DETERMINATION OF THE EFFECTS OF PULSE STARCHES VERSUS CORNSTARCH ON GROWTH PERFORMANCE AND GLUCOSE TOLERANCE IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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ABSTRACT

Carbohydrates are the major energy source for humans and terrestrial animals. However, in fish, protein and lipid are more efficiently used for energy than carbohydrates. Despite this, studies have shown that diets with an appropriate amount of carbohydrate improve growth in fish. Therefore, as a first step in examining what is already known about carbohydrate use in fish, a meta-analysis was performed. Three parallel meta-analyses were conducted to determine the effect dietary carbohydrate inclusion level on final weight (FW) of carnivorous finfish. The first meta-analysis examined the effect of starch as the primary component of carbohydrate from gelatinized and precooked cornstarch, gelatinized tapioca starch and gelatinized potato starch on fish growth. The overall effect size was 0.42 and *P* value was 0.65. The second meta-analysis examined the effect of glucose, maltose and dextrin on fish growth. The overall effect size was 0.39 and *P* value was 0.69. The third meta-analysis examined the effects of native starches (corn, tapioca and potato starches) on fish growth. The overall effect size was 0.79 and *P* value was 0.43. In conclusion, all three studies showed no significant difference in final weight between control and diets with carbohydrate inclusion up to 280 g/kg in most carnivorous fish species. Based on these meta-analyses, work in this thesis then used fish growth trials using the common commercial species, rainbow trout (*Oncorhynchus mykiss*).

The overall hypothesis of this thesis was inclusion of a better starch source like pulses would improve fish growth by improving post-prandial glycemic control. In this project, three experiments (digestibility, growth performance and glycemic index testing) were carried out to investigate utilization of pulse starches (pea, lentil and faba bean) versus modified cornstarch in rainbow trout diets. In the first experiment, there were significant differences in apparent digestibility coefficients (ADCs) of macronutrients among starch ingredients ($P < 0.05$). Protein digestibility was much higher than starch digestibility for pea, lentil and faba bean starches, while the ADC of crude protein in modified cornstarch was not measurable. Digestibility of most starches was low in rainbow trout (6% for pea starch, not detectable for faba bean, 36% for modified cornstarch), while lentil starch digestibility was inexplicably higher (55%). Similar trends were evident for protein, fat and energy digestibility in trout, with negligible digestibility for faba bean starch, low digestibility for modified cornstarch or pea starch (<30%), but high digestibility for lentil starch.

Since digestibility of faba bean starch was not detectable in trout, faba bean starch was not examined in the subsequent growth trial. The effects of modified cornstarch, pea starch and lentil starch on growth performance were investigated at control, 100 and 200 g/kg starch inclusion. After 8 weeks, fish had doubled their weight and regression model analysis showed that starch inclusion up to 200 g/kg did not significantly affect the average daily gain (ADG), specific growth rate (SGR), average daily feed intake (ADFI) or feed conversion rate (FCR). The one exception was the diet with lentil starch inclusion, which showed significant quadratic relationship (P = 0.03), with 100 g/kg inclusion having the best FCR at 0.90.

The third experiment was aimed at establishing glycemic index (GI) values for pulse versus cornstarch in fish maintained on normal commercial diets, but fasted for 48 hr. Baseline plasma glucose (pre-feeding) ranged from 4.5 ± 0.29 mmol/l and increased to a peak of 12.6 ± 1.41 or 9.8 ± 0.86 mmol/L, respectively at 24 hours after force-feeding glucose (a reference) or unmodified cornstarch. In contrast, plasma glucose levels continuously increased over 96 hours after feeding pulse starches or modified cornstarch, ranging from 20.3 mmol/l to 25.8 mmol/l at 96 h. There were significant differences among starches in peak, time to peak, and area under the curve or glycemic index among treatments. The glycemic index of unmodified cornstarch in rainbow trout was below 100 whereas GI values of pulse starches and modified cornstarch were all higher than 100, reflecting the severe and prolonged hyperglycemia that was observed.

Taken together, digestibility of raw pulse starches and modified cornstarch was poor in rainbow trout, but yet produced paradoxically high and prolonged hyperglycemia. The GI model from human and other related mammals does not fit rainbow trout. While the work in this thesis cannot explain benefits of using pulse starches in trout diets based on postprandial hyperglycemic or glycemic index, this thesis clearly showed that pea or lentil starches can be included in rainbow trout diets up to 200 g/kg. More studies are needed to find the upper tolerable limit for pulse starch inclusion in rainbow trout diets and to better characterize how trout utilize these starches for growth.

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LIST OF ABBREVIATIONS

ADC = Apparent digestibility coefficient

ADFI = Average daily feed intake

ADG = Average daily gain

ANOVA = Analysis of varies

ATP = Adenosine triphosphate

CP = Crude protein

DM = Dry matter

FAO = Food and agriculture organization

FCR = Feed conversion ratio

GE= Gross energy

GI = Glycemic index

GLUTs = Glucose transporters

IAUC = Integrated area under the curve

ND = Not detectable

SEM = Standard error of the mean

SGLTs = Sodium glucose transporters

SGR = Specific growth rate

WDDGS = Wheat distillers dried grains with solubles

1. INTRODUCTION

Over the past 30 years, aquaculture has been the fastest developing food production sector in the world (Stone, 2003). Aquaculture production has increased at the rate of 9.2% per year compared to 1.4% for wild fisheries and 2.8% for terrestrial farmed meat production (Stone, 2003). The increased production in aquaculture requires a parallel rise in the production of aquafeeds (Stone, 2003). Fishmeal is the primary protein source for aquaculture diets and aquaculture currently uses approximately 75% of total supply. As the demand for fishmeal increases, its price has also gone up (Stone, 2003). This lack of available supplies and increasing costs means aquaculture feeds must replace fishmeal to support the growth of the aquaculture industry. Vegetable ingredients are potential protein and energy sources in fish diets due to their large yields and lower market cost (Rodrigues et al., 2012). Carbohydrates are the main component of plant material (Stone, 2003) as well as the least expensive energy source in fish diets (X-J Cui et al., 2010). In general, carbohydrates are the major energy source in human and domestic animal diets (Kim and Kaushik, 1992). Wild salmonids consume high protein, low carbohydrate diets, with dietary protein used not only for growth but also as a major energy source (Refstie and Austreng, 1981). However, the inclusion of carbohydrates in fish diets would reduce the total feeding costs of fish production (Stone, 2003). Thus, maximizing the amount of carbohydrate in fish diets could be economically advantageous (X-J Cui et al., 2010). Salmonids, like rainbow trout (*Oncorhynchus mykiss*), are high trophic level, carnivorous fish species and desired by the market in Western countries, so their nutrition has been widely studied (Krogdahl et al., 2005; Furne et al., 2005).

Generally, the ability of teleosts to utilize carbohydrate is viewed as limited compared with mammals (Legate et al., 2001). Furthermore, the capacity of fish to utilize carbohydrates varies widely between and within fish species related to their feeding habits (Kamalam et al., 2012). These variations are also associated with the dietary formulation, water temperatures, the complexity of carbohydrate, the carbohydrate level in diets and fish size to name a few factors (Stone, 2003). Although carnivorous fish species are commonly thought to have a poor ability to using dietary carbohydrate (Kamalam et al., 2012), if provided at an appropriate level, carbohydrate can support good production in salmonid diets (Kim and Kaushik, 1992). In a previous study, the addition of 30% gelatinized starch in rainbow trout diets spared protein energy and reduced nitrogen egestion (Kaushik and Oliva-Teles, 1985). Omnivorous fish species can utilize a higher content of carbohydrate than carnivorous fish (Valente et al., 2010). The primary reason is unknown,

but could be due to higher amylase activity in the digestive tract or higher affinity of insulin receptors in the body of omnivorous compared to carnivorous fish (X-J Cui et al., 2010). Moreover, omnivorous fish tend to have longer intestinal tracts than carnivorous fish (Kramer and Bryant, 1995). A typical omnivorous fish species, Nile tilapia (*Oreochromus niloticus*), has a long intestine, which increases the capacity to digest various foods, including starches (Rodrigues et al., 2012). The optimal digestible level of starch inclusion for marine or cold-water fish is less than 20%, while warm-water fish can utilize higher levels (Wilson, 1994). There is a large variation in starch digestibility among fish species, particularly for native starches where digestion coefficients are often <40% (Bergot and Breque, 1983). Starch origin, starch physical state and dietary inclusion levels are three main factors influencing starch digestibility in fish (Stone, 2003). Processing methods such as extrusion or cooking which gelatinizes starches is known to improve starch digestibility in all fish species (Kim and Kaushik, 1992; Bergot and Breque, 1983). Despite this knowledge, exact values for digestibility of carbohydrates remain unclear in trout due to the persistent view that fish cannot utilize starches (Bergot, 1979).

The utilization of carbohydrate-rich diets affects hyperglycemia and depends on glucose load (Herme and Hansen, 1998). Carnivorous fish species like rainbow trout were previously considered to be glucose intolerance because of prolonged hyperglycemia observed after ingesting a carbohydrate-rich diet or a glucose load (Moon, 2001). Persistent hyperglycemia has also been demonstrated to negatively affect fish growth (Moon, 2001). Hyperglycemia can induce oxidative stress, which may adversely affect function of a variety of organs. Some of these adverse effects of prolonged hyperglycemia may be attributed to methylglyoxal, a toxic glucose metabolite, which was found to be elevated in the post-prandial period after ingesting simple, but not complex dietary carbohydrate and was associated with increased oxidative stress in dogs (Adolphe et al., 2012). Carnivorous fish exhibit weak competence in using absorbed glucose (Kaushik et al., 1989), while omnivorous fish not only absorb glucose more efficiently, but also clear glucose faster than carnivorous fish (Stone, 2003). Thus, the aim of this thesis is to examine whether using a better starch such as the slowly digestible pulse starches, can improve hyperglycemia and lead to greater tolerance of starch inclusion in rainbow trout diets.

The glycemic index is the relative rate of starch digestion in the bloodstream compared with a reference carbohydrate (Bell and Sears, 2003). Previous studies have illustrated that carbohydrates with a high glycemic index have negative effects on hunger, obesity, diabetes and heart

disease in humans (Bell and Sears, 2003). Research on the postprandial glucose response to carbohydrate-rich diets has also shown that low glycemic index foods have delayed carbohydrate absorption. This leads to reduced post-prandial blood insulin and glucose compared to high glycemic index foods in mammals (Frost and Keogh et al., 1996). In humans, corn and wheat are defined as high glycemic index (>70) foods, but these are two of the most widely used starch sources in feed formulations (Hamid et al., 2011). When rats were fed chronically with diets containing gelatinized wheat or corn, there was increased insulin and glucose blood levels in the post-prandial period, indicative of insulin resistance (Hemre and Hansen, 1998). In comparison, low glycemic index carbohydrates have a positive influence in reducing the risk of weight gain and chronic diseases such as cardiovascular disease and diabetes in humans (Bell and Sears, 2003). Certain legumes such as field peas, chickpeas and faba beans, all low glycemic index food in mammals, contain high concentration of slowly digestible or resistant starches, thus should be explored as a substitute for wheat or cornstarch in fish diets (Adamidou et al., 2009). Adamidou and Nengas et al. (2009) demonstrated that when legumes, including field peas, chickpeas and faba beans were fed to European seabass (*Dicentrarchus labrax*), all growth was improved in test diets compared with the control diet (wheat diet). Moreover, there were no adverse effects on body composition or pathology to organs such as liver and kidney in legume-fed seabass. Therefore, low glycemic index carbohydrate sources may play beneficial effects on both human and animal health. However, research focused on the determination of glycemic index of different carbohydrate sources in fish using the same rigourous methodology used to determine glycemic index values for humans has not been used in fish to date. Moreover, there is a need to systematically examine the sparse and inconsistent body of literature reporting on starch use in aquaculture diets. Therefore, the first aim of this thesis was to perform a meta-analysis of the scientific literature reporting on starch inclusion and effects on growth in aquaculture species. The second aim of this project was to perform laboratory experiments to measure digestibility of starches in rainbow trout and determine whether higher starch inclusion supported better growth performance, comparing pulse starches (pea, lentil and faba bean starches) to cornstarch. Finally, this thesis project utilized methods adapted to fish physiology, but based on rigorous methods employed in humans, to measure glycemic response of these starches to investigate whether glycemic index relates to digestibility or growth performance in fish.

2. LITERATURE REVIEW

2.1 Starch introduction

Starch is the primary source of stored carbohydrate found in all higher plants and also the second most plentiful biomass material in the world. (Thomas and Atwell, 1999; Bastioli et al., 2014; Dedeh and Sackey, 2002). It is also a major source of energy in animals and human (Naguleswaran et al., 2014). In nature, starch is produced through photosynthesis of plants and vegetables thus can be obtained from plant tissues, organs such as roots, stalks and crop seeds (Bastioli et al., 2014; Eliasson, 2004; Dedeh and Sackey, 2002). Crops are the most crucial starch used in food and feed industries. For instance, corn, wheat, potato, tapioca and rice are common crops used a major starch sources. Some non-starch byproducts can also be produced by refining of these crops, including oil, bran, simple sugars, gluten and ethanol (Bastioli et al., 2014). In North America, the primary source of starch used in the feed and food industry is currently corn at 82% of all starches, followed by wheat (8%), potatoes (5%) and cassava (5%). In 2010, the overall yield of corn was around 800 million tonnes worldwide. The largest producer was the USA at 331 million tonne and China the second largest producer at 158 million tonne. In the USA, 39.4% of corn was utilized as feed ingredients for animals and 34.9% was converted to ethanol for beverages or fuels in 2010 (Bastioli et al., 2014). In Asia, however, differing prices and availability of different starch sources led to food and feed industries utilizing mainly sago, tapioca or corn as starch sources for glucose production (Schenck and Hebeda, 1992). Thus, cornstarch is a leading starch source for conversion because of its outstanding yields of corn plant. It has been reported that 70% of the worldwide production of cornstarch is converted to glucose/fructose-containing sweeteners (Schenck and Hebeda, 1992).

Starch cannot only be used in food industries, but also widely utilized in pharmaceutical and paper industries (Bastioli et al., 2014). It has been reported that around 60 million tons of starch are extracted from various cereal, tuber and root crops every year. Of this, 60% is used in foods while 40% is used in pharmaceuticals as well as non-edible purposes such as paper, fabric and building materials (Copeland et al., 2009). Furthermore, byproducts of corn-derived glucose or fructose such as ethanol can be used as fuel sources (Bastioli et al., 2014). Generally, starch is an inexpensive, renewable and easily obtained resource. However, the price of corn fluctuates due to changes in commodity prices of its byproducts since ethanol competes with the wildly fluctuating markets for other natural and fossil fuel resources in recent years (Bastioli et al., 2014).

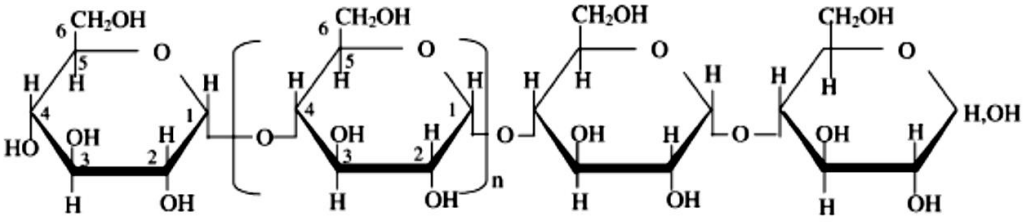
2.1.1 Starch structure

At the level of basic carbohydrate chemistry, starch is a polymer of the D-glucose (Thomas and Atwell, 1999). D-glucose is a six-carbon sugar, which is also known as the "building block" of starch (Thomas and Atwell, 1999). There are two structures of D-glucose: an open chain and a ring form. The ring form is also called pyranose such as D-glucopyranose (Thomas and Atwell, 1999). Starch is laid down in a semi-crystalline granular form in plants (Vermeylen et al., 2004). The diameter of granules in different starches varies, ranging from 0.5 to 175 μm and its shape can be spheres, ellipsoids, polygons, platelets or irregular tubules (Schenck and Hebeda, 1992). Early research that examined the structure of starch found that these semi-crystalline granules are primarily made of two components: amylose and amylopectin (Figure 2.1) (Jenkins and Donald, 1995; Tester et al., 2004). Both components consist of D-glucopyranose, which is linked together by α-1, 4 and α-1, 6 glycosidic bonds (Thomas and Atwell, 1999). The aldehyde group at carbon number 1 on D- glucose is highly reactive and makes it a reducing sugar. Therefore, in forming these linkages, the carbon number 1 on a D-glucopyranose reacts with the carbon number 4 or 6 from the next D-glucopyranose (Thomas and Atwell, 1999). As the aldehyde group on one end of starch polymer is always free, this is referred to as the reducing end and the other end of starch polymer is called non-reducing end (Thomas and Atwell, 1999). The glycosidic bonds in starch is alpha (α) form and this make certain starch polymers forming helical structures (Thomas and Atwell, 1999). The alpha configuration and helical structure of starch polymers make its distinct properties and play a vital role in enzyme digestibility (Thomas and Atwell, 1999).

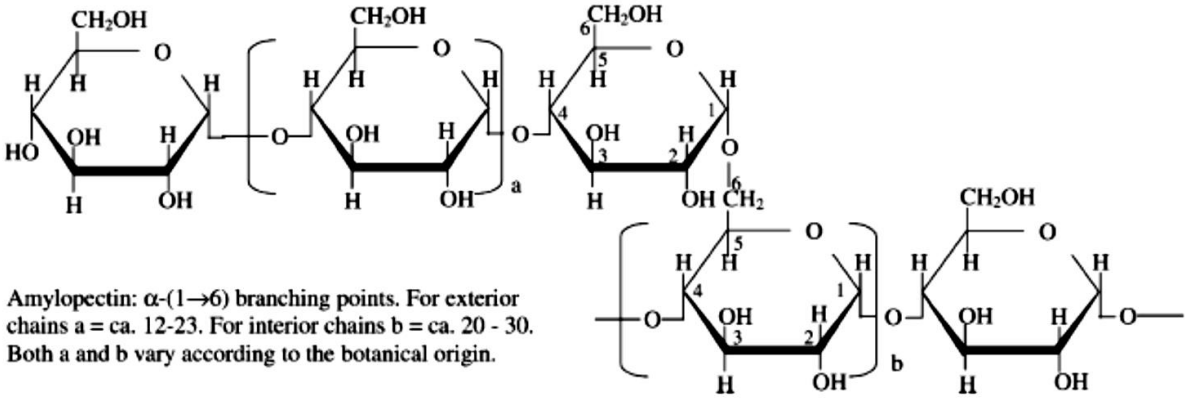
The molecular weight of amylopectin is 10^4-10^6 kDa while that of amylose is much smaller at 100 kDa (Svihus and Uhlen, 2005). Amylopectin is the principle component of most starch and the content is 80% in common native starches (Jenkins and Donald, 1995). Highly branched amylopectin is formed from a series of short chains involving α-1, 4 bonds that are linked together at their reducing end by α-1, 6 linkage (Eliasson, 2004; Garg et al., 2008). In contrast, amylose is a fully linear structure with little to no branching, consisting of either one or a few long chains of α-1, 4 linkage (Eliasson, 2004). In most common native starches, the content of amylose is 20-30%. Some types of plants contain less or even no amylose such as waxy starches while the level of amylose is higher in other types (Eliasson, 2004). The semi-crystalline form of starch granules relates to the amylopectin component. The short amylopectin chains form double helical structures and bond into clusters. These clusters combine to create a structure that has commutative

crystalline and amorphous lamellar composition (Figure 2.2) (Jenkins and Donald, 1995). This alternating amorphous and crystalline lamellar structure is ~9-10 nm in diameter (Gallant et al., 1997). The molecules in the crystalline regions form a lattice array while the molecules are arranged unordered in the amorphous regions (Radley, 1968). Swelling is limited in the crystalline regions and they are more resistant to chemical attack, while amorphous regions swell easily in water and are more reactive (Radley, 1968).

The type of starch granules can be classified depending on the content, structure and organization of amylopectin versus amylose and the degree of crystallinity (Copeland et al., 2009). In terms of X-ray diffraction, 26-45% of crystallinity is found in most common starches while those starches with high amylose content generally have only 10-15% crystallinity (Schenck and Hebeda, 1992). Based on the relationship between the crystalline structure and the length of amylopectin chains constituting the clusters (referred to as A-chain in Figure 1c), three types of crystalline structure are identified. A-type crystallinity is linked with short A-chains such as that found in cereal starches. B-type is linked with longer A-chains, for example potato starch, while C-type is linked with intermediate length A-chains such as legumes (Jenkins and Donald, 1995; Gallant et al., 1997). The greatest proportion of amylose is generally located in the amorphous regions, although some amylose may co-crystallize with amylopectin in crystalline regions (Jenkins and Donald, 1995; Eliasson, 2004). When amylose is synthetized in the presence of polar lipid, surfactants, normal alcohols or iodine, a fourth starch structure, type V, can also be detected. Type V starch occurs together with type A, B, C in high amylose content starches and in certain genotypic cornstarches (Schenck and Hebeda, 1992).



Amylose: α-(1→4)-glucan; average n = ca. 1000. The linear molecule may carry a few occasional moderately long chains linked α-(1→6).



Amylopectin: α-(1→6) branching points. For exterior chains a = ca. 12-23. For interior chains b = ca. 20 - 30. Both a and b vary according to the botanical origin.

Figure 2.1 The structure of amylose and amylopectin (Tester et al., 2004).

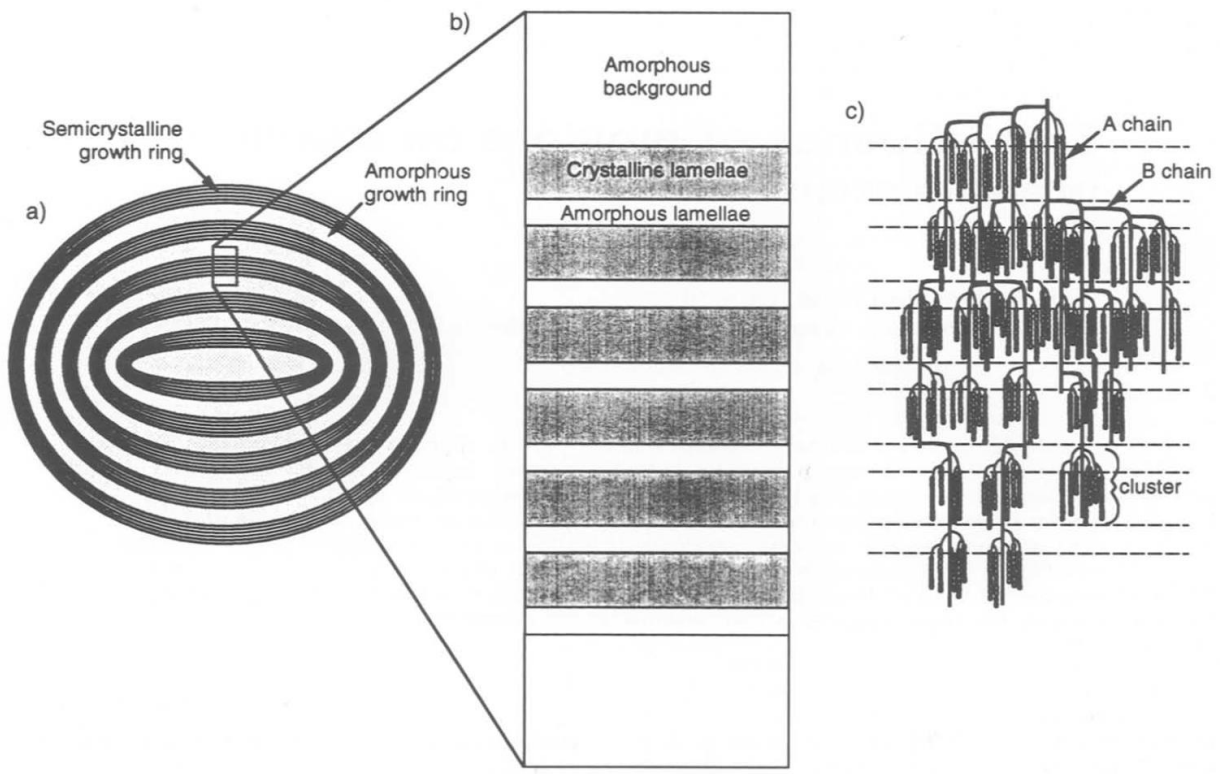


Figure 2.2 Graphical diagram of a starch granule structure. (a) A concentric rings of a starch granule consisting of commutative amorphous and semi-crystalline growth lamella. (b) (c) Expanded view of the internal structure. The semi-crystalline growth ring includes stacks of amorphous and crystalline lamella. Amylopectin with double helices (A chain) combine together to become clusters and those clusters further pack into crystalline lamellae. B chain of amylopectin provides a connection between clusters. Branching points for both A and B chains are predominantly located within the amorphous lamellae (Jenkins and Donald, 1995).

2.1.2 Starch properties

The structure and functionality of various starches are different, which causes the diversity of starch properties between and within plants (Copeland and Blazek et al., 2009). While amylose is described as a linear structure, it is found either as single helices or double helices in the native state (Svihus and Uhlen, 2005). Amylose is hydrophobic due to the hydrogen atoms in the helix (Thomas and Atwell, 1999). The helical form allows amylose to complex with lipid and this inclusion complex changes the properties of starch, including texture (Thomas and Atwell, 1999; Svihus and Uhlen, 2005). A noted capability of amylose is gelation, which is the consequence of re-organizing or retrogradation of solubilized starch polymers after starch has been cooked (Thomas and Atwell, 1999). Because of the highly branched and unique crystalline nature of am-

ylopectin, the properties between amylose and amylopectin are quite different (Thomas and Atwell, 1999). Delayed gelation and slow retrogradation happens after cooking amylopectin. Typically, non-gelling occurs in starch that consists of only amylopectin such as waxy maize (Thomas and Atwell, 1999). In general, the ratio of amylose and amylopectin in starch is of vital importance to its properties and also influences the shape of starch granules (Thomas and Atwell, 1999; Copeland et al., 2009). Gelatinization is one of the predominant characteristics of starch and happens under the combination of heat and sufficient water. In this process, the molecular order within starch granules is broken down, resulting in an irreversible disruption in properties of starch granules (Thomas and Atwell, 1999; Eliasson, 2004; Svihus et al, 2005). The changes occur in a predictable sequence of events, first granule swelling, then water absorbability, crystallinity melting, loss of birefringence, amylose leaching and finally starch solubilization (Eliasson, 2004; Thomas and Atwell, 1999). Gelatinization of starch depends on temperature, moisture, granule type as well as cooking conditions. Gelatinization temperature differs with respect to starch sources (Thomas and Atwell, 1999). In general, the gelatinization temperature ranges between 60 and 80 degrees, but there is a negative relationship between the amylose content and gelatinization temperature or peak viscosity in starch (Copeland et al., 2009).

The starch is referred to as “pasted” after gelatinization and this pasting process produces increasing viscosity (Thomas and Atwell, 1999). During the heating process, granules swell and water is absorbed into the amorphous regions of starch granule, which destroys the crystalline area until eventually the crystallinity of starch granules is lost (Copeland et al., 2009; Gallant et al., 1997). Amylose starts to leach from the granules and viscosity increases. The viscosity reaches to a maximum (peak viscosity) while the peak percentage of swelling is occurring in starch granules (Thomas and Atwell, 1999; Copeland et al., 2009). As heating continues, the viscosity decreases following peak viscosity because the starch granules break down as polymers are dissolved (Thomas and Atwell, 1999; Copeland et al., 2009). After pasting, when starch cools again, viscosity and texture are once again according to the proportion of amylose and amylopectin. There is a positive relationship between the content of amylose and gel formation (Leloup and Colonna, 1991). For example, dent cornstarch contains higher amylose (25%), so becomes a gel after cooling and has a lower peak viscosity. In contrast, waxy cornstarch contains only amylopectin, so the starch paste does not form a gel and upon cooling, it becomes soft and cohesive instead (Thomas and Atwell, 1999; Copeland et al., 2009).

The process of retrogradation happens after heating and is defined as the reassociation of starch chains in an ordered structure (Thomas and Atwell, 1999). On cooling, starch gels begin to retrograde and ultimately form crystalline aggregates (Thomas and Atwell, 1999). During this process, amylose is more likely to recompound and constitute hydrogen bonds than amylopectin (Thomas and Atwell, 1999). The reassociation of amylose is faster than that of amylopectin, occurring over minutes to hours versus hours to days, respectively (Copeland et al., 2009). With increasing cooling time, water is released from the gel, also known as syneresis, which plays an important role in refrigerated and frozen food products (Thomas and Atwell, 1999). The retrogradation of starch also differs with respect to starch sources. For instant, tapioca starch that contains 19% amylose becomes a soft gel while high amylose cornstarch (40% of amylose) forms a very firm gel after heating and cooling (Thomas and Atwell, 1999). Overall, amylose is primarily responsible for the property of starch gelation whereas amylopectin determines the structure of starch granules as well as starch swelling (Schenck and Hebeda, 1992).

2.1.3 Pulse starch versus cereal starches

Grains and pulses have served as staple foods for thousands of years. Cereals sales were $24.5 billion worldwide in 2008, with the USA being the largest cereal producer, accounting for 64.9% of the market value (Tsao et al., 2012). In Canada, pulse industries have been developing very fast in recent years. For example, in 2006, 2.8 million tonnes of pea were produced in Canada, with 75% from Saskatchewan. In 2005, more than 1.2 million tonnes of lentil were produced, with 95% from Saskatchewan. With increasing production of pulses in Canada, there is both great need and opportunity to expand markets for Canadian pulses. Furthermore, pulses are a crucial protein source in human diets outside North America as well as a good source of B-complex vitamins, minerals and carbohydrates (Huang et al., 2007; Hoover et al., 2010). Starch is the most plentiful carbohydrate in the pulse seed, which contains 22-45% starch (Hoover and Ratnayake, 2002). There are two methods for extracting starch from pulse seeds, either dry milling or wet milling. Dry milling is commonly used in industries for most starch sources, while wet milling is mainly used in the laboratory and produces a higher purity starch (Hoover et al., 2010). One exception to this restricted commercial use of wet milling is corn. Cornstarch production from maize using wet milling is almost exclusively and the highly purified cornstarch product is widely used for food, textile, paper and pharmaceutical industries (Sandhu et al., 2004).

Starch production from different pulse sources is increasingly common and the chemical composition of pulse starch products differs in total lipids, total amylose and nitrogen. Variation in the composition may be affected by the different analysis methods for determination of amylose or lipid content, cultivar differences and the physiological state of the seed. For example, pea starch from smooth peas contains 24-49% total amylose while pea starch from wrinkled peas contains 60.5-88% amylose. Faba bean starch contains 17-42% of total amylose while lentil starch contains 23.5-32.3% amylose (Hoover et al., 2010). In contrast, normal cornstarch generally contains 75% amylopectin and 25% of amylose (Sandhu et al., 2004). Moreover, cereal starches like corn and wheat possess significant morphological differences in starch granules compared to pulse starches. Most pulse starches granules are oval with smooth surfaces, with some spherical, round, elliptical or irregularly shaped granules also found. Generally, pulse starches are simple granules, although compound granules have been found in smooth and wrinkled peas (Hoover et al., 2010). In contrast, cereal starches are less smooth-surfaced granules and shapes differ among cereals. For instance, cornstarch granules are angular-shaped, while wheat starch granules are spherical and lenticular-shaped (Singh et al., 2003).

The crystallinity of pulse starches ranges from 17-34%. Most pulse starches are determined to be a C-type crystalline polymorphic form through X-ray diffraction compared with cereal starches that are A-type. Because of higher amylose content, pulse starches show significant differences in starch properties compared to cereal starches. For example, pulse starches show a restricted swelling and lower extent of amylose leaching, a high pasting temperature and fast retrogradation compared with cereal starches (Ambigaipalan et al., 2011). Moreover, the digestibility of native pulse starches is lower than that of cereal starches *in vitro*. The possible reasons have been attributed to a lack of pores on the granule surface of pulse starches, the higher content of amylose, B-type crystallites and strong interactions between amylose chains. Unfortunately, available information on the molecular structure of pulse starches is still limited. As a result, it is difficult to make a comprehensive comparison of cereal or tuber starch molecular structure with that of pulses (Hoover et al., 2010).

2.2 Starch metabolism in fish

2.2.1 Starch digestion

Starch digestion begins at the surface pores of starch granules and interior channels. After this, amylases enter into the granule center through channels to cause breakdown progressively from the inside out (Svihus and Hervik, 2016). Amylases, isoamylases and glucanosyltransferases are all critical enzymes for the degradation of starch (Rubsam and Krottenthaler, 2012). Amylases are divided into endo-acting α-amylases, exo-acting β-amylases and isoamylases or limit-dextrinases (Rubsam and Krottenthaler, 2012). α-Amylases and α-glucosidases participate in the digestion of starch found in the gastrointestinal tract after a meal (Eliasson, 2004). In monogastric animals, starch digestion happens largely in the small intestine via actions of α-amylases, dextrinase and glucoamylase (Svihus and Uhlen, 2005). While amylase is also found in saliva, the contribution of this amylase source in the digestion of starch is still unclear (Svihus and Hervik, 2016; Svihus and Uhlen, 2005). In non-ruminants, pancreatic α-amylase degrades starch in the duodenum, then α-amylase hydrolyzes α-1, 4 glycosidic bonds of starch molecules to produce free glucose monomers, oligosaccharides and dextrins (Tester and Karkalas, 2004). Some "brush border" enzymes produced in small intestinal epithelial cells include sucrase, lactase and isomaltase. These brush border-derived enzymes further convert disaccharides or available starch into glucose (Tester and Karkalas, 2004). Amylases breakdown α-1, 4 glycosidic bonds and endo-amylase (α-amylase) produce linear and branched oligosaccharides whereas exo-amylase (beta-amylase) produce maltose as well as limit dextrins (Tester and Karkalas, 2004). Glucose is the final product of starch digestion by the action of amyloglucosidase, which specializes in attacking α-1, 4 and α-1, 6 glycosidic bonds (Tester and Karkalas, 2004; Rubsam and Krottenthaler, 2012). Isoamylases breakdown α-1, 6 glycosidic bonds and release linear α-1, 4 chains, while glucanosyltransferases transfer α-1, 4 linkages to α-1, 6 glycosidic bonds (Rubsam and Krottenthaler, 2012).

Starch digestibility is related to several factors including the degree of crystallinity, structure of starch granule, amylose/amylopectin ratio, granule size and other substrates such as lipid and protein on the surface of starch granules (Svihus and Hervik, 2016). Studies have shown that there was a negative relationship between the degree of crystallinity and starch digestion (Bjorck et al., 2000). Research also indicates that a high ratio of amylose to amylopectin reduced the digestibility of starch (Svihud and Uhlen, 2005). In aquaculture, starch such as corn, wheat and pea

has been utilized as energy source in order to limit the utilization of protein as energy provider in fish feed (Bergot and Breque, 1983). Enzymes for starch digestion observed in fish are similar to mammals, but the digestive processes in fish are not as well characterized (Hidalgo and Urea, 1999). In mammals, amylase is produced by both salivary and pancreatic cells, while in fish, exocrine pancreas seems to be the major endogenous source of α-amylase (Krogdahl et al., 2005). Moreover, digestive α-amylase has been observed in the whole gastrointestinal tract of many fish species (Krogdahl and Hemre et al., 2005). Thus, fish appear to have a poor capacity to digest starch in contrast to other domestic animals. Moreover, the digestibility of starch in fish is lower than that in mammals, especially in carnivorous fish species such as rainbow trout (*Oncorhynchus mykiss*; Spannhof and Plantikow, 1983). In general, omnivorous fish species can tolerate higher levels of starch than carnivorous fish species (Wilson, 1994). This may be associated with the activity of amylase in different fish species, as Hofer and Sturmbauer (1985) found that the activity of amylase in the small intestine of carp (*Cyprinus carpio*) was 10-30 greater than that of rainbow trout (Wilson, 1994). Other studies also report that amylase activity in omnivorous fish species is higher than in carnivorous fish (Hidalgo et al., 1999).

The degree of starch polymerization also has an important effect on starch digestibility, with the digestibility of native starch known to be very low in fish (Spannhof and Plantikow, 1983). Furthermore, the level of starch incorporated into fish diets is inversely correlated with the starch digestibility (Spannhof and Plantikow, 1983). Spannhof and Plantikow (1983) showed that amylase activity was inhibited in the fish digestive system due to adsorption of amylase by native starch. This adsorbed amylase led to delayed passage of the starch-containing chyme through the intestine, thereby increasing the time needed for starch digestion and intestinal glucose absorption. These factors may lead a poor digestibility of starch in fish (Spannhof and Plantikow, 1983).

2.2.2 Absorption, transportation and metabolism

The absorption and metabolism of glucose has been widely studied in mammals. Glucose is transported across the mammalian luminal epithelial membrane by an energy-dependent process, specifically secondary active transport, which enables glucose molecules to move against their concentration gradient (Stokes and Fromm, 1964). The same mechanism appears to be present in cold-blooded species such as fish, but available information is limited (Stokes and Fromm, 1964). Once starch is degraded into glucose in the small intestine, glucose is absorbed from the small

intestine especially the terminal end of the mammalian duodenum and jejunum (Tester and Karkalas, 2004). Glucose transporters are separated into two groups based on structure and function: sodium/glucose cotransporters (SGLTs) and facilitative sodium-independent glucose transporters (GLUTs) (Terova et al., 2009). SGLTs can be found in the brush border membrane of mammalian intestinal or kidney epithelial cells and mediate the absorption of glucose. Absorbed glucose is subsequently transported across the basolateral plasma membrane of mammalian epithelial cells by facilitative glucose transporters (GLUTs) to the blood (Teerijoki and Krasnov, 2000).

GLUTs are found in most animal tissues (Terova et al., 2009). Different facilitative glucose transporter subtypes exhibit differences in tissue distribution, substrate selectivity, kinetic parameters and sensitivity to inhibitors (Krasnov and Teerijoki, 2001). There are five glucose transporters (GLUT1-5) that have been identified in mammals (Teerijoki and Krasnov, 2000). Of these, three GLUTs (GLUT1-3) have been identified in birds, but the available information for GLUT subtypes in other vertebrates is lacking (Planas et al., 2000). In fish, research indicates that glucose moves into a variety of cells via GLUT isoforms (Planas et al., 2000). Some fish species are reported to possess facilitative glucose transporters similar to those found in mammals (Mannerstrom et al., 2003). Fish GLUTs are expressed in tissues with high glucose utilization, including skeletal muscle, kidney and gill (Planas et al., 2000). Another study demonstrated that fish liver and brain were also efficient at absorbing exogenous glucose (Mannerstrom et al., 2003). GLUT 1 was found in heart and brain of tilapia (*Oreochromus niloticus*), rainbow trout, common carp and Atlantic cod (*Gadus morhua*) as well as in embryos of brook trout (*Salvelinus fontinalis*; Planas et al., 2000; Terova et al., 2009). GLUT2 is a low affinity transporter, with capacity to transport both D-glucose and D-fructose, making it distinct among GLUTs (Krasnov and Teerijoki, 2001). GLUT2 can be found in mammalina liver, pancreatic **β**-cells, small intestine and kidney, while also found in rainbow trout liver, kidney and intestine (Krasnov and Teerijoki, 2001). Finally, GLUT3 was observed in grass carp (*Ctenopharyngodon idella*) and Atlantic cod, while GLUT4 was found in brown trout (*Salmo trutta*) and Atlantic cod (Terova et al., 2009).

Liver is the main organ mediating glucose homeostasis in fish since it is involved with glucose production (gluconeogenesis), metabolism and storage (Polakof et al., 2010). GLUT2 is responsible for transporting glucose into or out of the liver, thus plays an important role in maintaining glucose homeostasis (Panserat and Plagnes, 2001). Reports indicate that the glucose metabolic

pathway in fish is similar to that in mammals, but occurs at a much slower rate (Figure 2.3) (Wilson, 1994). Glucose is completely catabolized into acetyl-CoA by glycolysis under aerobic conditions, followed by acetyl-CoA entry into Krebs cycle for either ATP production by the respiratory chain or the production of nicotinamide adenine dinucleotide phosphate by the pentose phosphate pathway (Enes et al., 2009; Polakof et al., 2012). Extra glucose could be stored as glycogen through glycogenesis or converted into lipids by lipogenesis (Enes et al., 2009; Polakof et al., 2012). Under fasting conditions or conditions where protein or fat are the primary foods consumed, glucose can be produced and released into the blood from endogenous sources. Glucose is derived from the breakdown of glycogen by glycogenolysis or *de novo* glucose synthesis (gluconeogenesis) from lactate, glycerol or some amino acids (Enes et al., 2009; Polakof et al., 2012). Critical enzymes involved in glucose homeostasis such as phospho*enol*pyruvate carboxykinase, fructose-1, 6-bishposphatase and glucose-6-phophatase, mediate glycolysis, glycogenolysis, lipogenesis and gluconeogenesis and have homologues identified in different fish species (Polakof et al., 2012). In addition, other organs such as kidney and muscle also participate in glucose homeostasis (Polakof et al., 2010).

Although some research exists showing nutrient transportation by the fish gastrointestinal tract is similar to that in other vertebrates, information in fish is incomplete (Polakof et al., 2010). Rainbow trout are capable of storing glucose as glycogen or metabolizing glucose similar to mammals (Polakof et al., 2010). Trout gut has been reported to regulate as well as participate in gluconeogenesis. Moreover, a large amount of intestinal glucose is oxidized to lactate, indicating an important role of glucose in gut metabolism (Polakof et al., 2010). A glucosensing mechanism has also been illustrated in several tissues of rainbow trout (pancreatic and brain tissues), similar to that in mammals, but the details of the fish gut glucosensing mechanism are still unclear (Polakof et al., 2010).

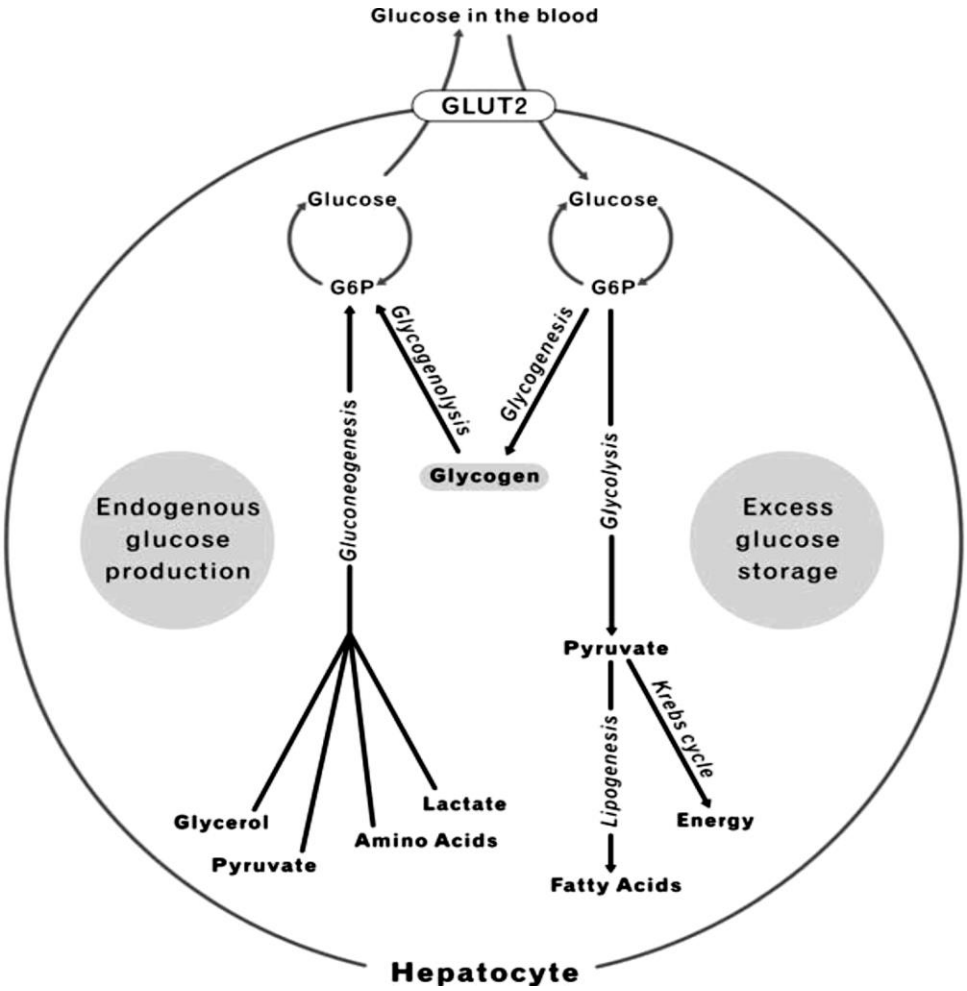


Figure 2.3 The metabolic pathways of glucose in the liver (Enes et al., 2009).

2.3 Glucose tolerance in fish

The term "glucose intolerance" is from the clinical diagnosis of diabetes mellitus in humans. All fish, but especially carnivorous fish species, are commonly considered to be glucose intolerant due to prolonged hyperglycemia observed after ingesting a high carbohydrate meal (Kaushik et al., 1989; Legate and Bonen et al., 2001). Oral and intravenous injections of glucose have been used to investigate glucose tolerance in fish (Legate et al., 2001). Rainbow trout, a strictly carnivorous fish species, is known to have a poor ability to digest carbohydrate. Moreover, several studies report that trout show prolonged hyperglycemia after ingestion of a carbohydrate-rich diet (Lee et al., 2003). Carbohydrate-rich diets not only cause hyperglycemia, but when fed chronically, also cause hepatomegalia and are reported to have a negative effect on growth (Blasco et al., 1996). However, mammalian hyperglycemia and glucose load are also known to be influenced by

carbohydrate source that differ in absorption rates of glucose by the small intestine (Hamid et al., 2011). Early studies in fish stated that the sluggish clearance of plasma glucose might be caused by insufficient secretion of insulin in response to the high plasma glucose or due to insulin resistance. However, later research indicated that the level of insulin secreted in fish is similar to that in mammals (Hrytsenko et al., 2010) and that insulin sensitivity is good in fish (Weber et al., 2016).

There are several possibilities to explain glucose intolerance in fish. First, dietary amino acids are the predominant stimulator of insulin release rather than glucose. Second, there may be a relatively low number of insulin receptors and glucose transporters in fish muscle, but a recent study examining whole body glucose flux in trout argues against this (Weber et al., 2016). Third, fish may have a poor capacity for glucose phosphorylation, leading to dysfunction of the liver in maintaining glucose homeostasis (Enes et al., 2009). In mammals, insulin is vital for regulation of glucose homeostasis whereas its responsibility in fish is controversial (Legate et al., 2001; Weber et al., 2016). Insulin receptors on fish hepatocytes and skeletal muscles are reported, showing that insulin or its receptors are not the problem producing apparent glucose intolerance (Legate et al., 2001). Another potential explanation is limited glucose transporter expression. However, mammalian-like glucose transporters have been found in several fish species, including GLUT 1-4 in Atlantic cod, GLUT 1-2 in rainbow trout and GLUT4 in brown trout. Moreover, these glucose transporters demonstrated similar functions in fish compared to mammals (Hrytsenko et al., 2010). Previous studies showed thatdifferences in kinetics of glucose transporters between fish and mammals were considered as a possible reason for glucose intolerance rather than the lack of a specific glucose transporter type (Hrytsenko et al., 2010). Hyperglycemia is known to have a negative effect on cardiovascular health among healthy and diabetic patients. The link is thought to be that hyperglycemia induces inflammation and produces oxidative stress, both of which could damage all tissues in the body, including the cardiovascular system, kidney, brain and adversely affect metabolism (Node and Inoue, 2009). Methylglyoxal is a reactive intermediate metabolite of glucose and also a precursor of advanced glycation end products, which can cause endothelial dysfunction, atherosclerosis, increase insulin resistance, promote obesity and cataracts (Adolphe et al., 2012). It has been reported that increased methylglyoxal was observed in the presence of chronic hyperglycemia such as diabetes (Adolphe et al., 2012).

2.3.1 Glycemic index

Glycemic index (GI) was first proposed in the 1980s for human foods and it is a simple way to measure the rate of starch digestion in a food as well as its influences on blood glucose responses (Jenkins et al., 1981). The classification of carbohydrates according to glycemic index is thought to be based on the rate of carbohydrate digestion in humans (area under the curve) compared to a standard test food such as pure glucose or white bread (GI value = 100% or 100). Low glycemic index foods are those with GI values below 40 in humans such as dried peas and other pulses, while a GI value between 40 and 70 is considered to be a moderate GI food. In contrast, a GI value above 70 is defined as a high glycemic index carbohydrate, including foods such as corn and wheat (Bell and Sears, 2003). High glycemic index foods generally induce higher postprandial blood glucose and insulin responses that are more rapid compared with low glycemic index foods (Frost et al., 1999; Powell et al., 2002). Based on *in vitro* starch digestion rates, different starches can also be divided into rapidly digested starch such as cooked starch, slowly digested starch like raw starch and resistant starch (Brown, 1996). It is assumed, but not proven, that *in vitro* starch digestibility corresponds to *in vivo* GI responses. In humans, it is known that ingesting large amounts of rapidly digested carbohydrates increases the risk of cardiovascular disease (Lavi et al., 2009). Also, consuming low glycemic index starch has been shown to have a positive effect on diabetes due to improvements in blood glucose regulation, reductions in serum triglycerides and increased duration of satiety after a meal (Granfeldt et al., 1994). Moreover, several human studies report that low glycemic index foods reduce insulin and glucose responses to carbohydrate-containing diets in contrast to high glycemic index foods for a long-term consumption (Frost et al., 1996).

Starch is generally made of two important components: amylose and amylopectin. As indicated earlier, high amylopectin starches are more digestible than high amylose starches. Thus, it is assumed, but not proven, that high content amylopectin starches lead faster, higher post-prandial glycemic and insulinemic responses compared to low amylose content starches (Chen et al., 2013). Studies investigating how starch type affects growth in terrestrial animals have produced mixed results. Some studies indicated positive influences of dietary amylose on growth in pigs, poultry and goats, while other studies showed negative effects in rats, pigs and poultry. In contrast, no influence on growth in rats and pigs were reported in other studies (Chen et al., 2013). In fish, one study reported improved growth of sunshine bass (*M. chrysops * M. saxatilis*) when fed

a high amylose/amylopectin ratio diet (Chen et al., 2013). Any starch in food that cannot be completely digested before fecal excretion is called resistant starch. In humans, resistant starch is considered beneficial due to slower absorption of glucose, thereby reducing the risk of postprandial hyperglycemia (Copeland et al., 2009). Sources of resistant starch in human diets include crude starches, physically enclosed starches or retrograded starch (Granfeldt et al., 1994). Also, covalent or physically-modified starches can reduce GI of carbohydrates and enhance food quality (Kumar and Prabhasankar, 2014). These observations imply low glycemic index carbohydrates, or starches with slow-digesting properties, could mitigate the detrimental effects of hyperglycemia in human as well as animals. The glycemic index of more than 400 foods in human science has been determined (Singh et al., 2006). However, GI values are not available for most other mammalian species. Moreover, in fish, no previous investigation has utilized the appropriate, standardized methodology for GI testing in humans due to large differences in physiology. Even with adaptation to account for species differences, the influence of different starch types on fish post-prandial glycemic response are not well characterized.

2.4 Anti-nutritional factors

Legumes including peas, faba beans and chickpeas, which contain high levels of starch, are potential replacements for wheat starch or wheat flour in fish diets (Adamidou et al., 2009). Previously, starch was added to salmonid diets only as a binder at inclusion rates below 10%, but increasingly is also used as a partial replacement for costly protein in aquafeeds. However, plant-based materials contain a number of antinutritional factors. Many studies focused on the effects of antinutritional factors have been reported in fish (Francis et al., 2001). Antinutritional factors can be divided into four groups: 1. affecting the use and digestion of protein such as protease inhibitors, tannin and lectins, 2. affecting the utilization of minerals such as phytate, gossypol pigments and oxalates, 3. Antivitamins and 4. Miscellaneous toxic agents such as mycotoxins, mimosine and cyanogens (Francis et al., 2001). Antinutritional factors that are heat labile include lectins, while other factors are heat stable such as saponins (Francis et al., 2001). Antinutritional factors can have adverse influences on the activity of digestive enzymes, minerals and absorption of other nutrients (Adamidou et al., 2009). For example, phytic acid is a heat-resistant antinutrient, which can reduce the utilization of phosphorus and some minerals like zinc or magnesium in fish, especially carnivorous species (Denstadli et al., 2007). In addition, the presence of antinutri-

ents may decrease the digestion of starch (Bjork et al., 1994). Potential interactions between different antinutritional factors should also be considered when using plant-based ingredients in aquafeeds. For instance, it has been demonstrated that the interactions of sapanon-tannin, tannin-lectin and tannin-cyanogen decrease the toxicity of individual antinutrient (Francis et al., 2001). Feed processing can have beneficial effects on the quality of antinutrient-containing foods (Fish and Thompson, 1991). Thermal processing can partially or completely eliminate several antinutritional factors (Venou et al., 2003). Processing techniques like soaking, boiling, cooking, dehulling, fermentation as well as extrusion are extensively used to remove some antinutritional factors, thereby improving the availability of plant-based materials such as legumes (Hady and Habiba, 2003). Of these techniques, extrusion has the most merit due to its versatility, large production capacity, but lower operating costs, energy efficiency and shorter cooking times (Hady and Habiba, 2003).

2.5 Feed processing

Raw starch is very useful ingredient in production of many feeds as a texture stabilizer and regulator. However, there are some restrictions such as low shear resistance, thermal resistance and thermal decomposition. Overall, there are three major methods utilized in starch modification: chemical, physical (such as extrusion and ultrasound) and enzymatic methods (Hu et al., 2014). Several chemical modifications have been applied in starch, for example acetylation, hydroxypropylation and cross-linking. Influence of these chemical modifications on starch properties from different plant sources has been examined. Moreover, modified starch with desirable characteristics are widely used in specific food applications in the food and feed industry (Singh et al., 2007).

Pulse and oilseed crops are considered to be promising plant-based ingredients for the aquafeed industry. Pulse products of interest for use in aquafeeds include whole meals as well as fractionated products after dehulling, air classification, aqueous and solvent protein purification to separate pulses into concentrated protein, starch fraction as well as fiber (Thiessen et al., 2003; Drew et al., 2007). Starch utilization is affected by feed processing since the structure of the starch granule or interaction with other components in the feed are changed (Burel et al., 2000). Currently, several feed processing methods are widely used in feed industries, including grinding, steam flaking, pelleting, extrusion and expander processing (Svihus and Uhlen, 2005). The effect

of steam conditioning and pelleting on the availability of starch is insignificant due to low gelatinization (Svihus and Uhlen, 2005). The influence of steam flaking on starch availability depends on the amount of steam added as well as treatment time (Svihus and Uhlen, 2005). Compared with steaming, more than 80g water/kg feed and high temperature (above 100 degrees) are needed during the processing of expander process and 22%-35% gelatinized starch can be produced without damaging nutrients in feeds (Svihus and Uhlen, 2005). Extrusion is the primary feed processing technique utilized in the production of fish feeds since it removes microbial load as well as decreases the content of heat-liable antinutrients in plant ingredients (Kraugerud and Svihus, 2011). Extrusion variables thought to affect starch utilization include retention time in the conditioner, screw speed, moisture level and throughput, die pressure, torque and shear (Kraugerud et al., 2011). During extrusion, sufficient water and higher temperature under high pressure are applied; this produces a large extent of gelatinization and disintegration of starch granules (Svihus and Uhlen, 2005).

Increased digestibility of starch after extrusion processing has been reported in several salmonids studies (Cheng and Hardy, 2003). A pretreatment before extrusion may be beneficial to the quality of plant based feeds for fish, as it could inhibit the activity of heat-liable or pressure resistant antinutrients present in plants such as phytate, trypsin inhibitors and saponins. In addition, pretreatment may improve the physical quality of feeds, including durability, hardness, expansion and the degree of starch gelatinization (Kraugerud and Svihus, 2011). The combination of heat and sufficient water causes starch gelatinization, which in turn causes starch to be more sensitive to amylases in the gastrointestinal tract (Bjorck et al., 1994). Moreover, feed processing may decrease the activity of α-amylase inhibitors. Even pelleting has been reported to decrease amylase inhibitors in wheat bran (Svihus and Uhlen, 2005). Retrogradation may occur in feeds after feed processing and this should be taken into account when feed processing methods are used. Amylose retrogradation is more important compared to amylopectin retrogradation because of the very slow retrogradation time for amylopectin (Svihus and Uhlen, 2005). However, current processing methods do not currently rely on retrogradation techniques to increase resistant starch content in aquafeeds (Svihus and Uhlen, 2005).

Although feed processing improves starch availability, it has some disadvantages. For example, processing might facilitate the development of amylose-lipid complexes that could decrease the starch digestibility. This issue has been reported during extrusion processing (Boekel et al.,

2010). Gelatinized starch is considered to be completely digested in non-ruminants even in carnivorous animals such as mink and fox. However, digestibility of starch, even in the gelatinized form, is still lower for carnivorous fish species like salmonids compared to omnivorous species and mammals (Svihus and Uhlen, 2005). In general, the digestibility of starch in animals can be improved by heat treatment and certain studies show that most fish species can utilize cooked starch better than native starch (Bergot and Breque, 1983). A reaction between reducing glucose and the amino group of amino acids happens during moist heat treatment and storage, known as the Maillard reaction (Deng et al., 2005). The Maillard reaction not only destroys essential amino acids, especially lysine, and causes slow digestibility, but also induces the production of antinutritional and toxic substrates. These Maillard products reduce the quality of dietary protein and feeds (Burel et al., 2000; Deng et al., 2005). However, the available information about the effect of Maillard reaction on the quality of fish feeds is scarce (Deng et al., 2005).

2.6 Hypotheses

Overall, we hypothesize that pulse starches are a suitable substitute for cereal starches as carbohydrate sources in aquaculture feeds. Based on these observations, I propose the following specific hypotheses:

1. That sufficient evidence exists in the literature to support inclusion of starch in aquaculture feeds without impairing growth performance of carnivorous fish species.
2. The digestibility of pulse starches (pea, lentil and faba bean) will be lower than modified cornstarch in rainbow trout.
3. Increasing inclusion of pulse starches (pea, lentil and faba bean) will support better growth performance in rainbow trout compared to modified cornstarch.
4. Glycemic index of pulse starches (pea, lentil and faba bean) will be lower compared to modified cornstarch.

2.7 Experimental approach

In order to address the above hypotheses, the following experimental approaches were undertaken. The first step was to perform a meta-analysis of existing literature reporting effects of dietary carbohydrate inclusion levels on final weight in carnivorous fish species (Chapter 3 of this

thesis). Next, I did three experiments including digestibility trial, growth trial and glycemic response testing trial in rainbow trout (Chapter 4 of this thesis).

3. META-ANALYSIS: THE EFFECT OF DIETARY CARBOHYDRATE INCLUSION LEVELS ON FINAL WEIGHT IN CARNIVOROUS FISH SPECIES

3.1 Publication fate and contribution

This chapter is a meta-analysis of the existing literature on the effects of starch on growth of carnivorous fish species. This analysis was needed to better synthesize and understand the potential utility of greater starch inclusion in aquafeeds. This chapter will not be published, but was needed before proceeding to formulating test diets with high carbohydrate content in rainbow trout in the subsequent chapter of this thesis. Ms. Ji contributed 100% of the literature search, meta-analyses and chapter writing. The defendant's co-supervisors provided help with study design and editing of this chapter.

3.2 Introduction

Feeding is the primary cost in the development of intensive fish culture and protein is the most expensive component of fish diets (Gallego et al., 1995). Because of this, a large amount of research has been performed to reduce the cost of feeding without causing adverse effect on production (Gallego et al., 1995). The replacement of protein by increasing the use of other cheaper energy sources is the most viable way to accomplish this goal (Enes et al., 2010). Many studies have shown that plant ingredients such as wheat, lupins and field peas can be used as a replacement of protein in fish industry (Stone et al., 2003). Starch is a major constituent of many plant ingredients and a commonly used, low cost, source of dietary energy in animals (Orire and Sadiku, 2014). The use of carbohydrates in fish diets is a controversial topic, because there are no deficiencies or symptoms observed when fish are fed diets without any starch (Abimorad et al., 2007). However, a few studies report a reduction in growth rates when some fish species were fed a carbohydrate free diet (Orire and Sadiku, 2014). The adequate inclusion level of carbohydrate in fish diets can maximize the utilization of dietary protein for growth (Abimorad et al., 2007). However, the ability to digest carbohydrate is limited in many carnivorous fish species (Krogdahl et al., 2004).

In recent years, there have been many published studies evaluating the optimal inclusion level of carbohydrate in carnivorous fish diets. Gallego et al. (1995) reported that the final weight and specific growth rate (SGR) of eels and rainbow trout decreased with an increase in the concentration of carbohydrate source (20%, 30%, 40%) in diets. Furuichi and Yone in 1980 also showed

the growth decreased with the increasing dietary dextrin concentrations (0%, 10%, 20%, 30%, and 40%) in yellow tail and red sea bream. However, they reported that growth was not significant affected ($P > 0.05$) by feeding 10% dextrin. Yamamoto et al. 2001 reported that rainbow trout fed with 18% gelatinized potato starch had the highest final weight or SGR compared to that of either the lower level or higher-level groups. This trend for an inverted U-shape for the inclusion-growth performance relationship was not statistically significant in this study, but suggests a promising approach. Based on these previous studies, a hypothesis for this thesis chapter is that a medium inclusion level (10%-20%) of carbohydrate in aquafeeds will be support the best growth performance in carnivorous fish species.

Despite previous research, it is difficult to come to a definitive conclusion on the effect of dietary carbohydrate on the growth of carnivorous fish based on individual studies. This is due to differences in the species of fish, carbohydrate source, basal diet and environmental conditions between the various studies. Meta-analyses can be used to combine results from past studies and make an overall conclusion on the body of research being studied. Therefore, we performed a systematic review and a series of meta-analyses to determine the effect of the dietary inclusion levels of carbohydrates on final weight in carnivorous fish species.

3.3 Materials and methods

3.3.1 Search strategy and inclusion criteria

Available data from all used studies were extracted and organized in excel. Review Manager (RevMan) Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014 was used for performing meta-analysis and conducting a systematic review of intervention. In July 2015, two databases: The Web Of Science (1926-2015) and Science Direct (1962-2015) were used and search terms were Topic = (carbohydrate OR starch) AND Topic = (growth) AND Topic = (trout* OR salmon* OR perch* OR cobia* OR sea bream* OR catfish OR flounder OR bass OR pompano). These studies were divided into three groups based on different carbohydrate sources: post-processing starch, monosaccharide/disaccharide (glucose, maltose and dextrin) and native starch. The post-processing starch means starch structure was physically or chemically changed by certain different feed processing methods such as extrusion. Native starch means starch that was not changed by any feed processing. The reason why I divided the meta-analysis into three groups was to examine the effect of starch sources under the same conditions. In order

to prevent selection bias, pre-specified inclusion criteria were: 1) language is English; 2) random allocation of experimental subjects; 3) use of carbohydrate not protein or oil; 4) growth trial; 5) use of carnivorous fish species; 6) presence of a control diet; 7) experimental diet is isoenergetic and isonitrogenous. Duplicate studies and review papers were removed. Results were analyzed separately when studies contained more than one test diet.

3.3.2 Data extraction

A standardized criterion was used to extract the available data from each study. The details of these data were study design, sample size, fish species, test carbohydrate sources and carbohydrate inclusion level. Final weight (FW) was an indicator of growth used in the meta-analysis and its precondition was the same initial weight. A standard deviation (SD) was reported for data sufficient to calculate SD.

3.3.3 Statistical analysis

Standardized mean differences between control and test diets were measured by using Hedges' g (Hedges, 1981). The following equation were used to calculate standardized differences:

$$g^* = J(n_1 + n_2 - 2)\ g \approx \left(1 - \frac{3}{4(n_1 + n_2) - 9}\right)\ g$$

$$g = \frac{\bar{x}_1 - \bar{x}_2}{s^*}$$

$$s^* = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}.$$

Where,

g^* is corrected standardized difference

g is standardized difference

S^* is pooled standard deviation

$\bar{x}_1$ is mean of test, $\bar{x}_2$ is mean of control

n_1 is test sample size, n_2 is control sample size

Chi-square was used to indicate the heterogeneity of these studies, with a random-effects model used to take into account study heterogeneity and sampling error in this meta-analysis. Weighted linear and quadratic regressions of inclusion rate on effect size were performed using SPSS (IBM SPSS Statistics 22). The weight of effect size is inversely proportional to variance in each study.

3.4 Results

3.4.1 Systematic review

The selection of studies is shown diagrammatically in Figure 3.1. A total of 2949 studies were initially identified during the search and 2869 were eliminated because they did not meet the defined selection criteria described above. Thus, 80 studies were reviewed thoroughly and 73 of these were excluded for the following reasons: 44 trials did not contain a control diet; the diet formulation in 21 studies was neither isoenergetic nor isonitrogenous; 3 papers were duplicates; 1 was a review paper and 1 trial had no replication of experimental units.

The inclusion criterion was met by 7 studies published from 1926 to 2015. A total of 177 experimental units from these trials conducted between 1998 and 2015 were reported. The studies were performed on six species of carnivorous fish including rainbow trout, gilthead sea bream (*Sparus aurata*) yellowfin sea bream (*Acanthopagrus butcheri*), golden pompano (*Trachinotus blochii*), sunshine bass (*M. chrysops x M. saxatilis*) and striped bass (*Morone saxatilis*). The inclusion rates of carbohydrate ranged from 3% - 28% in the post-processing starch meta-analysis, 10-25% in the monosaccharide/disaccharide (glucose, maltose and dextrin) meta-analysis, and 10-20% in the native starch meta-analysis.

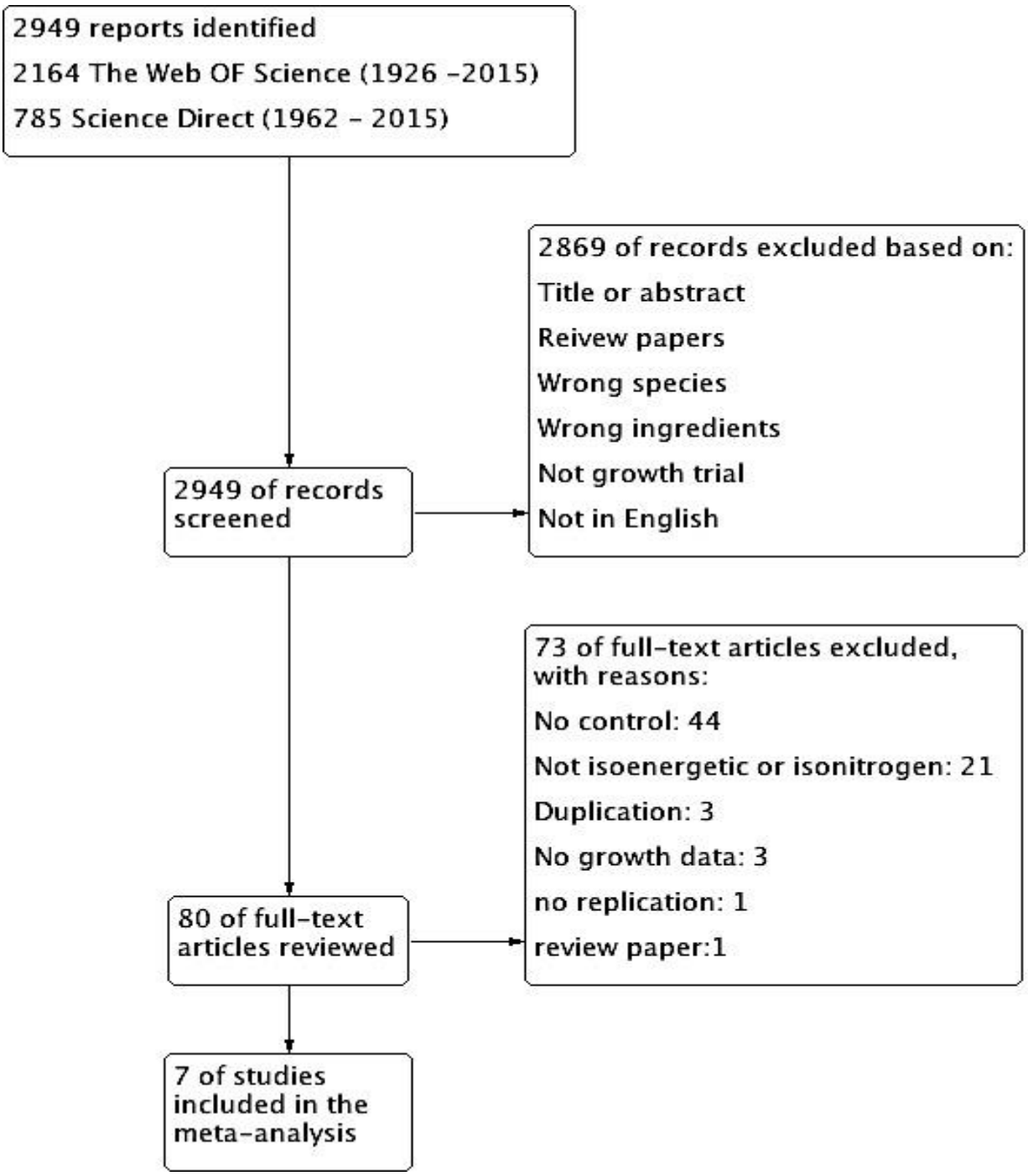


Figure 3.1 Flow diagram of study selection.

3.4.2 Post-processing starch

Only five studies included diets with post-processed starch. Therefore, fourteen data points from these five studies were included in this meta-analysis that examined effects of post-processing (Table 3.1). In these studies, rainbow trout, golden pompano and gilthead sea bream and yellowfin sea bream were used. Effect size ranged from -26.53 (180 g/kg) to 2.47 (230 g/kg) and the overall effect size was 0.42 with *P* value = 0.65 (Table 3.2 and Figure 3.2). The chi-square and *P*-value were 27.08 and 0.01, respectively. This showed the significant difference observed was heterogeneous in each study. As a result, a random effects model was used in the meta-analysis. Weighted linear regression showed no significant difference (Figure 3.3; $P = 0.98$).

Table 3.1 Data set for studies on the effect of dietary post-processing of starch sources from corn, tapioca and potato on the final weight (FW) of carnivorous fish.

Authors	Species	Inclusion (g/kg)	Test FW (g)	Test SD	Control FW (g)	Control SD	N
Gumus and Ikiz, 2009	Rainbow trout (c)	30	180.2	1.56	198.0	0.91	6
Zhou et al., 2015	Golden pompano (c)	56	40.2	1.91	38.0	5.28	6
Enes et al., 2008	Gilthead sea bream (c)	100	52.1	2.90	50.9	2.80	6
Zhou et al., 2015	Golden pompano (c)	112	41.8	4.07	38.0	5.28	6
Gumus and Ikiz, 2009	Rainbow trout (c)	120	188.9	2.44	198.0	0.91	6
Zhou et al., 2015	Golden pompano (c)	168	43.7	5.35	38.0	5.28	6
Gumus and Ikiz, 2009	Rainbow trout (c)	180	168.8	0.85	198.0	0.91	6
Enes et al., 2008	Gilthead sea bream (c)	200	58.2	2.90	50.9	2.80	6
Wu et al., 2007	Yellowfin sea bream (c)	200	12.8	0.39	15.5	1.14	6
Wu et al., 2007	Yellowfin sea bream (t)	200	13.7	0.29	15.5	1.14	6
Wu et al., 2007	Yellowfin sea bream (p)	200	13.1	0.52	15.5	1.14	6
Zhou et al., 2015	Golden pompano (c)	224	38.8	3.98	38.0	5.28	6
Peragon et al., 2000	Rainbow trout (c)	230	207.9	14.5	168.3	11.00	6
Zhou et al., 2015	Golden pompano (c)	280	33.9	5.87	38.0	5.28	6

c = corn; t = tapioca; p = potato. SD = standard deviation

Table 3.2 Meta-analysis details for effect size and weight for final weight when carnivorous fish were fed with post-processing starch in fish diets.

Authors	Species	Inclusion (g/kg)	Effect Size	Weight %	N
Gumus and Ikiz, 2009	Rainbow trout (c)	30	-11.18	0.7	6
Zhou et al., 2015	Golden pompano (c)	56	0.46	10.4	6
Enes et al., 2008	Gilthead sea bream (c)	100	0.34	10.5	6
Zhou et al., 2015	Golden pompano (c)	112	0.65	10.1	6
Gumus and Ikiz, 2009	Rainbow trout (c)	120	-3.95	3.8	6
Zhou et al., 2015	Golden pompano (c)	168	0.86	9.8	6
Gumus and Ikiz, 2009	Rainbow trout (c)	180	-26.53	0.1	6
Enes et al., 2008	Gilthead sea bream (c)	200	2.05	7.1	6
Wu et al., 2007	Yellowfin seabream (c)	200	-2.54	6.0	6
Wu et al., 2007	Yellowfin seabream (t)	200	-1.81	7.7	6
Wu et al., 2007	Yellowfin seabream (p)	200	-2.19	6.8	6
Zhou et al., 2015	Golden pompano (c)	224	0.14	10.6	6
Peragon et al., 2000	Rainbow trout (c)	230	2.47	6.2	6
Zhou et al., 2015	Golden pompano (c)	280	-0.57	10.2	6

c = corn; t = tapioca; p = potato

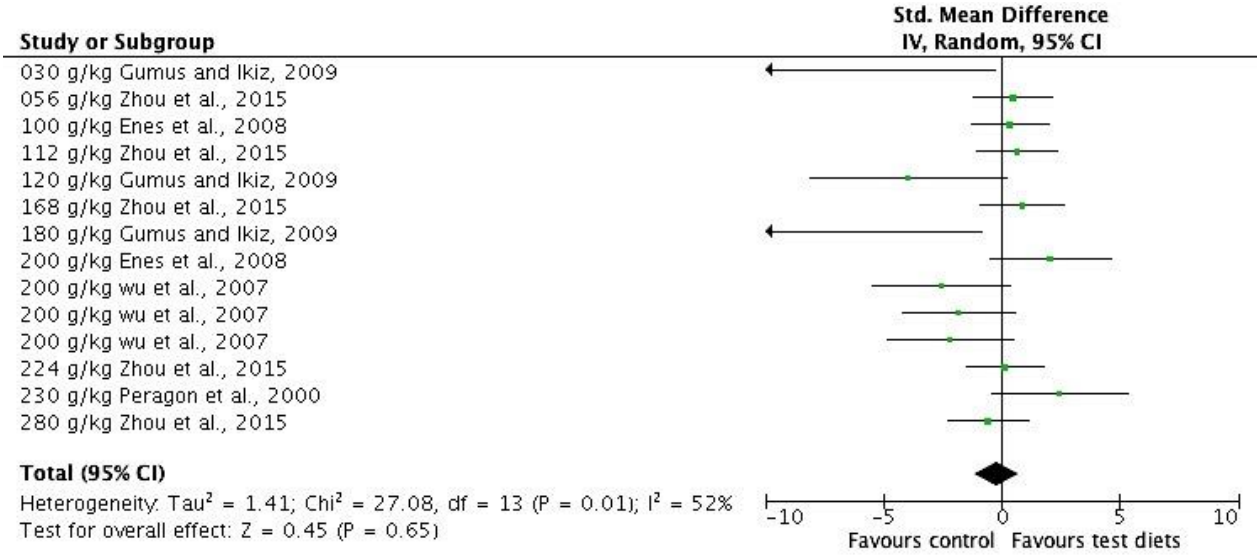


Figure 3.2 Forest plot of treatment effect size by post-processing starch inclusion level. Area of each square is proportional to inverse of study variance in the analysis. Horizontal lines represent 95% confidence interval (CI). In the study ID, the percentage means the inclusion level of carbo-hydrate in each study.

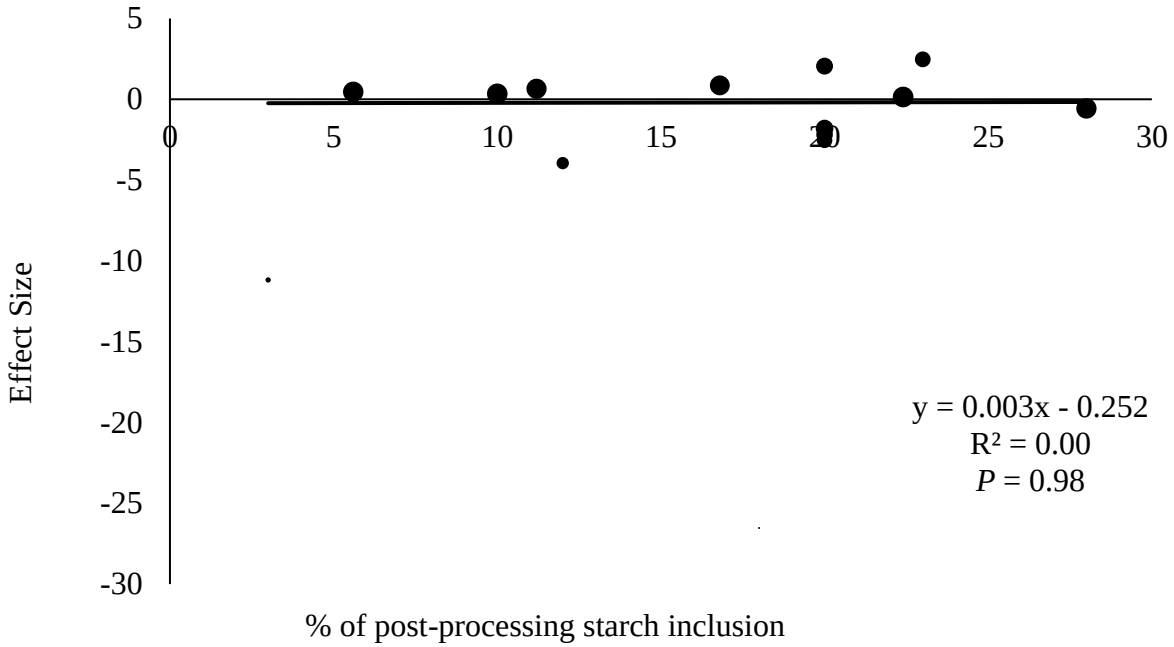


Figure 3.3 Weighted linear regression of effect size on dietary inclusion of post-processing starch on final weight.

3.4.3 Monosaccharide/disaccharide (glucose maltose dextrin)

Ten data points from two studies were included in this meta-analysis (Table 3.3). In these studies, striped bass and sunshine bass were used. Effect size ranged from -3.08 (200 g/kg) to 1.59 (250 g/kg) and the overall effect size was 0.39 with *P* value = 0.70 (Table 3.4 and Figure 3.4). The chi-square was 12.09 ($P = 0.21 > 0.05$) and this indicated the heterogeneity of differences in each study was non-significant. Weighted linear regression was not significance ($P = 0.78$) (Figure 3.5).

Table 3.3 Data set for studies on the effect of dietary monosaccharide/disaccharide sources from glucose, maltose and dextrin on the final weight (FW) of carnivorous fish.

Authors	Species	Inclusion (g/kg)	Test FW (g)	Test SD	Control FW (g)	Control SD	N
Small et al., 1999	Striped bass (g)	100	234.0	5.20	235.0	5.20	6
Small et al., 1999	Striped bass (g)	150	239.0	5.20	235.0	5.20	6
Small et al., 1999	Striped bass (g)	200	215.0	5.20	235.0	5.20	6
Small et al., 1999	Striped bass (g)	250	224.0	5.20	235.0	5.20	6
Rawles and Gatlin, 1998	Sunshine bass (g)	250	195.6	8.64	178.5	8.64	6
Rawles and Gatlin, 1998	Striped bass (g)	250	139.0	8.64	135.6	8.64	6
Rawles and Gatlin, 1998	Sunshine bass (m)	250	193.1	8.64	178.5	8.64	6
Rawles and Gatlin, 1998	Striped bass (m)	250	127.0	8.64	135.6	8.64	6
Rawles and Gatlin, 1998	Sunshine bass (d)	250	179.3	8.64	178.5	8.64	6
Rawles and Gatlin, 1998	Striped bass (d)	250	152.7	8.64	135.6	8.64	6

g = glucose; m = maltose; d = dextrin. SD = standard deviation

Table 3.4 Meta-analysis details for effect size and final weight when carnivorous fish were fed with monosaccharide/disaccharide from glucose, maltose and dextrin in fish diets.

Authors	Species	Inclusion (g/kg)	Effect Size	Weight %	N
Small et al., 1999	Striped bass (g)	100	-0.15	13.1	6
Small et al., 1999	Striped bass (g)	150	0.62	12.1	6
Small et al., 1999	Striped bass (g)	200	-3.08	4.0	6
Small et al., 1999	Striped bass (g)	250	-1.69	7.8	6
Rawles and Gatlin, 1998	Sunshine bass (g)	250	1.59	8.2	6
Rawles and Gatlin, 1998	Striped bass (g)	250	0.32	12.9	6
Rawles and Gatlin, 1998	Sunshine bass (m)	250	1.35	9.1	6
Rawles and Gatlin, 1998	Striped bass (m)	250	-0.79	11.4	6
Rawles and Gatlin, 1998	Sunshine bass (d)	250	0.08	13.2	6
Rawles and Gatlin, 1998	Striped bass (d)	250	1.59	8.2	6

g = glucose; m = maltose; d = dextrin

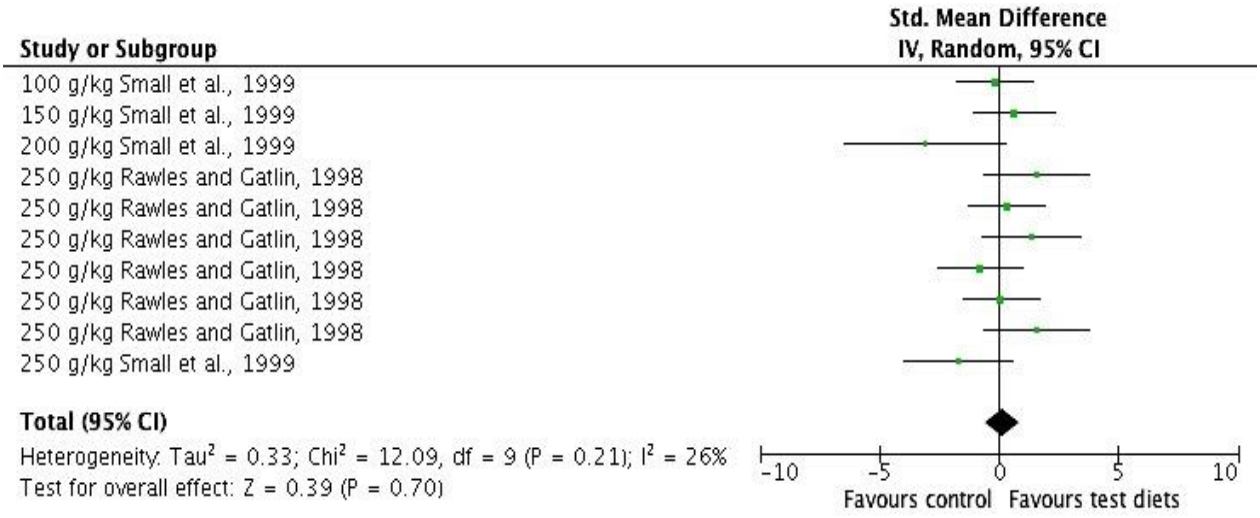


Figure 3.4 Forest plot of treatment effect size by carbohydrate inclusion level (sources from glucose, maltose and dextrin). Area of each square is proportional to inverse of study variance in the analysis. Horizontal lines represent 95% confidence interval (CI). In the study ID, the percentage means the inclusion level of carbohydrate in each study.

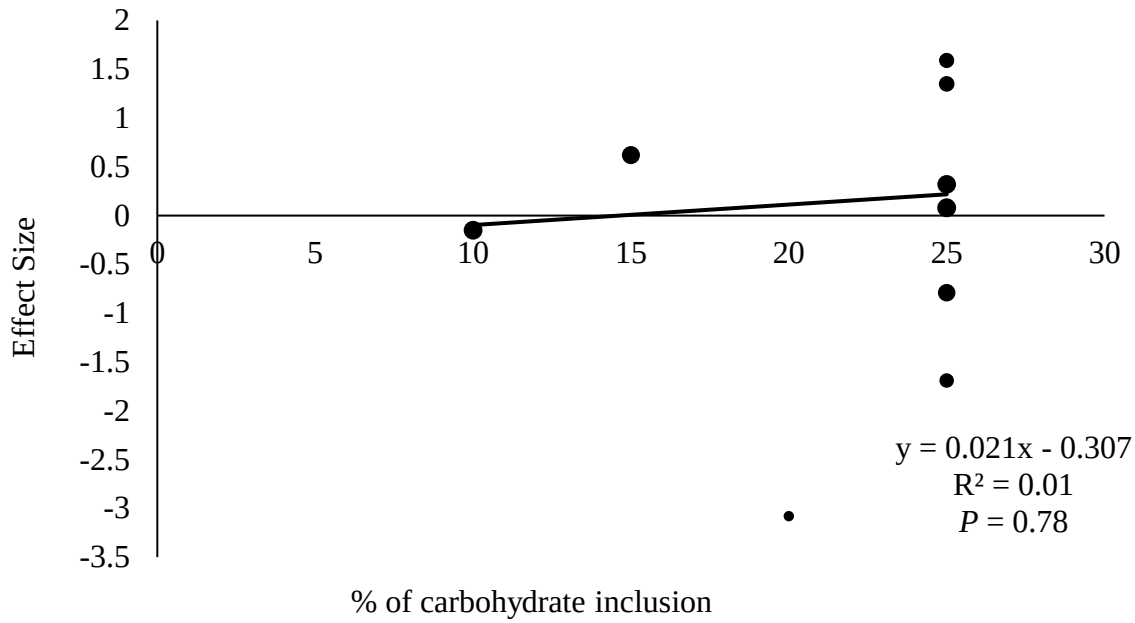


Figure 3.5 Weighted linear regression of effect size on dietary inclusion of carbohydrate sources from glucose, maltose and dextrin on final weight.

3.4.4 Native starch

Five data points from two studies were included in this meta-analysis (Table 3.5). Only gilthead sea bream and yellowfin sea bream were used in this meta-analysis. Effect size ranged from -0.18 (200 g/kg) to 1.13 (100 g/kg) and the overall effect size was 0.79 with *P* value of 0.43 (Table 3.6 and Figure 3.6). The chi-square was 1.96 ($P = 0.74 > 0.05$) and showed no significant heterogeneity among studies. Weighted linear regression indicated no significant difference with $P = 0.24$ (Figure 3.7).

Table 3.5 Data set for studies on the effect of dietary native starch sources from corn, tapioca and potato on the final weight (FW) of carnivorous fish.

Authors	Species	Inclusion (g/kg)	Test FW (g)	Test SD	Control FW (g)	Control SD	N
Enes et al., 2008	Gilthead sea bream (c)	100	55.3	3.40	50.9	2.80	6
Enes et al., 2008	Gilthead sea bream (c)	200	55.9	4.50	50.9	2.80	6
Wu et al., 2007	Yellowfin seabream (c)	200	15.2	1.51	15.5	1.14	6
Wu et al., 2007	Yellowfin seabream (t)	200	15.4	0.55	15.5	1.14	6
Wu et al., 2007	Yellowfin seabream (p)	200	15.6	0.31	15.5	1.14	6

c = corn; t = tapioca; p = potato. SD = standard deviation

Table 3.6 Meta-analysis details for effect size and weight for final weight when fish fed with native starch in fish diets.

Authors	Species	Inclusion (g/kg)	Effect Size	Weight %	N
Enes et al., 2008	Gilthead sea bream (c)	100	1.13	15.6	6
Enes et al., 2008	Gilthead sea bream (c)	200	1.07	16.2	6
Wu et al., 2007	Yellowfin seabream (c)	200	-0.18	22.6	6
Wu et al., 2007	Yellowfin seabream (t)	200	-0.08	22.8	6
Wu et al., 2007	Yellowfin seabream (p)	200	0.08	22.8	6

c = corn; t = tapioca; p = potato

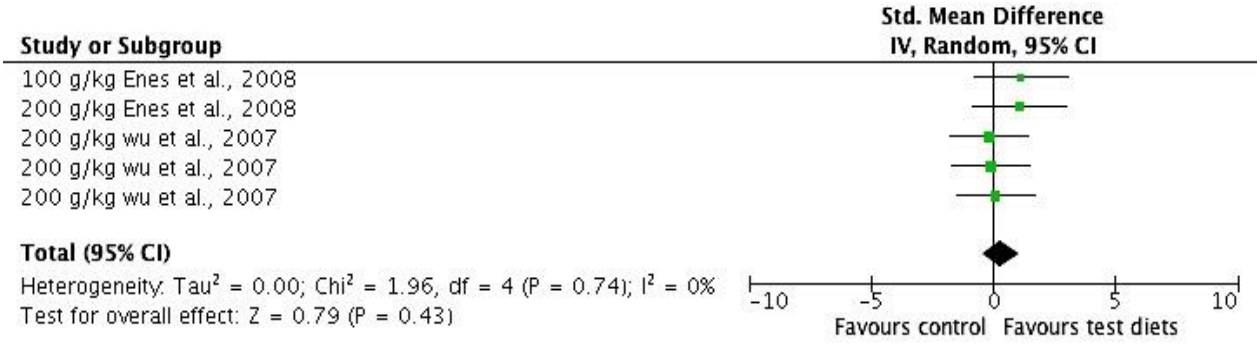


Figure 3.6 Forest plot of treatment effect size by native starch inclusion level. Area of each square is proportional to inverse of study variance in the analysis. Horizontal lines represent 95% confidence interval (CI). In the study ID, the percentage means the inclusion level of carbohydrate in each study.

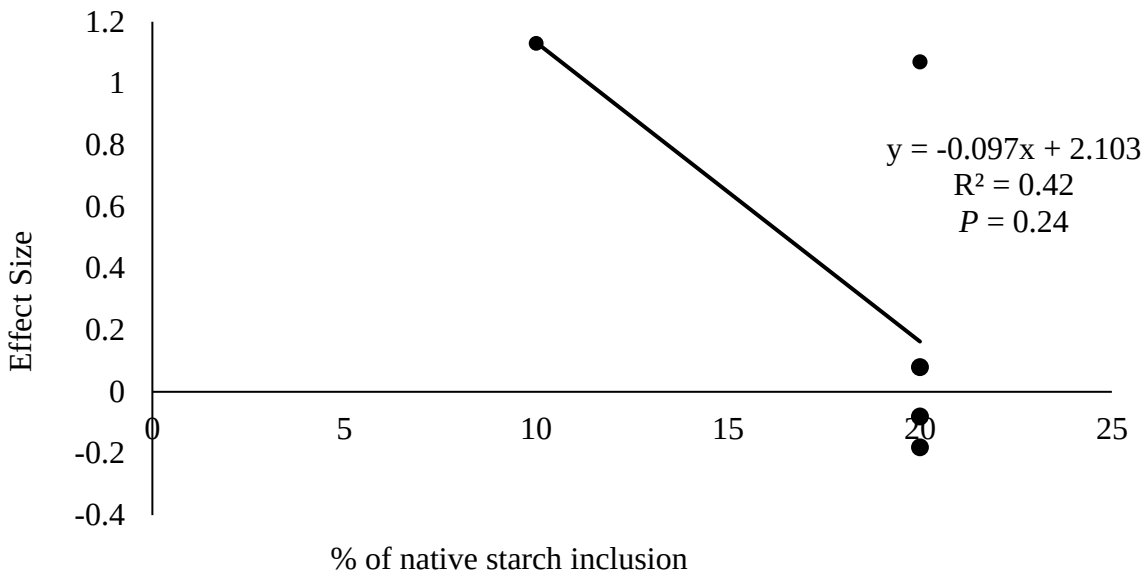


Figure 3.7 Weighted linear regression of effect size on dietary inclusion of native starch on final weight.

3.4.5 Overall description for total studies

The forest plot described the effect size of 29 data points from all seven studies in the meta-analysis (Figure 3.8). The Chi-square was 41.58 with *P* value = 0.05, which indicated the heterogeneity of among studies was not significant. The overall effect size was 0.36 (*P* = 0.72). It illustrated there was no significant difference in the final weight between treatment and control in the meta-analysis. Weighted linear regression also showed no significant difference (*P* = 0.94) (Figure 3.9).

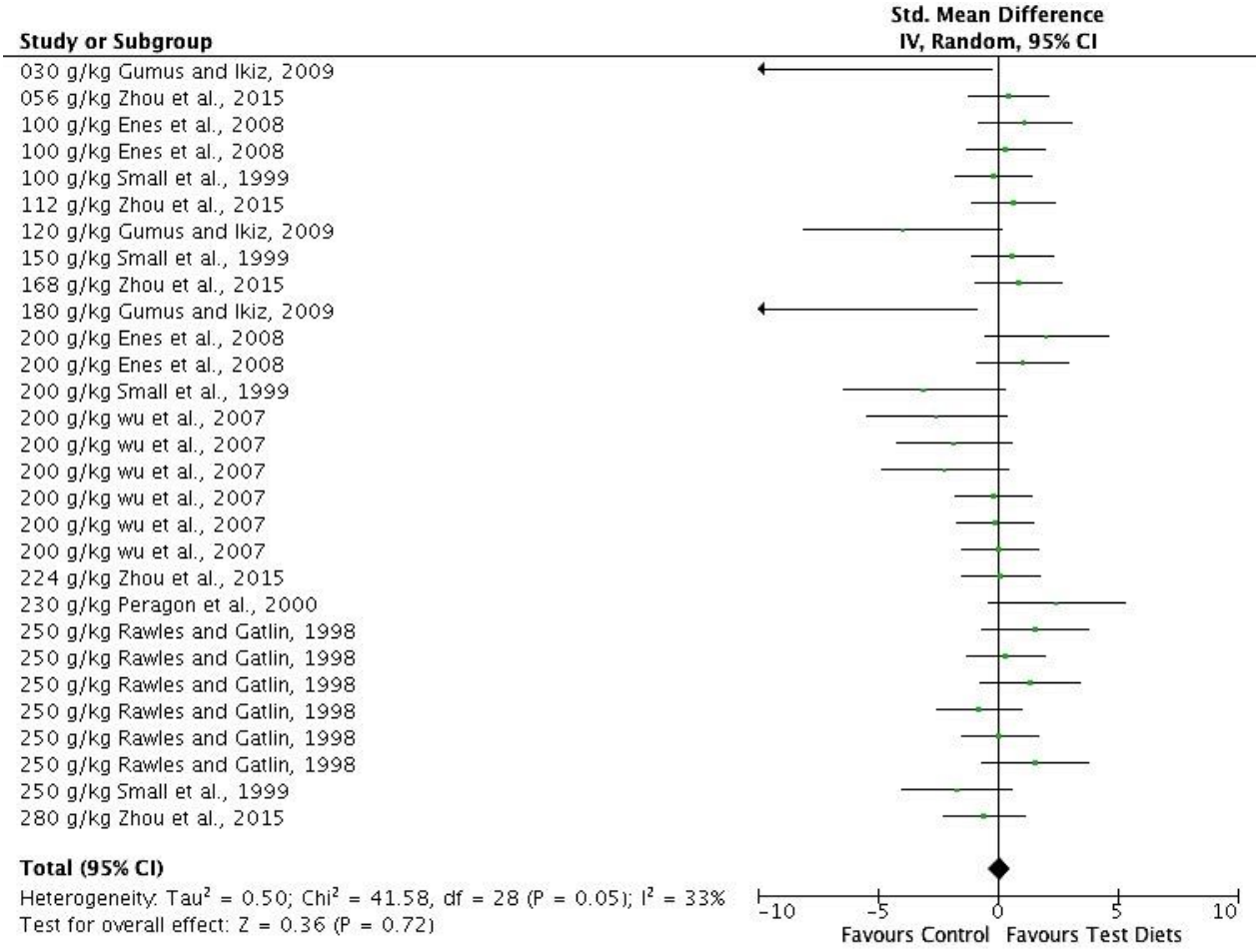


Figure 3.8 Forest plot for effect size by the dietary carbohydrate inclusion level in carnivorous fish species. The area of each square represents the weight of each study with in the meta-analysis. Horizontal lines represent the 95% confidence interval (CI). In the study ID, the percentage means the inclusion level of carbohydrate in each study.

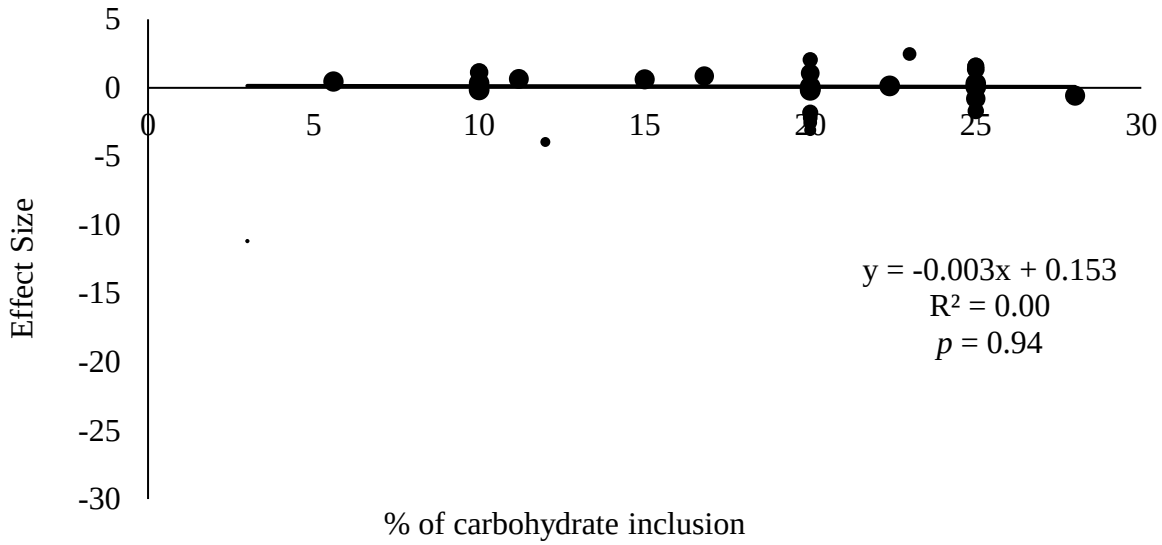


Figure 3.9 Weighted linear regression of effect size from various dietary carbohydrate inclusion on final weight.

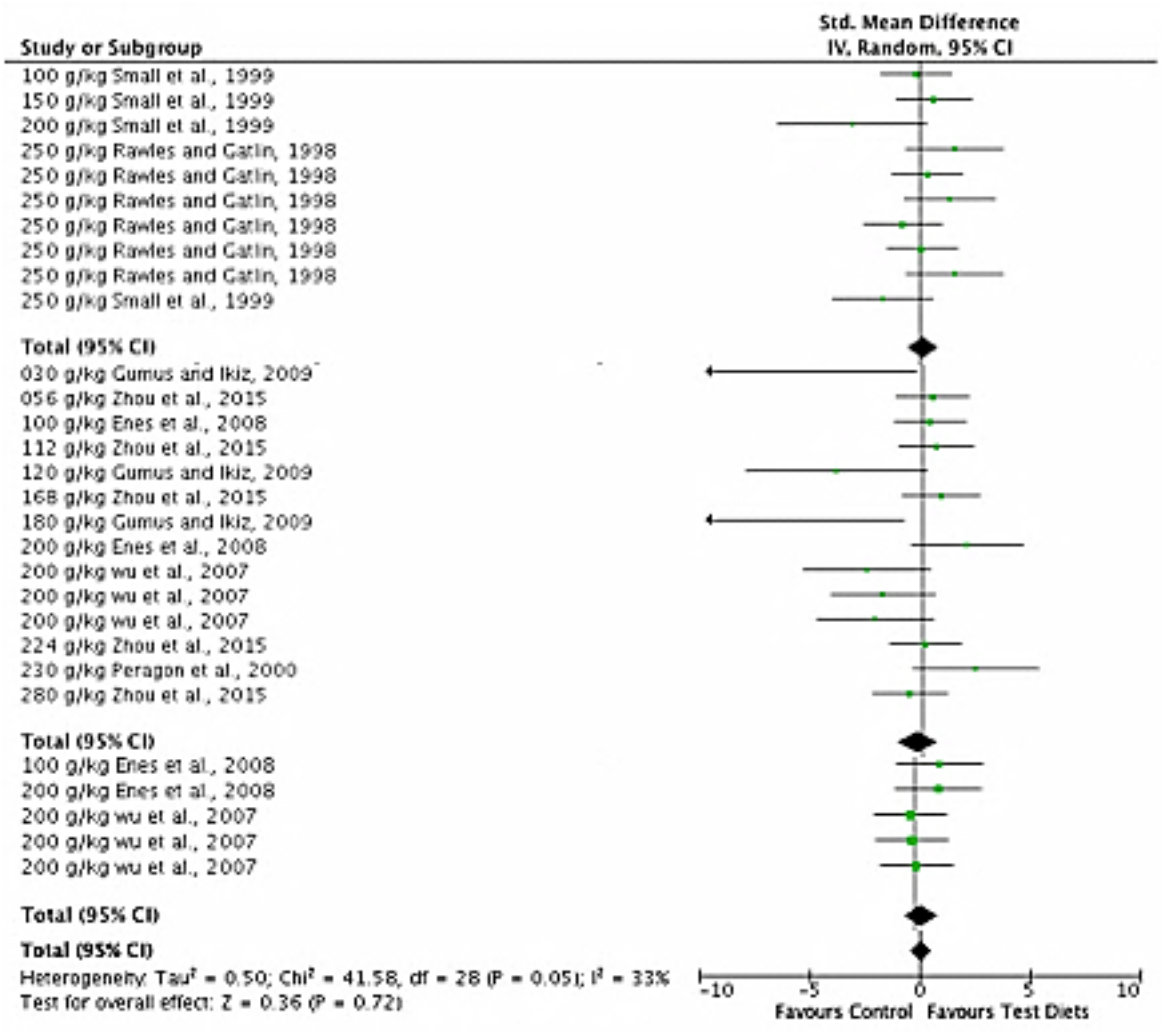


Figure 3.10 Combined forest plot of the effect size.

3.5 Discussion

The ability of carnivorous fish species to digest and absorb carbohydrates varies according to the fish species and type of carbohydrate being fed. From the first group of this meta-analysis, the final weight of test diets did not show significant difference with that of control diets in each study (Table 1). The same result was found when fed 150 g/kg of glucose to striped bass in the second group of the meta-analysis and (Table 3). Also, the study from Enes et al., 2008 in the third group of the meta-analysis showed the same tendency as group 1 and 2 (Table 5). It was also interesting to note that the study from Wu et al., 2007 showed an opposite result with the study from Enes et al., 2008 while fed 200 g/kg of post-processing starch to carnivorous fish (Table 1). The different fish species and carbohydrate sources they used may cause this different. Moreover, two studies in Table 1 found that when fed fish with 220 g/kg or 230 g/kg of post-processing starch, the fish final weight of test diet was higher than that of control diet but was lower than that of control diet when fed fish with high (280 g/kg) level of post-processing starch. In Table 2, five studies indicated that the final weight of test diet was higher than that of control diet when fed 250 g/kg of glucose, maltose or dextrin to bass while other two studies showed the opposite results. This may be caused by different bass species and also different carbohydrate sources. Overall, optimal fish growth performance tended to be observed around 200 g/kg of carbohydrate regardless of fish species and carbohydrate sources. Some of fish species grew better while fed with even higher carbohydrate in this meta-analysis. This finding is consistent with the literature. In the study of Hutchins et al. (1998), fed sunshine bass with 400 g/kg of the same monosaccharide/disaccharide carbohydrate demonstrated a negative effect on weight gain compared to 200 g/kg. However, in 1979, Bergot studied use in rainbow trout, showing that rainbow trout fed with 300 g/kg glucose had the best results, while carbohydrate inclusion from 150 g/kg to 300 g/kg had no adverse effect on weight gain. This is not in accordance with the finding in this meta-analysis. Young et al. (2006) used pre-cooked extruded carbohydrate to replace fish oil in salmon diets. In this trial, overall results showed that growth was unaffected by carbohydrate source, but there was a reduction in SGR as fish size increased.

3.6 Other factors

3.6.1 Feed processing conditions

In the recent years, fish production technologies have been improved and post-processing of starch has been developed that modify the digestibility of starch. Carnivorous fish are not able to digest carbohydrate efficiently, but technological processing including heat and moisture may improve starch digestibility (Moreira et al., 2008). In the meta-analysis, the overall effect size of post-processing starch was 0.45 compared to that of native starch (0.79), which indicated raw starch performed better than post-processing starch in final weight. Besides, Fu (2007) found that there was no significant difference in final weight between southern catfish (*Phractocephalus siluriformes*) fed with raw cornstarch versus precooked cornstarch. This may because the use of different feed processing methods. Extrusion processing has been found to be very efficient in improving starch digestion (Venou et al., 2003). Venou et al compared raw wheat or raw corn to extruded wheat or extruded corn in gilthead sea bream. This sea bream study found that raw corn produced the lowest final weight, while extruded wheat or corn produced better growth. Rather than being related to degree of starch gelatinization or digestibility as previously thought, the degree of plant ingredient processing may instead play a vital role in reducing the activity of several anti-nutritional factors to improve total nutritional value.

3.6.2 Feeding regime

In this meta-analysis, fish were fed twice a day in all trials. Some excluded papers pointed toward the effect of feeding strategy on growth. Bergot (1979) indicated that rainbow trout fed with diets containing 30% glucose four times a day had the highest weight gain compared to fed fish twice a day versus six times a day. Moreover, Hung and Storebakken in 1993 demonstrated that lower SGR and higher final body weight were observed in the continuously fed fish than in the meal-fed fish. Feeding fish until satiation is required in growth trial, but Bergot (1979) also observed that fish fed with high carbohydrate up to 6 meals per day also resulted in a high plasma glucose level, generally considered an adverse effect. Therefore, the suitability of the feeding regime or feeding methods are also very important to consider in a growth trial, but was not examined in this current meta-analysis study.

3.6.3 Environmental factors

Environment factors such as water temperature, photoperiods, salinity plays an important role in growth trial. Temperature is the most important factor for determining the growth rate of fish (Amin et al., 2014). Some trials conducted a factorial experimental design with different temperatures while studying the effect of carbohydrate inclusion level on growth. In the study of Young et al., (2006), temperature (which varied with season), showed a clear relationship to fish growth. Moreover, changes in photoperiod were also a strongly influential factor. In the study of Moreira (2008), starch digestibility and growth were improved as temperature increased (from 18 °C to 25 °C) in European sea bass. Amin et al. (2014) studied the effect of temperature and carbohydrate level in brook trout. They found that SGR was better at 15°C than 19°C, while incorporating pregelatinized cornstarch (18-26%) did not have a negative effect on growth. Based on previous research, the use of optimal temperature for different fish species can prevent difficulties in interpreting growth results, since the maintenance of healthy environmental conditions is required during the experimental period.

3.7 Conclusion

All available growth data from literature in carnivorous fish species fed with varying dietary carbohydrate inclusion levels was compared through this meta-analysis. The analysis of effect size and regression models showed that there was no significant difference in carnivorous fish species fed with carbohydrate up to 280 g/kg inclusion level compared to those fed control diets. If there were an optimal inclusion level, this would likely be 20%, but we cannot say this for sure based on no significant effects found. A deficiency of this meta-analysis is that no study included in the met-analysis tested a high percentage carbohydrate diet, which in turn may be the reason for non-significant findings. Despite this, trends suggest that a medium inclusion level (~ 200 g/kg) showed better growth than low percentage of carbohydrate (< 100 g/kg). Based on this, 200 g/kg carbohydrate is recommended for inclusion for greatest growth efficiency in carnivorous fish. Another deficiency was that there were not enough papers available that met all criteria for a reliable meta-analysis. Most papers were excluded because of a lack of control diet tested or un-

balanced diets. For the further research in this area, I suggest feeding trials include both a control diet and formulate balanced diets (isocaloric and isonitrogenous).

In conclusion, the optimal inclusion of carbohydrate used in fish diets not only depends on the carbohydrate sources but also depends on fish species, fish body size or age. With improvements in technology, the equipment used for research has also changed and improved. This may be a reason for the differences among these studies that spanned many decades of research. For example, advanced feed processing methods have improved feed efficiency in aquaculture. Also, advanced culturing systems improve fish welfare and keeps environmental factor such as water temperature, oxygen level and ammonia more constant. Overall, planning the most suitable experimental strategy and keeping conditions constant can be very important in the future research and also more research need in this area.

4. DETERMINATION OF THE EFFECTS OF PULSE STARCHES (PEA, LENTIL AND FABA BEAN) VERSUS CORNSTARCH ON DIGESTIBILITY, GROWTH PERFORMANCE AND GLYCEMIC INDICES IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

4.1 Publication fate and contribution

This chapter is to determine the digestibility of pulse starches (pea, lentil and faba bean) and cornstarch in rainbow trout. Thus, the digestible nutrients will be used for diet formulation in the subsequent growth trial. The growth trial is to examine growth performance and feed efficiency of rainbow trout at 0, 100 and 200 g/kg starch inclusion levels. The glycemic responses of test starches will also be determined in rainbow trout. This study provides information for fish nutritionist to predict the fish growth when formulating carbohydrate in fish diets. This chapter will not be published by itself, but will be combined with work by Yue Guo on tilapia and would be published in the journal of Aquaculture Nutrition. Ms. Ji contributed 100% of all experiments and chapter writing. The defendant's co-supervisors, Dr. Lynn Weber and Dr. Murray Drew provided help with study design and editing of this chapter.

4.2 Introduction

Carbohydrates, particularly starch from cereal grains are the major energy source for terrestrial livestock species (Chen et al., 2013). In contrast, the major sources of energy in salmonid feeds are protein and lipids, derived from fishmeal and fish oil (Stone, 2003). Since the late 1990s, the world production of fish meal and oil have plateaued at 6 million and 950 thousand metric tonnes, respectively, and aquaculture is currently using all of the available supplies of these commodities (Tacon and Metian, 2008). In order to ensure the sustainability of salmonid aquafeeds, it is essential to increase the use of carbohydrates as energy source in aquafeeds (Chen et al., 2013).

Carbohydrates, primarily starch, are considered to be a feasible replacement for fishmeal or fish oil in aquafeeds because of their wide availability and low cost (Krogdahl et al., 2005). To date, grains have been the primary starch sources used in salmonid diets with some use more recently of soymeal. The major issue with grain carbohydrates in fish, especially carnivorous fish species like rainbow trout, is limited digestibility (Krogdahl et al., 2005). Moreover, soymeal

causes enteritis and decreases fish growth performance (Krogdahl et al., 2005). However, the use of feed processing methods such as extrusion has been shown to improve the digestibility of carbohydrates in fish (Burel et al., 2000). Studies conducted by Inaba et al. (1963) in rainbow trout found that the digestibility of cooked wheat starch was 48% whereas that of native wheat starch was 22%. A study also showed that the digestibility of gelatinized cornstarch was 95% in rainbow trout. Smith reported similar findings in 1971 (Hofer et al., 1985). In addition, improved growth has been shown in rainbow trout, Atlantic salmon (*Salmo salar*), European eel (*Anguilla anguilla*), cod and carp when fed diet with starch compared to diet without starch (Hemre et al., 2001). Despite this, the use of carbohydrates in the diets of carnivorous fish is limited and the recommended inclusion level of dietary carbohydrates in many carnivorous fish like rainbow trout is a maximum of 20% (Stone, 2003). Despite this, other studies have reported that higher incorporation levels of extruded cereal starch can be used without an adverse effect on the growth of rainbow trout (Kim and Kaushik, 1992).

Another factor affecting the digestibility and metabolism of dietary starches is their absorption rate. Starches that are rapidly absorbed from the gut, such as cereal starches, may induce prolonged hyperglycemia after a meal (Hemre and Hansen, 1998). The glucose absorption rate after a meal containing starch varies depending on starch source, processing methods and the species of fish. Unprocessed starches are poorly digestible and the glucose absorption rate is therefore slow. However, commercial aquafeeds are extruded and extrusion can increase the digestibility and rate of glucose uptake from dietary starch (Venou et al., 2003).

Since prolonged hyperglycemia has been reported when fish are fed carbohydrate-rich diets, it is assumed that salmonids have a poor ability to catabolize/dispose of glucose absorbed in the postprandial period (Marandel et al., 2016). Based on mammalian literature, slowly digestible starch should reduce postprandial hyperglycemia and may improve the ability of salmonids to regulate glucose metabolism. While starches from cereals are rapidly digestible, pulse starches are more slowly digested and absorbed. This is due to several factors including a high content of amylose compared to cereals. Amylose is digested slower than amylopectin due to its helical structure (Svihus et al., 2005). Hoover and Sosluski (1986) reported that the amylose contents of lentils, faba beans and peas were 38.5%, 32% and 34.2% respectively. In comparison, cereal

starches are typically composed of 25–28% amylose. Thus, pulse starches might be better utilized as energy sources than cereal starches in salmonid fish.

Glycemic index (GI) is a simple way to measure the rate of both absorption and metabolism of dietary glucose *in vivo* and is therefore a useful measure of the nutritional quality of dietary starch. GI is defined as the incremental area under the glycemic response curve after a test food compared to a control food. Values are expressed as the percentage of the area under the curve after ingesting the same amount of carbohydrates of reference food, usually pure glucose or white bread in humans (Chung et al., 2008). While GI has been extensively used in human and terrestrial, omnivorous animal studies (Jenkins et al., 1981; Frei et al. 2003, Drew et al., 2011), it has never been used in fish or carnivorous salmonids. In humans, it has been reported that low GI diets have a positive effect on the control of postprandial hyperglycemia (Jeckins et al., 2008). This suggests that GI might also be a useful measure for determining the nutritional value of starch sources in salmonid diets.

I hypothesize that increasing inclusion of slowly digested starches such as pulse starch will produce good growth compared to a control diet (0% test starch inclusion). Moreover, I hypothesize that pulse starches and other poorly digested starches will have a low GI rainbow trout. Therefore, the aim of this study was to test digestibility, growth performance and glycemic response of pea, lentil and faba bean starch compared to modified cornstarch in the carnivorous rainbow trout. Digestibility and growth trials in trout were conducted using a hydroxypropyl-modified cornstarch. From similar trials in beagle dogs, the modified cornstarch had a lower digestibility and glycemic index than unmodified cornstarch. This was considered desirable in order to eliminate differences in digestibility between the pulse starches and cornstarch as a confounding factor leading to differences in growth performance and glycemic index in trout. For comparison of glycemic index only, unmodified cornstarch was also used alongside pulse starches and modified cornstarch in glycemic testing in rainbow trout.

4.3 Material and Methods

4.3.1 Starch sources

Pulse starch concentrates (pea, lentil and faba bean) were provided by the Alliance Grain Traders (Saskatoon, SK). These starch products were produced using dry processing. Hydroxy-

propyl modified cornstarch (waxy corn; Thik-N-Thin 99; Tate-Lyle, Saskatoon, SK) and commercial wet processed, unmodified cornstarch (Fleischmann's, ACH Food Companies, Mississauga, ON) were the cereal starch sources used in these studies. The proximate analyses of the raw starches are shown in Table 4.1.

Table 4.1 Proximate analysis of raw starch ingredients.

Ingredient	DM (g/kg)	CP (g/kg)	GE (kcal/kg)	Starch (g/kg)
Pea starch	899	150	4327	715
Lentil starch	899	168	4354	735
Faba bean starch	905	218	4412	685
Modified cornstarch	917	5	4133	811
Cornstarch	877	5	3750	984

DM = dry matter; CP = crude protein; GE = gross energy

4.3.2 Fish husbandry

All animal protocols were approved by the University of Saskatchewan Committee on Animal Care and Supply and followed the Canadian Council on Animal Care guidelines (2005). Female triploid rainbow trout (*Oncorhynchus mykiss*) were cultured in 360 L tanks at the Prairie Aquaculture Research Centre (University of Saskatchewan). The rearing system was a recirculating system with biological filter. Water temperature was maintained at 12 ± 1℃ and the photoperiod was a 14 h light/10 h dark cycle. Temperature and dissolved oxygen were recorded daily and water pH, chlorine, ammonia, nitrate and nitrite were measured weekly. Mortalities were recorded daily, with dead fish were weighed and removed. Fish were fed twice a day to satiation.

4.3.3 Experiment 1: Digestibility of starch ingredients

4.3.3.1 Experimental diets

Digestibility was determined using the indirect method with Celite (Celite 545, Celite Corporation, World Minerals Co., Lompoc, CA) as a nonabsorbable marker. A fishmeal reference diet (Table 4.2) was formulated according to Bureau and Cho (1994). The experimental diets consisted of 700 g/kg of the reference diet and 300 g/kg of the starch ingredients. Diets were mixed using a Hobart mixer (Model L-800, Ohio, U.S.) with approximately 100 g/kg water added to pro-

duce a dough. The resulting dough was cold extruded through a 5-mm die. After extrusion, the diets were dried in a forced air oven (55°C, 12 h), chopped and screened to obtain a suitable pellet size. The diets were stored at –20°C prior to feeding. Feed samples were randomly subsampled for proximate analyses.

Table 4.2 Ingredient compositions of experimental diets for the digestibility trial in rainbow trout.

Ingredient (g/kg)	Control	Pea starch	Lentil starch	Faba bean starch	Modified cornstarch
Fish meal[1]	300	210	210	210	210
Soybean protein concentrate	170	119	119	119	119
Corn gluten meal	130	91	91	91	91
Wheat flour[2]	280	196	196	196	196
Vitamin premix[3]	4.750	3.325	3.325	3.325	3.325
Mineral premix[3]	4.750	3.325	3.325	3.325	3.325
Vitamin C	0.50	0.35	0.35	0.35	0.35
Celite	10	7	7	7	7
Fish oil[4]	100	70	70	70	70
Pea starch	0	300	0	0	0
Lentil starch	0	0	300	0	0
Faba bean starch	0	0	0	300	0
Modified cornstarch	0	0	0	0	300

[1]South American Aquagrade; EWOS Canada Ltd.
[2]Robin Hood All-Purpose Flour; Robin Hood Multifoods Corporation, Markham, ON, Canada.
[3]Vitamin/mineral premix is a commercial premix; EWOS FISH-STR VIT PX, Surrey, BC; proprietary formulation.
[4]Mixed variety fish oil; EWOS Canada Ltd.

4.3.3.2 Experimental design

Rainbow trout (225 fish total; start weight 353 ± 6.8 g) were randomly assigned to 15 tanks (15 fish per tank; 3 replicate tanks per test diet). Fish were adapted to the experimental diets for 7 days before collecting fecal samples. Fish feces were collected using a modified Guelph system (Randall and Drew, 2011). Fecal samples were collected prior to feeding every morning. The collected fecal samples were centrifuged at 3000 rpm at 4 ºC for 10 minutes (Beckman Coulter J6-MC Centrifuge, Mississauga, ON), supernatant removed and fecal pellet frozen, then freeze dried before chemical analysis. The apparent digestibility coefficient (ADC %) for macronutrients and energy in the individual diets was calculated using the equation of Forster (1999).

4.3.4 Experiment 2: The effects of different starches on growth performance

4.3.4.1 Experimental diets

As the starch digestibility of faba bean starch was not detectable in the experiment 1, the effect of faba bean starch was not examined further in growth trials or glycemic response studies. An 8-week feeding trial was performed to assess the effects of feeding three starch sources (pea and lentil versus modified cornstarch) on the growth performance of rainbow trout. The 7 diets consisted of a control diet and diets containing 100 or 200 g/kg of pea, lentil or modified cornstarch (Table 4.3). The diets were formulated to meet or exceed the nutritional requirements of rainbow trout, were isoenergetic (4100 kcal/kg) and isonitrogenous (380 g/kg crude protein). Diets were extruded in the Saskatchewan Food Industry Development Centre (Saskatoon, SK). The extruder preconditioner speed was between 85.8 rpm and 86.4 rpm, total moisture was set between 33% and 36.6%, pressure was set at 24 bar and the screw speed was 396 rpm. Die size was 5mm and die temperature ranged from 122°C to 129°C. Feed was stored in sealed containers at 4 °C until use.

Table 4.3 Ingredient compositions of experimental diet for 8 weeks growth trial in rainbow trout.

	Diets						
Ingredient (g/kg)	Control	10% Modified corn starch	10% Pea starch	10% Lentil starch	20% Mod-ified corn starch	20% Pea starch	20% Lentil starch
Mineral premix[1]	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Vitamin premix[1]	5.00	5.00	5.00	5.00	5.00	5.00	5.00
WDDGS[2]	42.57	26.00	26.00	26.20	10.00	10.00	10.00
Soybean meal	200.00	105.00	160.00	200.00	10.00	119.13	200.00
Cellulose	70.00	40.00	40.00	47.00	10.00	10.00	24.11
DL-methionine[3]	1.35	1.23	1.57	1.67	1.13	1.80	2.00
Wheat gluten	1.00	10.50	10.50	10.40	20.00	20.00	20.00
Canola oil	125.00	123.10	124.93	104.00	121.24	125.00	83.67
Poultry byproduct meal	200.00	107.40	115.00	137.73	14.92	30.86	74.68
Corn Gluten Meal	10.00	26.77	15.30	10.00	43.38	20.62	10.00
Wheat Flour[4]	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Fishmeal[5]	260.10	275.00	275.00	273.00	289.33	289.00	285.54
Blood meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soy Protein Concentrate	9.98	105.00	51.70	10.00	200.00	93.60	10.00
Modified corn starch	0.00	100.00	0.00	0.00	200.00	0.00	0.00
Pea starch	0.00	0.00	100.00	0.00	0.00	200.00	0.00
Lentil starch	0.00	0.00	0.00	100.00	0.00	0.00	200.00
Digestible Energy (kcal/kg)	4,166	4,133	4,134	4,134	4,100	4,101	4,104
Digestible Protein (g/kg)	380	380	380	381	380	380	381

[1]Vitamin/mineral premix is a commercial premix; EWOS FISH-STR VIT PX, Surrey, BC; closed formulation.
[2]WDDGS = Wheat distillers dried grains with solubles
[3] DL-methionine, feed grade. Degussa Corporation, Theodore, AL, USA.
[4]Robin Hood All-Purpose Flour; Robin Hood Multifoods Corporation, Markham, ON, Canada.
[5]South American Aquagrade; EWOS Canada Ltd.

4.3.4.2 Experimental design

Rainbow trout (350 fish total; average initial weight 284 ± 2.6 g) were randomly assigned to 21 tanks (15 fish per tank; 3 replicate tanks/diet) and diets randomized to a given tank. Fish were acclimated for 7 days to the test diets before the start of the feeding trial. Fish were fed to satiety twice a day, with the amount of feed consumed recorded twice a week. At the end of the 8-week growth period, the final weight of each tank was measured and specific growth rate (SGR), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. Regression analyses were performed to determine the effect of starch inclusion rate on growth performance and feed utilization of the fish.

4.3.4.3 Calculation of growth indicators and feed utilization

Average daily gain (ADG), specific growth rate (SGR), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated by the following equations:

ADG (g) = (Average final weight-Average initial weight)/days

SGR = (ln final weight – ln initial weight) / days*100

ADFI (g) = Total feed consumption/ (fish number* days)

FCR = Average Daily Feed Intake/ Average Daily Gain

4.3.5 Experiment 3: Glycemic response testing

4.3.5.1 Experimental design

Methods for glycemic testing were adapted from those already established in our laboratory in dogs (Adolphe et al., 2012). After finishing the growth trial, fish were reassigned to basic husbandry tanks and fed commercial feed for a minimum of two weeks before starting the glycemic test. Individual fish were weighed, randomly assigned to individual tanks and fasted for 48 hours before starting the administration of each dietary treatment. The meal size used in this experiment was 0.25 g of available carbohydrate per kg of fish determined using a commercial kit (Table 4.4; Megazyme International, Wicklow, Ireland). Fish were fed glucose (Sigma-Aldrich, Inc., Missouri, USA) (control, N=10), pea starch (N=5), lentil starch (N=6), faba bean starch (N=5), modified cornstarch (N=5) or cornstarch (N=5). Each meal was force-fed in a gelatin capsule (Double

"00" Vcaps; Bloomingdale, IL 60108) after fish were anesthetized with 10 mg/l Aquacalm (metomidate hydrochloride, Western Chemical, Ferndale, WA) for each blood sampling or force-feeding time, with recover in an individual, normal husbandry tank between sampling time points. At time 0 h, fish blood was first sampled from the caudal vein of rainbow trout by using a 1ml syringe (BD, Franklin Lakes, NJ 07417 USA) and blood glucose immediately measured using a glucometer (OneTouch® Ultra® 2 Meter, Code25, LifeScan Canada Ltd., Burnaby, BC). Fish were then force fed with the test meal and recovered. Subsequent blood samples were taken at 6, 12, 24, 48 and 96 h after force-feeding. Fish continued to be fasted during this subsequent period of 96 hours.

Table 4.4 In vitro available carbohydrate content of pulse starch (pea, lentil, faba bean), modified cornstarch and cornstarch used in glycemic response testing in rainbow trout.

Treatment	Available carbohydrate (g/kg)
Glucose	1000
Pea starch	753
Lentil starch	759
Faba bean starch	712
Modified cornstarch	660
Cornstarch	1000

4.3.5.2 Calculation of glycemic index

The area under the glycemic response curve (AUC) of starch ingredients were calculated using the following formula:

GI = Integrated AUC $_{treatment}$ *100 / Integrated AUC $_{glucose}$

4.3.6 Sample analytic methods

All diets and fecal samples were ground using a ZM 100 Retsch Mill (Retsch GmbH, Haan, Germany) with a 0.5 mm screen. All samples were sent to the Central Testing Laboratory Ltd. (Winnipeg, MB) for proximate analysis. The moisture, crude protein, gross energy, lipid, ash and

fibre content of ingredients, and experimental diet were determined by standard methods. Starch analysis was conducted by using AOAC (Method 996.11) and AACC (Method 76.13) Megazyme total starch assay procedure (K-TSTA, 100 assays per kit, Burlington, ON).

4.3.7 Statistical analysis

Digestibility and glycemic response results were analyzed using the General Linear Models procedure of SPSS (Version 24, SPSS Inc., Chicago, IL, USA). The Ryan-Einot-Gabriel-Welsh F was used to separate means and differences were considered significant when $P < 0.05$. The effect of starch inclusion rate on fish growth parameters was analyzed by fitting linear and quadratic regression models to the data. Regressions and treatment comparisons were considered significant when $P < 0.05$.

4.4 Results

4.4.1 Experiment 1 – Digestibility of starch ingredients

Nutrient composition for diets and ingredients are shown in Table 4.5. The pulse starch ingredients used in the study contained 150-200 g/kg of crude protein. In contrast, both cornstarches used in this study (modified and unmodified) contained negligible concentrations of crude protein e.g. 5 g/kg for the modified cornstarch. For digestibility, there were significant differences in dry matter, crude protein, gross energy and starch digestibilities among the different starch ingredients ($P < 0.05$) (Table 4.6). The ADC% of dry matter for lentil starch was significantly higher than for pea starch or modified cornstarch and all were significantly higher than faba bean starch. The ADC% of crude protein of lentil starch was also significantly higher than that of pea or faba bean starches ($P < 0.05$). The ADC% of crude protein for the modified cornstarch was not detected due to the negligible crude protein concentration. The ADC% of gross energy of lentil starch was significantly higher than those of pea or modified cornstarch. All starches were significantly higher than the ADC% for gross energy of faba bean starch ($P < 0.05$). The ADC% of starch for lentil starch was significantly higher than that of modified cornstarch which was significantly higher than that of pea starch ($P < 0.05$). Most important for this study was the observation that the starch ADC% of faba bean starch was undetectable. Because faba bean starch was not digestible, this starch was not tested in the rainbow trout growth trial that followed next.

Table 4.5 Proximate analysis of each diet for digestibility trial in rainbow trout.

Diets	DM (g/kg)	CP (g/kg)	GE (kcal/kg)	Starch (g/kg)
Control	940	446	5250	219
Pea starch	931	371	5064	326
Lentil starch	932	375	5081	359
Faba bean starch	929	383	5098	307
Modified cornstarch	929	328	5007	332

DM = dry matter; CP = crude protein; GE = gross energy

Table 4.6 ANOVA outputs of apparent digestibility coefficients (ADC %) of the starch sources for dry matter (DM), crude protein (CP), gross energy (GE) and starch in rainbow trout.

Ingredient	DM (%)	CP (%)	GE (%)	Starch (%)
Pea starch	32^{b}	87^{a}	37^{b}	6^{a}
Lentil starch	73^{c}	102^{b}	74^{c}	55^{c}
Faba bean starch	1^{a}	79^{a}	12^{a}	ND
Modified cornstarch	30^{b}	ND	33^{b}	36^{b}
P value	<0.01	<0.01	<0.01	<0.01
SEM	45.9	15.3	34.5	51.4

ND = not detectable
SEM = Standard error of mean
Mean values with the different superscripts within columns are significantly different ($P < 0.05$).

4.4.2 Experiment 2 – The effects of different starches on growth performance

Growth performance in rainbow trout was tested in diets formulated with increasing starch inclusion. Isocaloric and isonitrogenous diets contained 0% (control), 10% or 20% lentil starch, pea starch or modified cornstarch and were tested an 8-week feeding trial (Table 4.7). There were no significant linear or quadratic relationships between starch inclusion level and ADG (Figure 4.1), SGR (Figure 4.2), ADFI (Figure 4.3) or FCR (Figure 4.4). The only exception was that a significant quadratic relationship was found between starch inclusion level and FCR for lentil starch (P = 0.03) (Table 4.8). The relationship between FCR and starch inclusion was U-shaped, with the best (lowest) FCR observed at 100 g/kg lentil starch.

Table 4.7 Proximate analysis of diets for growth trial.

Nutrient Composition	Control	10% Pea starch	10% Lentil starch	10% Modified cornstarch	20% Pea starch	20% Lentil starch	20% Modified cornstarch
Dry Matter (g/kg)	919	969	969	969	957	960	953
Gross Energy (kcal/kg)	5468	5305	5232	5487	5232	5132	5260
Crude Protein (g/kg)	483	495	489	430	407	472	408
Crude Fibre (g/kg)	84	76	73	84	44	61	52
Fat (g/kg)	187	154	150	196	154	127	156
Ash (g/kg)	101	100	110	91	88	98	84

Table 4.8 Linear or quadratic regression parameters of average daily gain (ADG), specific growth rate (SGR), average daily feed intake (ADFI) and feed conversion rate (FCR) of rainbow trout fed with diets containing 0, 100 and 200 g/kg pea starch, lentil starch and modified cornstarch.

	Regression parameters	Inclusion ^{2}	Inclusion	Constant	r^2	*P*
ADG	Pea starch	NA	0.076	3.52	0.29	0.14
	Lentil starch	-0.009	0.25	3.45	0.52	0.11
	Modified cornstarch	-0.008	0.21	3.45	0.40	0.22
SGR	Pea starch	NA	0.015	0.93	0.30	0.13
	Lentil starch	-0.002	0.048	0.91	0.55	0.09
	Modified cornstarch	-0.001	0.040	0.91	0.41	0.21
ADFI	Pea starch	NA	0.037	3.77	0.14	0.31
	Lentil starch	-0.005	0.11	3.78	0.19	0.53
	Modified cornstarch	NA	0.047	3.83	0.23	0.19
FCR	Pea starch	NA	-0.010	1.08	0.43	0.05
	Lentil starch	0.001	-0.032	1.11	0.70	0.03
	Modified cornstarch	0.001	-0.033	1.11	0.47	0.15

NA = not applicable factor since linear fit was better than non-linear for this end-point; Inclusion^2 refer to where inclusion rate is squared

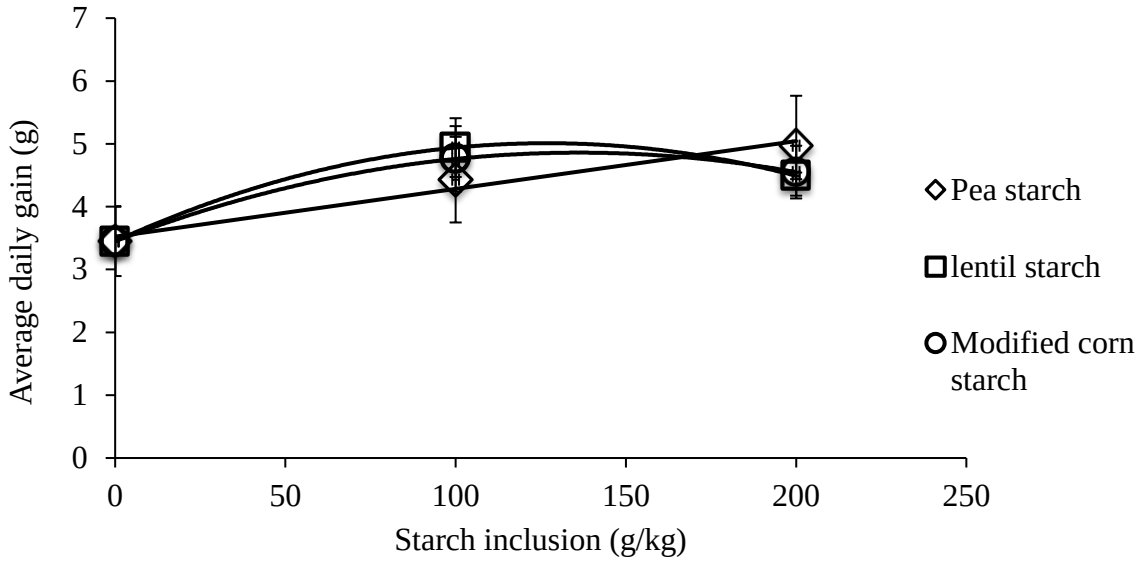


Figure 4.1 Regression models of average daily gain in rainbow trout fed by 0, 100 and 200 g/kg three starches (pea, lentil and modified corn). Each point shown is the mean of three replicates ± standard error. See Table 4.8 for fit statistics.

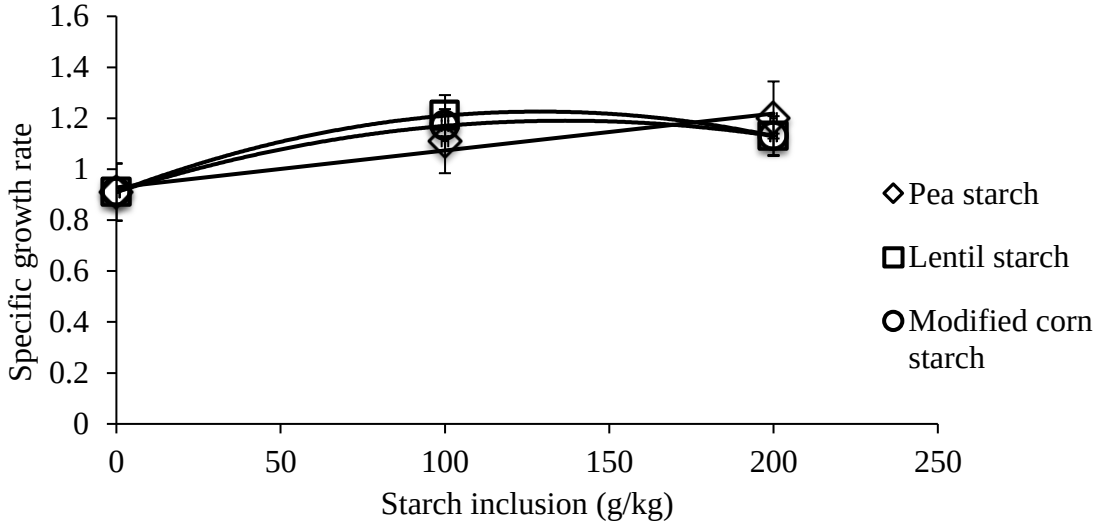


Figure 4.2 Regression models of specific growth rate in rainbow trout fed by 0, 100 and 200 g/kg three starches (pea, lentil and modified corn). Each point shown is the mean of three replicates ± standard error. See Table 4.8 for fit statistics.

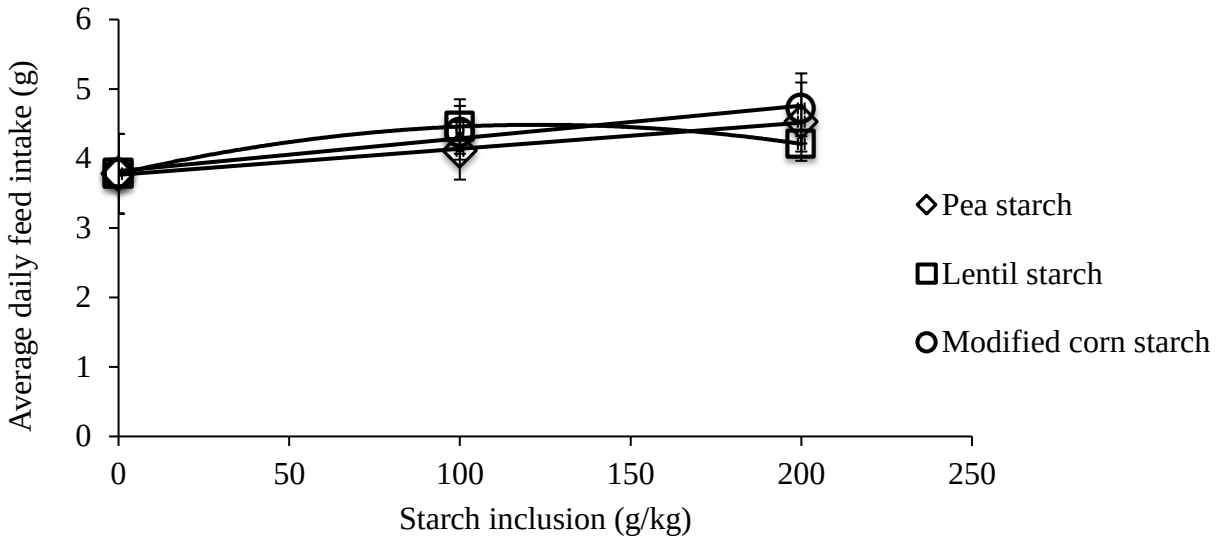


Figure 4.3 Regression models of average daily feed intake in rainbow trout fed by 0, 100 and 200 g/kg three starches (pea, lentil and modified corn). Each point shown is the mean of three replicates ± standard error. See Table 4.8 for fit statistics.

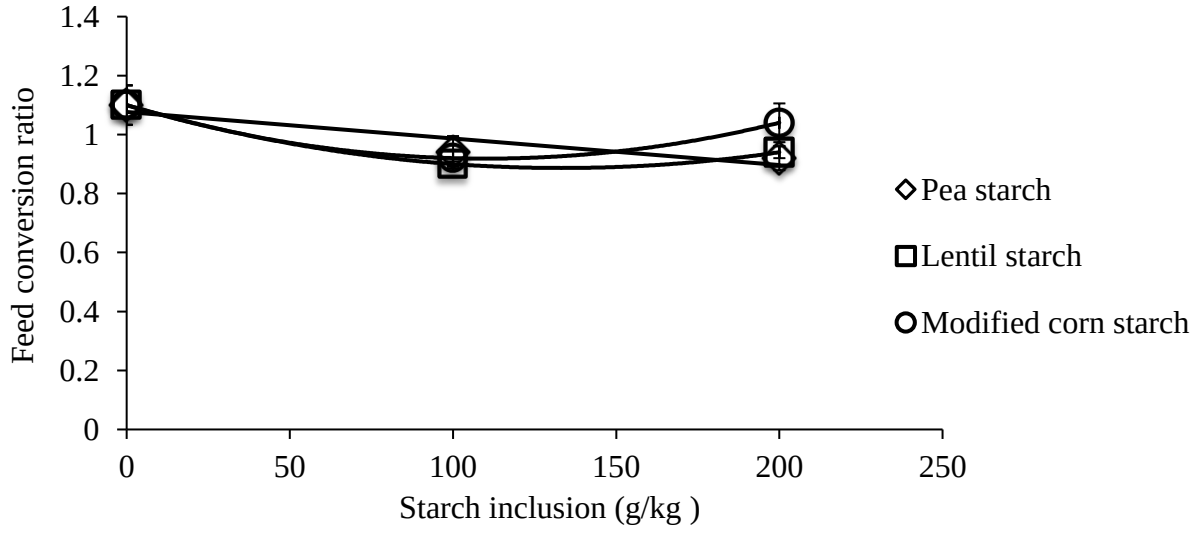


Figure 4.4 Regression models of feed conversion ratio in rainbow trout fed by 0, 100 and 200 g/kg three starches (pea, lentil and modified corn). Each point shown is the mean of three replicates ± standard error. See Table 4.8 for fit statistics.

4.4.3 Experiment 3 – Glycemic response testing of starch ingredients

Glycemic response testing was conducted using methods adapted to rainbow trout from dogs and humans. After 48 hr of fasting, fish were force-fed 0.25 g/kg body weight of available carbohydrate in the form of the starch ingredient alone. Blood glucose was measured at 0, 6, 12, 24, 28 and 96 hr after force-feeding (with no additional feeding during this period). At 0 h, the plasma glucose of the fish fasted for 48 hr ranged from 5.5 ± 0.40 mmol/l (Figure 4.5). Overall, blood glucose increased after feeding all carbohydrate sources, then fell to near baseline levels after fish were fed glucose or unmodified cornstarch (Figure 4.5). In contrast, blood glucose in fish fed unmodified cornstarch or any of the pulse starches (pea, lentil or faba bean) continued to rise during the 96-hr period examined (Figure 4.5). Thus, the time to peak for postprandial plasma glucose was significantly ($P < 0.05$) shorter after being fed glucose or unmodified cornstarch, while modified cornstarch, lentil starch or faba bean starch fed fish had peaks that occurred at or close to the end of the 96-hr period of testing (Table 4.9). The peak plasma glucose concentration in fish fed with glucose or unmodified cornstarch was not statistically different from each other, but was significantly lower than that of pea starch, lentil starch, faba bean starch or modified cornstarch ($P < 0.05$; Table 4.9). Surprisingly, the AUC for the glycemic response to feeding unmodified cornstarch was similar to that of glucose, but significantly lower than that after feeding modified cornstarch or pulse starches (Table 4.9). This in turn led to the GI of all starches except unmodified cornstarch being greater than 100 which defies the definition of glycemic index as originally proposed for humans. The GI value obtained in trout was lowest for unmodified cornstarch and this was not significantly different from glucose (Table 4.9). While GI values for modified cornstarch and all pulse starches tended to be higher than that of either glucose or unmodified cornstarch, only the difference between unmodified cornstarch and lentil starch was significantly different (Table 4.9).

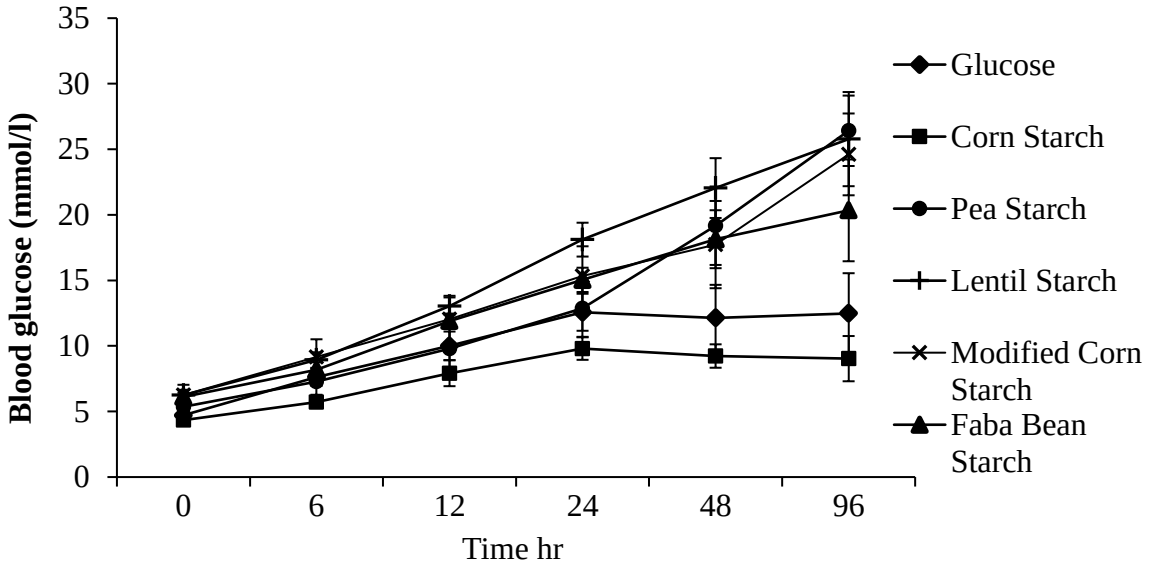


Figure 4.5 Glycemic responses in fasted rainbow trout following a single feeding of 0.25 g/kg available carbohydrate in the form of glucose, pulses starches (pea, lentil or faba bean), modified cornstarch and cornstarch over 96 hours. Each point is shown as mean ± standard error. Glucose n = 8 fish; Lentil n = 6; all other groups n = 5.

Table 4.9 Postprandial glycemic responses in fasted rainbow trout following a single feeding of glucose, pulse starches (pea, lentil and faba bean), modified cornstarch and cornstarch.

	Glucose	Pea starch	Lentil starch	Modified corn-starch	Faba bean starch	Cornstarch
Peak (mmol/L)	15.0 ± 2.4 ^{ab}	26.4 ± 2.7 ^{c}	27.1 ± 2.7 ^{c}	24.6 ± 3.1 ^{bc}	21.0 ± 3.5 ^{bc}	11.2 ± 1.3 ^{a}
Time to peak (hr)	54 ± 13 ^{ab}	96 ± 0 ^{c}	88 ± 8 ^{bc}	96 ± 0 ^{c}	77 ± 12 ^{abc}	48 ± 13 ^{a}
Area Under the Curve (mmol/L hr)	660 ± 174 ^{ab}	1189 ± 196 ^{b}	1329 ± 170 ^{b}	1086 ± 232 ^{ab}	1000 ± 213 ^{ab}	427 ± 51 ^{a}
Glycemic Index	100 ± 26 ^{ab}	180 ± 30 ^{b}	201 ± 26 ^{b}	164 ± 35 ^{ab}	151 ± 32 ^{ab}	65 ± 8 ^{a}

Results are showed as mean ± SEMs; SEM = standard error of mean.
Glucose n = 8 fish; Lentil n = 6; all other groups n = 5.
Values in a row with the same superscript indicate no significant difference. Differences were detected using Ryan-Einot-Gabriel-Welsh F test ($P < 0.05$) after 1-way ANOVA.

4.5 Discussion

4.5.1 Digestibility of starch ingredients

General starch digestibility of native legumes was reported ranged from 36.8 – 42.0% in human (Rehman and Shah, 2005). However, the ADC% of starch in dry processed faba beans and lentils has not been previously reported in rainbow trout. A previous study reported that the ADC% of starch for extruded pea was increased to 41-75% in rainbow trout (Thiessen et al., 2003). In this thesis, all starches were tested in native form, as cold-pelleted digestibility diets. The starch ADC% of dry processed pea starch was only 6% in rainbow trout in this thesis. The ADC% of faba bean starch in this thesis in rainbow trout was even lower, being non-detectable or 0%. In contrast, lentil starch digestibility was surprisingly high at 55% in rainbow trout in this thesis. Compared with mammals, Rehman and Shah, 2005 showed native lentil starch ADC% was 41% in humans and it was increased to 76% after cooking. ADC% of faba bean starch, pea starch and cornstarch was determined in pigs, which were 99.8%, 100% and 100%, respectively (Gunawardena et al., 2010). Moreover, Fleming and Vose (1979) reported also that the digestibility of all legume starches except high-amylose wrinkled pea was close to 100% in rats. Clearly fish, especially salmonids such as rainbow trout, cannot digest native starch as efficiently as mammals. As the available information on the digestibility of lentil starch in rainbow trout is absent, it is difficult to make a reasonable explanation for the relatively high ADC% of starch for lentil starch compared to other starch ingredients in this study.

However, the above digestibility values were all apparent total tract digestibility, which does not take into account microbial fermentation in the hindgut or colon. This can cause overestimation of starch digestibility. In present study, the method of total tract digestibility was also used to determine the digestibility of starch in rainbow trout, but the ADC% of starch for pulse starches or modified cornstarch in rainbow trout was very low. In contrast, tilapia showed a higher starch digestibility (50.5% - 60.9%) for pulse starches or modified cornstarch than rainbow trout (Guo, 2016). This can be attributed to longer intestine and higher amylase activity in omnivorous fish than carnivorous fish like rainbow trout (Wilson, 1994; Hidalgo et al, 1999). Rainbow trout is a strictly carnivorous fish with low amylase activity that has been used extensively in research (Furne et al., 2005). Hidalgo and Urea et al. had conducted a comparison of amylase and proteo-

lytic activities in different fish species in 1999. The finding showed that the lowest amylase activity was found in the digestive tract of rainbow trout compared with omnivorous such as seabream and carp. This can also be used to explain the higher ability of starch utilization in omnivorous fish.

Volatile fatty acids have been identified and quantified in the hindgut of only a few of marine herbivorous fish species such as *Kyphosus cornelii, K. sydneyanus* and *Medialuna californiensis* (Kandel et al., 1994). Clements and Choat (1997) reported that the concentration of short–chain fatty acids in the posterior intestine was less than 11.4 mM in *Girella* spp. (omnivorous fish) and more than 39.3 mM in *Kyphosus* spp. (herbivores) after ingestion of algal diets. This indicates that microbial fermentation may play a more important role in herbivorous fish than omnivorous fish species. White and Coveny (2010) also showed that mannitol was fermented to short-chain fatty acids in the hindgut of eight marine herbivorous fish species. While implications are that fermentation plays a role in the hindgut of herbivores or omnivores, similar fermentation is assumed to be insignificant in carnivorous fish species like rainbow trout. Starch digestibility can be affected by the methods of fecal sample collection. The Guelph system was used in present experiment. The Guelph system and the Choubert method with mechanically rotating screens have been showed to be highly reliable methods for collecting feces from salmonids compared to other methods such as stripping, anal suction and intestinal dissection (Hajen et al., 1993).

In addition, it has also described that there is an inverse relationship between starch digestibility and the inclusion levels of carbohydrates, with maximum inclusion level of crude starch thought to be 20% in salmonids (Spannhof and Plantikow, 1983). In this thesis, 30-35% total starch was present in the diets testing digestibility and this may have falsely decreased the apparent digestibility of the starch sources. Bergot and Breque (1983) reported that rainbow trout fed with 31% native cornstarch had 38% starch digestibility. Bergot in 1993 also showed that starch digestibility of maize starch (28%) was 34% in trout. These findings are consistent with the ADC of starch for cornstarch observed in this thesis. Moreover, high inclusion levels of both native and gelatinized starches can depress starch digestibility (Hua and Bureau, 2009). Brauge and Medale (1994) showed that the ADC% of starch for crude wheat starch decreased from 81.1% to 35.4% as the inclusion level increased from 8% to 24.4% in rainbow trout. It also reported that rainbow

trout fed with 14.4% of gelatinized wheat starch had 98.3% of ADC for starch while ADC% of starch was 82.8% when trout fed diet with 22.8% of extruded pea (Burel et al., 2000).

In the present thesis, digestibility diets were prepared using cold extrusion and no gelatinization would have occurred. All test starches are likely to be in native form. However, hydrothermal treatment has been reported to improve the digestibility of starch in rainbow trout (Drew et al., 2007). Slow digestion of raw starch is attributed to the ordered structure of starch granules with alternating crystalline and amorphous layers. Hydrothermal treatments causes starch granules disintegrated that increases their susceptibility to enzymatic degradation (Svihus and Hervik, 2016). It has been stated that rainbow trout could use gelatinized starches very well rather than native starches (Degani and Viola, 1986). Feed processing like hot extrusion enhances starch digestion in several fish species such as rainbow trout and gilthead sea bream (Venou et al., 2003). It has been reported that the digestibility of extruded wheat starch was higher (48%) than native wheat starch (22%) in trout. Similar findings were observed when rainbow trout were fed cornstarch. It showed the digestibility of raw cornstarch was between 30% and 40% while that of gelatinized cornstarch ranged from 87% to 90% (Bergot and Breque, 1983). Hua and Bureau (2009) conducted a model to elucidate variation in starch digestibility across studies and this model indicates that dietary inclusion levels negatively influence the digestibility of starch and the regression model showed as following: digestible starch content (%) = 0.560 raw starch – 0.011 raw starch^2 + 0,904 gelatinized starch – 0.009 gelatinized starch^2 (only considering starch as a factor). Krogdahl et al. (2004) fed Atlantic salmon and rainbow trout diets with precooked corn at a low carbohydrate level (7%) and high carbohydrate level (23%). Both species showed a negative correlation between starch digestibility and inclusion levels. At 7% starch, starch digestibility was 86.4% for Atlantic salmon and 99.1% for rainbow trout whereas; starch digestibility was 58.1% for Atlantic salmon and 97.5% for rainbow trout at high starch diet with 23% starch (Krogdahl et al., 2004). Stone 2003 also demonstrated that the ADC% of native wheat starch decreased from 79% to 41% as the inclusion level was increased from 30% to 60% in silver perch (*Bidyanus bidyanus*). A similar pattern was observed when gelatinized wheat starch was used in diets for silver perch (89% of ADC for diet with 30% gelatinized wheat starch and 70% of ADC for diet with 60% gelatinized wheat starch).

Starch source is one of the major factors affecting the digestibility of starch in vitro. In this project, the ADC% of modified cornstarch was higher than pea starch or faba bean starch, consistent with the hypothesis that cereal starches would be more readily digested than pulse starches. However, the ADC% of modified cornstarch was lower than lentil starch, which was unexpected. Starch granules in pulse starches and cereal starches are significantly different and thought to be related to the ratio of amylose and amylopectin (Kraugerud et al., 2011). Starch granules of corn are angular-shaped and less smooth surfaces with pin holes or fissures, which facilitates enzymes entering into the starch granules (Singh et al., 2003). In contrast, starch granules of most pulse starches are oval and their surfaces are smooth without pin holes or grooves. Lower digestibility of pulse starches has been attributed to a lack of holes on the granule surfaces (Hoover et al., 2010). Moreover, pulse starches have a higher amylose concertation compared with cereal starches. Apparent amylose content of pea, lentil and faba bean starch were 24-88%, 23.5-32.3% and 17.9-42%, respectively (Hoover et al., 2010). In contrast, amylose concentration of normal cornstarch ranged between 22.4-32.5% (Singh et al., 2003). Amylose is a linear structure, consisting of α-1, 4 linkages (Eliasson, 2004). This alpha (α) form causes some starch polymers to form helical structures, restricting enzymatic hydrolysis. In comparison, the branched structure of amylopectin is more easily attacked by digestive enzymes (Thomas and Atwell, 1999). Therefore, high amylose content is considered a more resistant starch, which is slower to be digested by starch enzymes (Wang and Copeland, 2013). Chemically modified corn with hydroxypropyl groups improves certain properties such as clarity of starch paste, viscosity and reduced syneresis (Singh et al., 2007). Thus, the chemical modification was expected to also alter digestibility. However, a previous study showed that the starch ADC% of unmodified cornstarch in rainbow trout was 38.1% (Thiessen et al., 2003), a value remarkable similar to that observed in this thesis for the hydroxypropyl modified cornstarch (36%).

The methods for starch isolation also affect starch digestibility. Pulse starches used in this study were obtained through dry milling and still contain 15-22% crude protein. The purity of pulse starches from dry milling is lower than that from wet milling. Moreover, the protein particles cannot be completely separated from starch granules by air classification (Hoover et al., 2010). Other macro-nutrients such as fat and protein on the surface of starch granules may act as

physical barriers, reducing the susceptibility to enzymatic hydrolysis and lowering starch digestion (Yu et al., 2004; Svihus et al., 2005). Moreover, the presence of antinutrients including phytic acid, condensed tannins, protease inhibitors and **α**-amylase inhibitors can restrict the protein and starch digestibility in different animal species (Alonso et al., 2000). This might also explain the relatively low digestibility coefficients of each nutrient for pulse starches in rainbow trout in this thesis. Feed processing not only reduces or even removes antinutritional factors present in botanical material, but also increases the susceptibility of starch granules to enzymatic breakdown and then improves the digestibility of nutrients in diet (Kraugerud and Svihus, 2011; Wang and Copeland, 2013). Further research is suggested to examine whether processing such as thermal extrusion improves not only starch utilization, but also increases utilization of dry matter, gross energy and protein in rainbow trout.

4.4.2 The effect of different starches on growth performance

In this thesis, there were no significant linear or quadratic relationships between starch inclusion level and average daily gain or specific growth rate when rainbow trout were fed up to 200 g/kg of starch ingredients. This is in agreement with the results of meta-analysis performed in Chapter 3 where it was concluded that there were no significant differences in final weight between control and carbohydrate-enriched diets fed carnivorous fish species at up to 280 g/kg of carbohydrates, regardless of sources. Therefore, based on both meta-analysis and growth trials in this thesis, pulse starches can be safely included up to 20% in rainbow trout diets without compromising growth or feed conversion ratio.

Similar findings were observed in other fish species such as golden pompano (Zhou et al., 2015) and gilthead sea bream (Enes et al., 2008). However, this result is opposite with the findings from Gumus and Ikiz (2009) in rainbow trout where impaired growth performance occurred at higher dietary starch inclusions. Wu et al. in 2007 also reported that the growth of yellow sea bream fed diets without carbohydrate was better than that of diets with carbohydrates. In the meta-analysis from Chapter 3 of this thesis, a 200 g/kg carbohydrate inclusion level was suggested as an optimal inclusion for maximum growth in most carnivorous fish species. Findings from the current chapter of this thesis do not support this optimal inclusion since there was no significant

difference in growth performance detected among treatments. As discussed earlier, hydrothermal treatment of starch through processes such as extrusion improves both digestibility as well as growth performance in rainbow trout (Podoskina et al., 1997). Boccignone et al., in 1989 observed that the FCR of diets containing extruded corn was 17% better than that of native corn in rainbow trout after a 30 day feeding trial. In other carnivorous fish like eels that have the similar dietary habits to rainbow trout, Degani and Viola (1986) showed 1.05% of SGR when eels were fed a diet containing 27% of soluble cornstarch, while 0.48% of SGR was observed on a native potato starch diet in a 79 day feeding trial. This indicates that eels also use gelatinized starch more efficiency than native starch, similar to rainbow trout. The growth diets (extruded) used in this thesis were formulated based on the digestibility values obtained by feeding uncooked starch (cold pelleted). Thus, the actual digestible nutrient content of the growth diets might have differed significantly from the calculated values. Growth diets were formulated to be isocaloric based on the cold pelleted digestibility diets, but the potential increase in available carbohydrate after extrusion may have made the high starch diets have higher caloric density. However, given that the growth performance parameters did not differ significantly among diets tested, this suggests that first that caloric density was unlikely to have changed much and second that the effect of hydrothermal processing similarly affected all ingredients.

As discussed above, carbohydrate inclusion level is one of the major factors affecting starch utilization. Starch digestibility decreases with increasing dietary carbohydrate inclusion level in many carnivorous fish species such as rainbow trout, cod, Atlantic salmon and silver perch (Stone, 2003). Adamidou et al evaluated three legumes (field pea, chickpeas and faba bean) at two different inclusion levels (17% and 35%) in European seabass in 2009. In their study, wheat was used as control and all diets (extruded) showed improved growth compared to the control. They concluded that these legumes could be included up to 35% to replace wheat in the feed of European sea bass without adverse effects on growth performance (Adamidou et al., 2009). In this study, three inclusion levels of starch ingredients had no significant effect on growth or feed utilization and no significant difference was observed between treatments. Based on this study and the results of the current thesis, it can be concluded that pea starch or lentil starch could be

used as a substitute instead of cereal starches up to 200 g/kg of starch ingredient without compromising growth performance of rainbow trout.

4.5.3 Glycemic response of starch ingredients

While many studies report post-prandial glucose responses in fish (Palmer and Rynan, 1972; Polakof et al., 2012), glycemic testing using the strict methodology employed in humans for human food testing has never been done previously in fish. In preliminary glycemic tests, force-feeding rainbow trout with 0.5 g glucose/kg fish body weight produced prolonged elevations in blood glucose even at 96 hours postprandial. Levels greater than 33.3 mmol/L blood glucose (a level associated with severe, lethal diabetes in mammals) were observed at 48 hours postprandial, agreeing with earlier research in rainbow trout (Palmer and Rynan, 1972). Therefore, the amount of available carbohydrate fed to fish in this thesis was reduced to 0.25 g/kg body weight. In this thesis, the glucose levels ranged from 4.34-6.11mmol/L at the baseline among individual fish. This finding is in agreement with a previous study, which defined the normal range of plasma glucose in salmonids as between 3-10 mmol/L (Hemre and Mommsen, 2001). Legate and Bonen et al. in 2001 conducted an intravenous glucose tolerance test in rainbow trout. After intravenous injection of glucose, plasma glucose increased rapidly within 1 min and levels decreased slowly until returning back to baseline by 24hr. Other studies have reported that plasma glucose can return to baseline within 24 hours after an oral gavage of glucose in rainbow trout (Bergot, 1979; Hemre and Hansen, 1998), which does not agree with findings in this thesis. The major difference between the work in this thesis and other studies is that all other studies introduced glucose as a solution, while this thesis force-fed powdered glucose or pulse starches in a capsule. The reason for this is that preliminary work in tilapia in our group using glucose lavage solutions laced with dye revealed that a good portion of lavaged fluid was vomited or leaked out of the fish's mouth after gavage (Guo, 2016). Since loss of the force-fed meal would falsely lessen the post-prandial glycemic response peak and duration, a capsule was considered a suitable compromise. While digestion of glucose or starch from a capsule would delay the glucose peak until after the capsule is dissolved, all treatments would be similarly delayed and thus comparisons among carbohydrate sources still valid. Blood glucose levels of rainbow trout following feeding of pulse

starches (pea, lentil and faba bean) or modified cornstarch increased continuously, while glucose or unmodified cornstarch produced a lower, shorter glycemic response. This difference must be related to the starches themselves, not the gelatin capsule. Also, blood glucose levels after feeding pulse starches and modified cornstarch were generally higher than that after glucose treatment, a finding that is opposite to what has been reported in humans and dogs (Adolphe et al., 2012). Complex starches are known to take longer to be digested and absorbed compared to simple starches like glucose (Stone, 2003). In fact, low starch digestibility of pulse starches (pea, lentil and faba bean) and modified cornstarch was found in this study in rainbow trout. Low digestibility was predicted to produce lower glycemic responses.

Prolonged postprandial hyperglycemia after consumption of complex carbohydrates was instead observed in rainbow trout in this thesis. Literature in this area has previously suggested fish are glucose intolerant (reviewed in Moon, 2001). Moreover, several possible factors unique to fish may explain prolonged hyperglycemia, namely a high susceptibility of fish to handling stress and high gluconeogenic capacity in carnivorous fish (Hemre and Mommsen et al., 2001). Stress during handling induces an elevation of epinephrine and cortisol in the blood, both of which increase blood glucose levels in fish (Stone, 2003). Although fish were anesthetised in this thesis before taking each blood sample, stresses from catching and allocating fish prior to sampling cannot be avoided. Persistent high plasma glucose in rainbow trout may also be attributed to gluconeogenesis. Furuichi and Yone in 1982 revealed an additional increase in gluconeogenesis in omnivorous (carp) and carnivorous fish species (red sea bream and yellowtail) after a glucose load, with yellowtail having the highest increase compared to carp. Elevated blood glucose caused solely by gluconeogenesis has also been observed in rainbow trout fed diets lacking dietary carbohydrate after 30 hours (Brauge and Medale et al., 1994). Protein is also a potent stimulator of gluconeogenesis in fish where glycogenic amino acids are converted into glucose (Polakof et al., 2012). Harmon and Eliertson in 1991 also pointed out that somatostatin is released after a glucose load in rainbow trout from somatostatin-secreting cells. Since these cells are very sensitive to glucose and this pancreatic hormone inhibits insulin secretion, this may also provide an explanation for hyperglycemia in rainbow trout. With the experimental design used in this thesis, exogenous versus endogenous sources of glucose cannot be distinguished. Other possible ex-

planations in previous research include an imbalance between hepatic glucose phosphorylation versus glucose-6-phosphate hydrolysis, the fact that amino acids are better insulin secretagogues compared to glucose in fish, low numbers of muscle glucose transporters, low insulin receptors and low glucose phosphorylation in muscle (Polakof and Panserat et al., 2008). Diet adaption can affect the degree of starch utilization in fish (Hemre et al., 2001). However, in this thesis, fish used for glycemic testing were not acclimated to the test diet and instead were fed high fishmeal protein-containing commercial diet immediately prior to testing. Thus, it would be interesting to have compared results in unacclimated trout to those in fish at the end of the growth trial. Further studies examining postprandial glucose metabolic responses, the role of gluconeogenesis and insulin dynamics are warranted to provide explanations for observations in this thesis.

This thesis is the first study to determine GI of feed ingredients in rainbow trout, so there is no data available in fish with which to compare. Glycemic indices for pulse starches were general higher than cornstarch in rainbow trout in this study. In human, pulse is defined as low GI food ($\leq$55) but the GI of pulses determined in this study is opposite to that. Interesting, the GI for unmodified cornstarch in rainbow trout was 65 in this thesis, which is similar with the GI of normal cornstarch determined in humans (Kumar and Prabhasankar, 2014). The original prediction was the low digestibility starches would produce low GI values. Thus, pea starch, which had a low digestibility in Chapter 3 of this thesis, was predicted to have a low GI, the opposite was observed. This combined with the observed prolonged hyperglycemia, the glycemic index model derived from mammal does not work for rainbow trout. Glucose utilization in rainbow trout appears more complicated and available information is limited. More research on glucose tolerance in rainbow trout is needed and needs to be related to other parameters such as insulin, glucose transporters or glucosensing system in fish.

4.6 Conclusion

The most important finding of this study is that dietary starches can be added up to 20% without adverse effects on growth and feed utilization. Importantly, pulse starches can be used as a starch source in the feed of rainbow trout to support good growth performance. Starch digestibility for pulse starches or modified cornstarch was low in present study. As a result, feed pro-

cessing techniques such as extrusion are highly recommended for aquafeeds. Extrusion not only improves digestibility of nutrients, but also provides more valuable information on digestible nutrients before formulation of growth diets. Polakof et al. (2008) demonstrated that dietary carbohydrates play an important role as a regulator in the glucosensing system (similar to mammalian models) of rainbow trout. Despite this acknowledge role, GI values for pulse starches and cornstarch in rainbow trout refute the mammalian model of glycemic responses and the mechanisms of starch utilization and glucose metabolism in rainbow trout remains unclear.

5. GENERAL CONCLUSION AND FUTURE PERSPECTIVES

Rapid expansion of aquaculture in recent decades has led to a crucial and inevitable need for replacement of costly fishmeal with alternate energy sources such as carbohydrates in fish feeds. In the meta-analysis and the growth trial of this project, results indicated no adverse effects on the growth of rainbow trout when fed with high carbohydrates diets. Cereals starches like corn and wheat are commonly used as carbohydrates source in aquafeeds and use of cereal starches has been widely studied in fish. In contrast, utilization of other starches such as pulses in aquafeeds is limited. In Canada, pulses have become one of the largest crops produced. Pulse Canada in 2010 reported that Canada was the second largest producer of pulses with 4.4 million tonnes in the world. Around 70% of pulses are exported from Canada every year, with major areas for pulse yields being Manitoba, Alberta and Saskatchewan (Ambigaipalan et al., 2011). Despite high local production, North American and European consumption of pulses by humans is low. Pulse industries need an expanding, reliable market such aquaculture to expand and sustain pulse sales. In this project, pulse starches (pea, lentil and faba bean) were examined and compared with modified cornstarch in rainbow trout. The digestibility experiment showed low starch digestibility of raw pulse starches as well as modified cornstarch in rainbow trout. Rainbow trout is a strict carnivorous fish species that uses protein more efficiently than carbohydrates. The low capacity to digest native starch in fish has been described and reviewed by Wilson in 1994. Although low digestibility of raw starch is observed in fish, processing technology has improved to remedy this problem. The extrusion technique with a combination of heat and moisture greatly improves carbohydrate digestibility. Because the utilization of native starch can be improved by feed processing, it seems the level of incorporation plays a more important role in the determining rationale diet formulations for fish compared to starch sources.

Recommended levels of digestible carbohydrates are less than 20% for coldwater fish while higher levels can be used in warmwater fish (Wilson, 1994). Carbohydrate digestibility has an inverse relationship with inclusion level regardless of processing and this has been indicated in many studies. For example, digestibility of cooked potato starch in rainbow trout was 69% when the inclusion level was 20% in the diet, but 26% digestibility was found when inclusion level increased to 60% (Singh and Nose, 1967). This also happens in others species like channel catfish

and common carp (Chiou and Ogino, 1975). Growth diet formulation is based on digestible nutrients. In order to maximize the amount of carbohydrates while reducing nutrient waste in fish diets, the appropriate inclusion level of carbohydrates and processing methods should be taken into account to produce more valuable digestible nutrients using digestibility trials. In this project, ADG, SGR, ADFI or FCR were not affected by starch inclusion levels (0%, 10% and 20%). This implies the pulse starches or modified cornstarch can be included in the diet of rainbow trout up to 20% without adverse influences on growth and feed utilization. In order to maximize the incorporated levels of carbohydrates in the diet of rainbow trout, higher levels (> 20%) of pulse starches versus cornstarch needed to be investigated.

On one hand, maximizing the content of carbohydrates in fish diets can reduce feed costs. On the other hand, it may cause health problems to fish, especially for carnivorous fish such as rainbow trout. Feeding carbohydrate-rich diets has negative impact on fish liver. Increased liver size has demonstrated in salmonids and other carnivorous such as red sea bream and yellowtail (Phillips et al., 1948; Furuichi and Yone, 1971; Shimeno et al., 1979). Persistent hyperglycemia was also observed when fed rainbow trout diets with 15% and 30% glucose (Bergot, 1979a). From the GI trial of the project, prolonged postprandial hyperglycemia was observed in rainbow trout after single feedings of glucose, pulse starches and cornstarch. However, this thesis tested starch ingredients by themselves in unacclimated rainbow trout instead of whole diets in acclimated fish. Whether responses would be different if whole meals were fed or if GI results would change after acclimation to starch-containing diets is unknown. The original thought was to use GI model from human to determine the GI of food in rainbow trout, and see whether GI of pulse starches or cornstarch has a similar process to mammals. However, the results of this thesis clearly indicate that the GI model does not fit rainbow trout. Although many of the mammalian components of glucose metabolism and regulation are present in fish, metabolic rate is much slower than homeothermic animals (Wilson, 1994). For better use of carbohydrates in fish, it is first necessary to better understand how glucose is metabolized and regulated in fish. In order to compare the effects of different starch sources in fish, I suggest using whole diets to test glucose tolerance. Moreover, parameters such as plasma insulin, glucagon and somatostatin are also important to measure in future research. In addition, better characterization of carbohydrate digesting; glucose

transporting and metabolic enzyme gene expression throughout the gut of fish is needed. This information could provide a basis for better comparing how different starch sources are utilized in fish. Polakof et al. (2012) pointed out that rainbow trout fed diets containing carbohydrates could improve the ability of regulate blood glucose. According to this, glucose tolerance testing following an 8-week growth trial would be good to test.

While dietary carbohydrates have been examined in many fish species including salmon, carp and catfish using diets with simple carbohydrates, effects are varied among studies. In contrast, the use of dietary fibre in fish feeds has not yet been well studied. Monogastric animals including fish are not capable of digesting fibre due to a lack of suitable enzymes in the intestinal tract, but fibre is known to be important for gut health and microbial ecology. In contrast, some studies indicate that rainbow trout may have limited ability to degrade cellulose, depending on the intestinal bacterial population (Davies, 1985). The role of gut microbial community in fish is relatively unknown. In conclusion, starch has become a preferable energy source used in fish diets. Using the combination of heat and moisture through the process of extrusion is very important for starch utilization in fish. This is especially true in carnivorous fish such as rainbow trout. Finally, and most important, a 20% starch inclusion level for rainbow trout has no negative impacts on growth and feed efficiency, thus should be recommended for wide use in aquaculture.

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