shown to be successful in human disease prevention and alleviation, currently no research exists indicating that low GI diets are capable of reducing companion animal obesity and related disease. Future studies need to look into the impact of low and high glycemic diets on weight regulation, satiation and metabolic disease risk factors. In order for low GI diets to be feasible for companion animals, research must prove their efficacy on disease. At this time, no recommendation can be made to whether decreased carbohydrate availability in companion animals diets is beneficial. However, the use of low GI diets has the potential to improve the length and quality of life in dogs.

An inadequacy of this study was that field pea was used a single ingredient during processing. Processing and extrusion of peas alone is not indicative of the chemical processes that would occur during conventional pet food processing. Pet food ingredients typically included in formulations would have a definite effect on pea starch gelatinization, retrogradation and corresponding glycemic response in animals. As mentioned, nutrients such as fat and protein are capable of forming complexes with

carbohydrates that are known to affect digestibility. Future studies need to address full diet processing and the interactions of ingredients on glycemic response.

Current validated *in vitro* techniques to measure *in vivo* physicochemical characteristics of feeds are few and far between. Animal research is already very controversial and is by no means getting any cheaper. High levels of variability between animals in the current study make it hard to support the use of small sample size canine trials. However, with most of the human population owning at least one companion animal, there is a huge economic factor involved with developing diets to promote the health and longevity of our faithful companions. With technology such as near infrared spectroscopy at our fingertips, the time has never been greater to develop models and calibrations between *in vitro* and *in vivo* testing.

Our understanding of how the chemistry and structure of starch affects glycemic responses is also quite rudimentary. More research is required to improve our understanding of the physiological effects of starch digestion rates in dogs. This research is likely applicable to human and agricultural animal nutrition as well. Furthermore, while the use of low-GI ingredients is one way to control glycemic responses, we also need to improve our knowledge of how to alter the GI of dog food by changing processing methods. Most dog food is extruded. Modern extruders provide a versatile and tool for altering the chemistry and physical form of dog food and may allow fine control of starch degradability rates. This would allow the use of a wide variety of starch sources in dog food while still maintaining desirable GI in canine diets.

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