DETERMINATION OF THE EFFECTS OF CORN, PEA, LENTIL AND FABA BEAN STARCHES ON THE DIGESTIBILITY, GROWTH PERFORMANCE AND GLYCEMIC INDICES IN NILE TILAPIA *(OREOCHROMIS NILOTICUS)*.

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ABSTRACT

A meta-analysis was performed using five random effects models to compare the growth performance of Nile tilapia *(Oreochromis niloticus)* fed different carbohydrate sources (raw starch versus glucose; raw starch versus dextrin; hydrothermally processed starch versus glucose; hydrothermally processed starch versus dextrin; and starch sources with different amylose content). The standardized mean difference (SMD) of growth indicators was calculated using Hedges' g (HG). The growth performance of fish fed raw starch in the diet was significantly higher than glucose (SMD = -0.64; $P < 0.05$), but significantly lower than dextrin (SMD = 0.61; $P < 0.05$). The starch source with amylose content under 10% was considered as the control group to interpret the effects of different amylose and amylopectin content to growth. There was a significant quadratic relationship between effect size and amylose content in starch source ($P < 0.01$, $r^2 = 0.545$).

Following the meta-analysis, three experiments were performed to determine the 1) digestibility, 2) growth performance and 3) glycemic indices (GI) in Nile tilapia fed different pulse starches. In Experiment 1, 405 mixed sex Nile tilapia (mean weight 336 g) were fed one of five diets containing 700 g kg^{-1} of the reference diet and 300 g kg^{-1} of the experimental ingredient (modified cornstarch, pea, lentil or faba bean starch). The apparent digestibility coefficients (ADCs) for dry matter, crude protein, gross energy and starch varied between the four starch ingredients. The ADC of starch in the four ingredients ranged from 50.5 to 60.9. Lentil starch was significantly higher for all digestibility parameters compared to the other starch sources.

An 8-week growth trial was then conducted where Nile tilapia (mean weight 531 g) were fed diets containing either 0, 150 or 300 g kg^{-1} modified corn, pea, lentil or faba bean starch (306 g kg^{-1} digestible crude protein and 3600 kcal kg^{-1} digestible energy based on the digestibility results from Experiment 1. There was a significant quadratic relationship found between starch inclusion levels and average daily gain (ADG), specific growth rate (SGR), or feed conversion ratio (FCR) in fish fed diets formulated with faba bean starch. The highest growth performance was achieved when faba bean starch inclusion was approximately 200 g kg^{-1} ($P < 0.05$), with lower growth performance at inclusion rates both above and below this level. In contrast, no significant relationship was found between faba bean starch inclusion and average daily feed intake (ADFI). A significantly positive linear relationship was seen between the remaining starch inclusion levels and ADG or SGR ($P < 0.05$). Lastly, a negative linear relationship was found between pea starch or modified cornstarch inclusion and FCR, but no significant difference was found between lentil starch inclusion and FCR.

In Experiment 3, fasted Nile tilapia (6 per ingredient) were anesthetized and force fed 0.5 g available carbohydrate per kg body weight of glucose, pure corn, pea, lentil or faba bean starch. Blood samples were taken from the caudal vein at 0, 3, 6, 12, 24, and 48 h after force-feeding. All starch sources produced a glycemic response that peaked around 12 hours after feeding. Using the glycemic response to pure glucose as a control index at 100, the GI of corn, pea, lentil and faba bean starches were 61, 54, 69, and 76, respectively, with no significant differences found between them.

In conclusion, Nile tilapia was able to digest pulses starch and was able to absorb and effectively handle glucose. These results suggest that pea and lentil starch can be included in Nile tilapia diets up to 300 g kg^{-1} without negatively affecting fish growth performance but faba bean starch should not be fed above 200 g kg^{-1}. Therefore, pulse starches were recommended in tilapia diets to reduce the use of ecologically and economically costly fishmeal, which contribute to the sustainable development of aquaculture.

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ABBREVIATIONS

ADC: Apparent digestibility coefficient
ADFI: Average daily feed intake
ADG: Average daily gain
AUC: Area under curve
CI: Confidence interval
df: Degree of freedom
FCR: Feed conversion ratio
FW: Final weight
GI: Glycemic index (indices)
GLUT: Glucose transporter
HG: Hedges’s g
I^2: Heterogeneity
IV: Inverse variation
SD: Standard deviation
SGLT: Sodium-dependent glucose transporter
SGR: Specific growth rate
SMD: Standard mean difference
WG: Weight gain

1 INTRODUCTION

Global fish consumption per capita increased tremendously from 9.9 to 19.2 kg between 1960 and 2012 (FAO 2014). However, the capture fishery production has remained almost constant at 90 million tonnes per year for the last 25 years (FAO 2014). To fill the growing gap between supply and demand, aquaculture has expanded rapidly and now represents nearly 50% of total global seafood production (FAO 2014).

Fish feed is a major cost in the aquaculture industry accounting for approximately 50-70% of total production costs. Protein is the most expensive component of aquafeeds and has been mainly supplied by fishmeal. However, fishmeal is derived from marine capture fisheries and so, its supply has also remained relatively constant. Combined with the growing demand for fishmeal, the price of fishmeal has increased dramatically from approximately $500/tonne in 2003 to $1900/tonne in 2013 (FAO 2014). Moreover, all wild fish stocks, including those used for fishmeal are increasingly scarce and continued reliance on them is ecologically unsound. Thus, identifying an alternative ingredient for decreasing the use of fishmeal in aquafeeds has become one of the most important research areas to sustainably satisfy the rapid expansion of aquaculture. Plant protein sources are the obvious choice as substitutes for fishmeal owing to their availability and stable costs. However, plant protein sources have lower nutrient value than fishmeal and most of them also contain anti-nutritional factors, which can result in the negative impacts on fish growth and health (Drew et al., 2007). Thus a large amount of research has been done to improve the nutritional value of plant ingredients in finfish.

While most research on the use of plant ingredients in aquafeeds has concentrated on protein, most plant seeds contain high amounts of another energy substrate: starch. Starch is the main polysaccharide found in grains and pulses, and is the primary source of dietary energy in most mammalian and avian livestock diets. However, it is generally accepted that starch is poorly tolerated in teleost fish (Moon 2001). Polakof et al. (2012a) reported that fish tend to develop hyperglycemia more readily than mammals after being fed diets high in starch. However, there appear to be species differences. Glucose tolerance test results are quite variable in different fish species, but more studies to date have been conducted in carnivorous species, particularly salmonids (Polakof et al., 2012a). Increased glycosylated haemoglobin and retinopathy were found in hyperglycaemic Indian perch (Barma et al., 2006) and zebrafish

(Gleeson et al., 2007), respectively, which are the same symptoms seen in diabetic mammals with prolonged elevations in blood glucose. Despite these observations, there are some suggestions that omnivorous fish can tolerate higher dietary starch concentrations compared to carnivorous fish species (Legate et al., 2001; Polakof et al., 2012a).

The digestion and metabolism of glucose is determined by the type and magnitude of intestinal enzymes, glucose transporters and key glycolytic enzymes. Excess glucose can result in accumulation of the toxic glucose metabolite, methylglyoxal, which can bring about oxidative stress and inflammation in mammals (Dhar et al., 2008). The glucose tolerance levels vary widely in teleost fish (Polakof et al., 2012a), which may relate to the nutritional niche of the species (carnivores versus omnivores versus herbivores). Krogdahl et al. (2005) reported that salmonids have a poor ability to digest starch even if the starch is gelatinized. Some studies have indicated that herbivorous and omnivorous fish such as common carp and Nile tilapia can have optimal growth on diets with up to 50% glycemic carbohydrate (Wilson 1994; Hemre et al., 2002; Polakof et al., 2012a). However, carnivorous species such as Atlantic salmon do not digest or metabolize dietary starch well and have a prolonged glycemic response after ingesting starch (Krogdahl et al., 2005).

The metabolism of dietary starch depends on several factors. Starch sources vary in starch structure: amylose versus amylopectin. Amylopectin is metabolized more rapidly than amylose and this may reduce its nutritional value in some species of finfish. The starch granule Type (A, B and C) can also result in the different rates of digestion (Schafer and Drew 2007). Type A granules are rapidly digested, while Types B and C granules are more slowly digested. Based on these observations, a starch, which is high in amylose and has Type B or C starch granules, might be the best starch source for finfish, especially carnivorous fish species due to the slower rate of digestion and thus lesser likelihood of producing hyperglycemia.

Presently, extruded aquaculture diets use wheat or cornstarch only as pellet binders and are generally not included as a major source of energy (generally <10% inclusion by weight). Thus, aquaculture fish consume only small amounts of starch and this is generally rapidly digestible starch. Pulses are the edible grain seed harvested from an annual legume (*Fabaceae* family). They include pea, lentil and faba beans and the production of these crops in Saskatchewan has expanded rapidly in the last few decades. These crops are high in starch, have a higher amylose to amylopectin ratio than cereal starches and the starch occurs as Type C starch granules (Schafer and Drew 2007). Thus, pulse starches are slowly digested compared to wheat or corn. Moreover, these ingredients have been used for glycemic control in human diets supporting the notion that they are slowly digested and produce a lowered glycemic response compared to cereal grains (Atkinson et al., 2008). Based on these observations, pulse starches might be better tolerated by finfish than cereal starches.

The objectives of the following studies for this thesis are to: 1) determine the digestibility of corn, pea, lentil and faba bean starch in Nile tilapia; 2) determine the effects of increasing inclusion levels of corn, pea, lentil and faba bean starch on the growth performance of Nile tilapia; and 3) determine the glycemic responses of Nile tilapia after consuming corn, pea, lentil and faba bean starches.

2 LITERATURE REVIEW

2.1 Starch Structure Introduction

2.1.1 Amylose and amylopectin

Starch is the primary dietary source of energy in most animal diets and includes two different chemical forms: amylose and amylopectin. Amylose is an essentially linear polymer, which is made up of glucose units connected by α (1,4) linkages with few branch linkages. In contrast, amylopectin is a branched chain polymer, which is made up by glucose units with about 95% α (1,4) and 5% α (1,6) linkages, respectively. Hizukuri et al. (1997) indicated that branch points only appear in less than half of amylose molecule and the numbers of branch points are less than 20 in each amylose molecule. Amylose has a molecular weight around 10^2 to 10^3 kDa in cereals and pulses, while amylopectin has a much higher molecular weight between 10^4 to 10^6 kDa (Biliaderis, 1991). In native starch, amylose tends to be hydrolyzed and digested more slowly compared with amylopectin owing to the essentially linear structure of amylose and more branch structure of amylopectin (Schafer and Drew, 2007). The content of amylose in cereal starches such as corn and rice is between 200 and 300 g kg^{-1}. Pulse starches tend to have a higher amylose to amylopectin ratio than cereal starches, with an amylose content ranging from 35-39% in pea, lentil and chickpea (Chung et al., 2008). Ratnayake et al. (2002) indicated the amylose content ranged from 30-40% in smooth pea starch and 60-76% in wrinkled pea starch. However, faba bean starch has a similar amylose content compared with cereals starch, ranging from 17% to 29%. Waxy starch contains all amylopectin or negligible amylose content. High amylose starch varieties include amylose up to 700g kg^{-1}. Svihus et al. (2005) indicated that higher amylose content starch is related to lower digestibility.

2.1.2 Structure of starch granules

Starch is aggregated in granules in endosperm. The granules consist of amylose and amylopectin polymers, which form a semi-crystalline matrix (Smith 2010). The structure of amylopectin can be described as a cluster model. The clusters connect together to form alternating crystalline and amorphous layers. The amylopectin chains have been classified into different types (A, B and C; Hizukuri, 1986). Short chain A (degree of polymerization 6-12) is connected by a single α (1,6) linkage to the amylopectin. B chains can be classified as B1, B2, B3 and B4 according to their length and number of clusters. Both chains A and chains B1 form double helices and crystalline layers in clusters. B2, B3 and B4 work as connecting chains to link the clusters. The possible areas of amylose in the granule may combine into amorphous growth rings, amorphous lamellae or interspersed and co-crystallized with amylopectin molecules, but the exact structure is still under debate (Jane et al., 1992; Kasemsuwan and Jane, 1994; Jenkins and Donald, 1995; Atkins et al., 1999; Hoover et al., 2010).

2.1.3 Starch granule types

The three granules types found within starches can be identified by X-ray diffraction. Starch granule types A, B and C has average chain lengths of amylopectin from 23 to 29, 30 to 44, and 26 to 29 glucose unit, respectively (Sajilata et al., 2006). Type A consists of an open structure, which is rapidly digested compared to Type B owing to the compact structure (Cummings and Englyst, 1995; Schafer and Drew, 2007). Type C granules consist of Types A and B, which tend to be digested intermediately among these three different types (Wursch et al., 1986). Type A, B and C starch granules are primarily found in cereals, potatoes and legumes, respectively (Wursch et al., 1986; Cummings and Englyst, 1995; Schafer and Drew, 2007). Starch granule type is not the only factor that affects the rate of starch digestion. Yu et al. (2004) reported that a variety of slowly digestible barley starch had a higher level of protein associated with starch granules, which decreased the rate of digestion in ruminants and monogastrics.

2.1.4 Glycemic index of starch source

The glycemic index (GI) method is an in vivo method for measuring the appearance of glucose in the bloodstream after a defined amount of starch is ingested in a fasted animal. In humans, the GI of food is defined by comparing the blood glucose response between a test food and a standard food (Otto and Niklas, 1980). The standard food consists of either a pure glucose solution or white bread, both of which have a GI of 100 by definition. Foster-Powell et al. (2002) indicated that in humans, corn and rice starch have higher GI between 100 and 130 compared to pulse starches that generally have GI values between 40 and 60. In recent years, some research has been conducted to determine the GI of starch in domestic animals. Adolphe et al. (2012a) reported that dogs had GI levels of 29 and 47 for pea and corn, respectively. Drew et al. (2012)

indicated that the GI of corn was 105 in pigs. The GI of these ingredients was lower in dogs than pigs or humans, which may be due to a reduced ability to digest and absorb starch compared with humans and pigs. A limited number of studies have been conducted to examine the glycemic responses of finfish. Deng et al., (2005) indicated that there was no significant difference in GI between cornstarch (GI=40) and potato starch (GI=35) in white sturgeon when fed 1g of carbohydrate per kg of fish body weight. Figueiredo-Silva et al., (2013) indicated Nile tilapia had higher ability to regulate blood glucose than rainbow trout when fed the same diets. Paradoxically, Nile tilapia had higher peak glucose and more prolonged elevations in blood glucose, resulting in much higher area under the blood glucose curve in response to an oral glucose challenge compared to any other fish species examined including rainbow trout or carp (Polakof et al., 2012a). In general, high GI meals result in a large increase of blood glucose and insulin in all species (Bell and Sears, 2003). When blood glucose is excessively elevated or remains high for too long, this causes oxidative stress and subsequent negative impacts on vascular function (Adolphe et al., 2012b). Based on the hypothesis that excess glucose causes similar effects in all species, adverse effects are predicted to be greater for animals that have poor tolerance for starch, such as carnivorous mammals and finfish. Greater understanding of the relationship between the post-prandial glucose response and dietary tolerance to starches is needed for all fish species, particularly omnivorous fish such as Nile tilapia before we can recommend incorporating starches at higher levels into aquafeeds.

2.2 Starch Processing

2.2.1 Dry milling and wet milling

Pulse seeds contain 22-45% of starch on a dry matter basis (Hoover et al., 2010) and the starch can be isolated by two extraction methods: dry milling and wet milling. In dry milling, the first step is dehulling. This is accomplished using an abrasive dehuller followed by sieving. Next, the dehulled seed is ground using a hammer mill or pin mill and the resulting meal is air classified to separate starch and protein fractions (Hoover et al., 2010; Figure 2.2.1.1). The air classification of re-milled flour cannot completely separate the starch and protein fractions, even in the situation of repeated air classification of re-milled flour (Meuser et al., 1995). The protein content of legume starch produced by the dry milling process is approximately 15% (Alliance Grain Traders, personal communication).

A water washing method (Reichert and Youngs, 1978) is required to further reduce the protein concentration to 2.5 g kg^{-1}. The process of wet milling results in a higher purity of starch than dry milling (Hoover et al., 2010; Figure 2.2.1.2). The protein content of legume starch produced by wet milling process is between 0.01 and 0.5% (Hoover and Sosulski, 1991; Davydova et al., 1995). While most commercially available corn and pea starches are wet processed and thus virtually devoid of protein content, no wet processed lentil or faba bean starches are currently available on the market. The company that partnered with our research group on this project produces dry processed pea starch in addition to similarly dry processed lentil and faba bean starch. Thus these relatively protein-rich, dry processed pulse starches were used in this thesis, while the cornstarch used was wet processed.

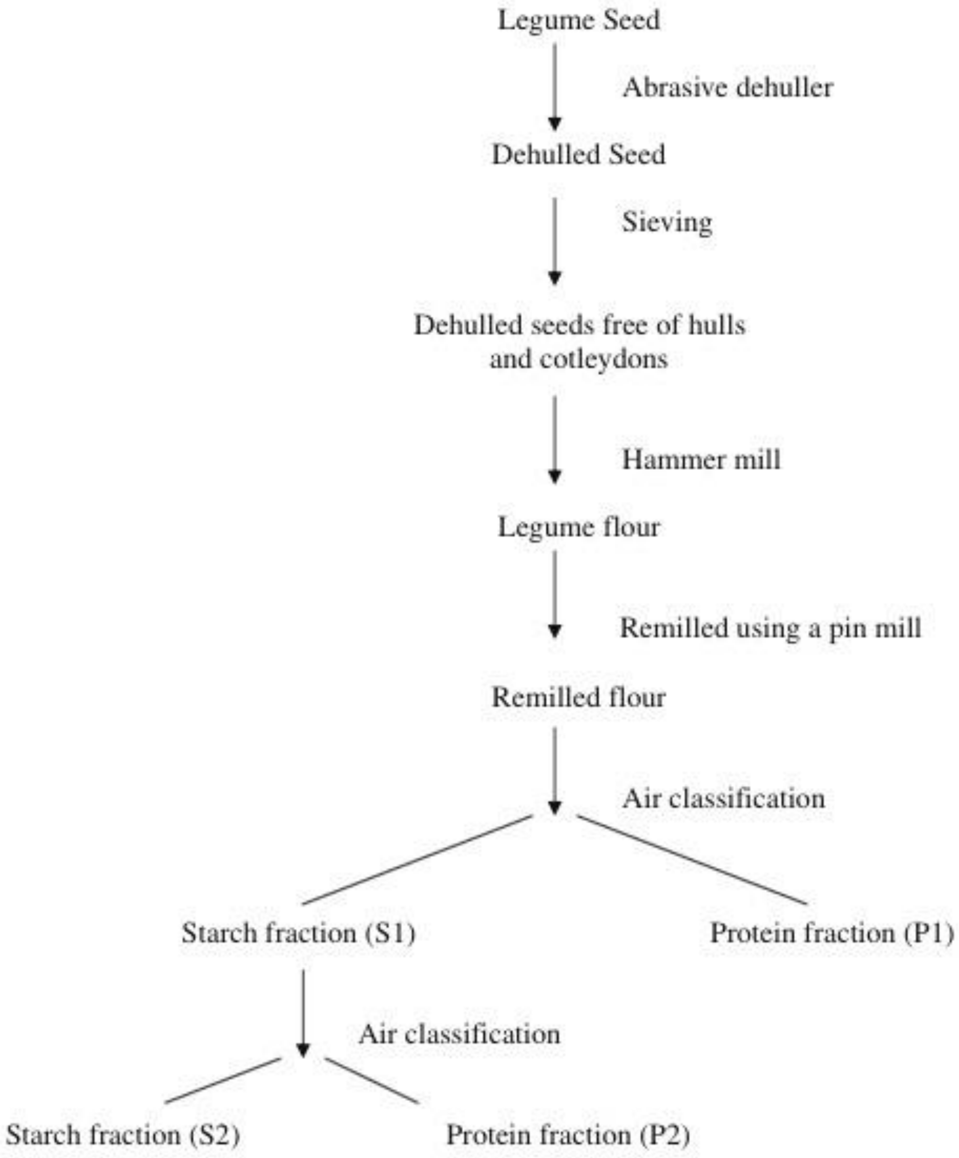


Figure 2.2.1.1 Dry processing of legume seeds to produce a starch concentrate starch (Adapted from Hoover et al., 2010; reproduce with permission from Food research international).

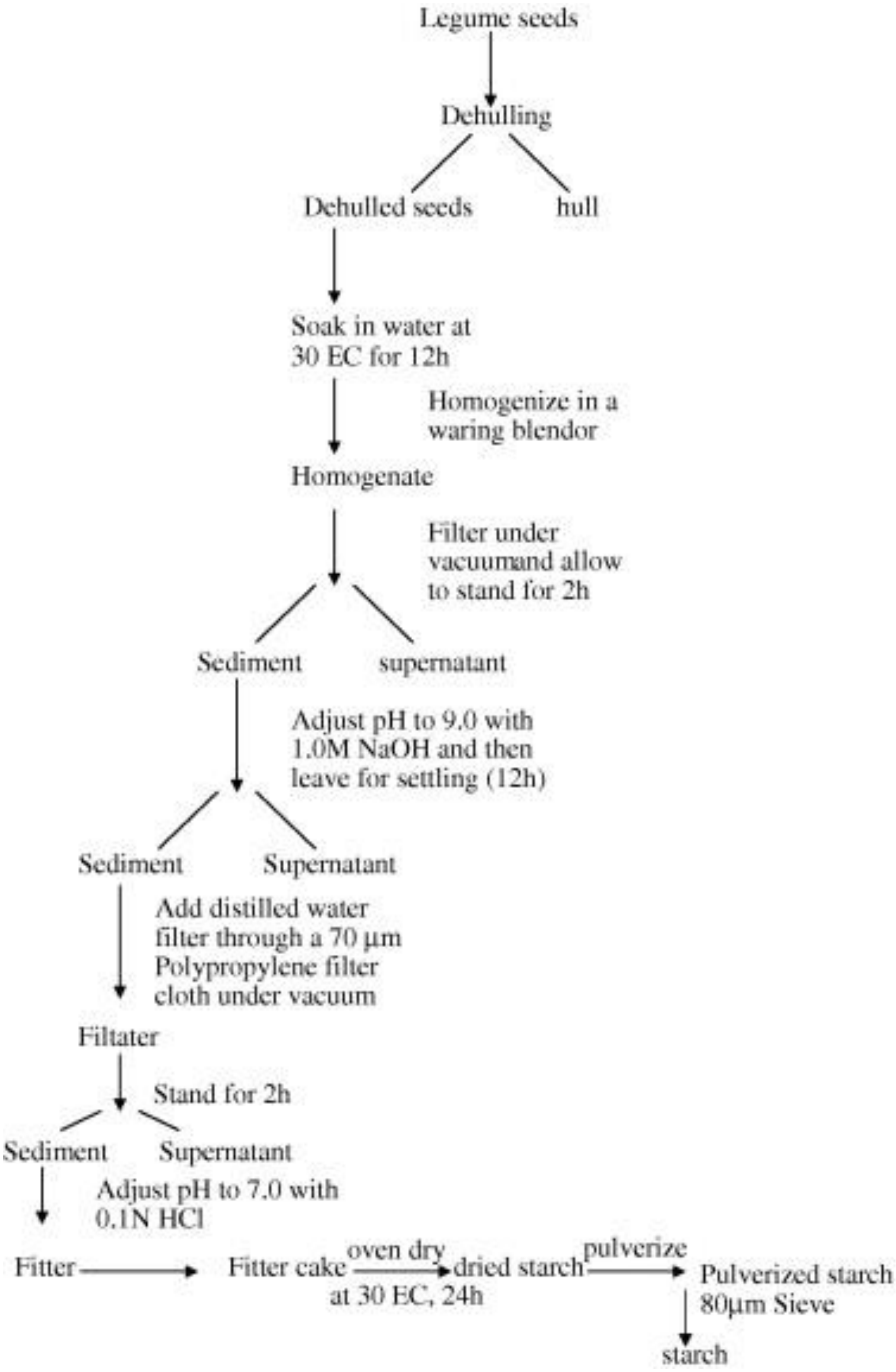


Figure 2.2.1.2 Wet processing of legume seeds to produce a starch concentrate (Adapted from Hoover et al., 2010; reproduce with permission from Food research international).

2.2.2 Gelatinization and retrogradation of starch

Gelatinization of starch consists of the following changes: granule swelling, double helices dissociation, and amylose leaching (Donovan, 1979; Hoover and Hadziyev, 1981; Jenkins and Donald, 1998; Waigh et al., 2000). First, excess water diffuses into amorphous regions of the starch granule in the conditions of heat and moisture, which can lead to hydration and radial swelling. Next the water molecules bound to double helical structures of amylopectin can diffuse into crystalline regions under heated conditions, which dissolve the amylose chain and decrease the number and size of crystalline areas. Finally, the soluble amylose leaches into the surrounding water. The transition temperatures in onset, peak and conclusion (To, Tp and Tc, respectively), and the enthalpy of gelatinization (ΔH) influence the distribution of amylopectin short chains (chain A) in the crystalline area and amylose and amylopectin ratio in crystalline areas (Noda et al., 1996; Hoover et al., 2010). Therefore, the gelatinization of starch disintegrates the structure of the granule, which results in more rapid digestibility than native starch and thus should increase the peak blood glucose response along with the GI.

Gelatinization of starch can result in a dispersed form of both amylose and amylopectin, which enable them to be hydrolyzed readily by amylase. However, the starch source with higher amylose content tends to swell granules less extent during hydrothermal process compared with starch with higher amylopectin content (Ai, 2013). Moreover, starch having higher amylose content would form new amylose-lipid complexes during extrusion, which also resulted lower hydrolysis rate compared with gelatinized starch with higher amylopectin content (Bhatnagar and Hanna, 1994). The linear molecules of gelatinized starch in a dispersed form after cooling randomly recrystallize to adjacent starch molecules to form double helical structure, which was termed starch retrogradation (Mishra et al., 2012). During the retrogradation, both amylose and amylopectin can be retrograded, but amylose has a much higher retrogradation rate than amylopectin. Moreover, amylose tends to be re-crystallized nearly irreversibly, which make it to be almost resistant to amylolytic enzymes digestion (Mishra et al., 2012). In summary, the hydrolysis rate of gelatinized starch with higher amylose content is relatively lower than starch source with higher amylopectin content.

2.3 Digestion and Metabolism of Starch in Finfish

2.3.1 Glucose homeostasis

Fish have lower blood glucose and glucose turnover rates than other vertebrates (Polakof et al., 2012a). The glucose metabolism of fish suggests that they can adapt to long-term starvation, but are very sensitive to hyperglycaemia. Polakof et al. (2012a) reported that glucose tolerance tests, feeding rich carbohydrate diets, and change of seasonal osmoregulation could result in more than 100% increase of fish glycaemia. Furthermore, a significant increase (50-100% above baseline) of glycemia can occur through the action of hormones such as cortisol, glucagon, glucagon-like peptide, glucose hormone, melatonin, serotonin, and cholecystokinin, or by the treatment of specific metabolites such as 2-deoxy-D-glucose. In contrast, hormones (insulin, insulin-like growth factor-1), drug (metformin) treatments or food starvation can result in the decrease of glycemia in fish. While glucose tolerance tests give highly varied results in fish, in general, fish remain hyperglycemic for a longer period of time than mammals. However, there are species differences in glycemic responses and herbivorous and omnivorous fish species can return to basal blood glucose level faster than carnivores.

2.3.2 Glucose metabolism

Pilkis and Granner (1992) indicated that the activity and gene expression of key enzymes in glycolysis, glycogenolysis, lipogenesis and gluconeogenesis maintain the glucose metabolism through the regulation of glucose storage and production. Excess glucose can be stored as glycogen or converted to lipid by glycogenesis or lipogenesis, respectively. The lack of glucose in starvation can be regulated by glycogenolysis (glycogen depletion to glucose), or by gluconeogenesis (glycerol and amino acid conversion to glucose). Enes et al. (2009) reported that glycogen phosphorylase (GPase), which is rate-limiting enzymes of glycogen depletion, and phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase and glucose-6-phosphatase (G6Pase) which are three important enzymes in glucogenolysis are found in most of fish species.

The liver is a key organ for glucose metabolism. Glucose can be metabolized to produce ATP or converted into glycogen and fatty acids in muscle tissue. However, when glucose is in excess, especially in trout, it causes the accumulation of fructose-1,6-bisphosphate (Fru-1,6-P2; Enes et al., 2009). Fru-1,6-P2 can combine with aldolase and triose phosphate isomerase, which results in the accumulation of methylglyoxal (Dhar et al. 2008). Methylglyoxal may result in the increased stress of oxidation and inflammation (Dhar et al., 2010). However, more studies are required to fully understand this mechanism in finfish.

2.3.3 Amylase and glucose transporters

Glucose, from the breakdown of complex carbohydrates by pancreatic amylase, is transported into the bloodstream by a sodium-dependent glucose transporter (SGLT) and a glucose transporter (GLUT). Knowledge of SGLT and GLUTs in fish is limited. Enes et al. (2009), indicated that fish do not have salivary amylase, and have lower levels of pancreatic amylase compared to mammals. Polakof et al. (2012a) stated that SGLT was found in trout intestine. Loewen and Subramaniam (unpublished data, 2016) found that SGLT also exists in Nile tilapia intestine. Enes et al. (2009) reported that the GLUT2 and GLUT4 are found in the liver and muscle, respectively. GLUT2 is the major glucose transporter in the liver, which maintains the balance of glucose concentration between the liver and bloodstream (Krasnov et al. 2001; Hall et al. 2006; Castillo et al. 2009; Terova et al. 2009). However, feeding different carbohydrates does not change the number of both GLUT2 and GLUT4 (Enes et al., 2009).

2.3.4 Carbohydrate tolerance in fish species

Carbohydrate metabolism of teleosts varies markedly due to species diversity. Fish are markedly more insulin-resistant than mammals and birds (Bever et al., 1981; Garin et al., 1987; Lin et al., 1978). Glucose in excess results in persistent hyperglycemia in fish, which may bring about reduced growth rate and fatty liver (Enes et al., 2011; Hemre et al., 2002; Panserat et al., 2009). Therefore, the concentration of dietary carbohydrate in aquafeeds is generally recommended to be under 20% (Polakof et al., 2012a). Fish may have a high ability to tolerate pulses. Collins et al. (2012) indicated that pea meal did not have negative effects on rainbow trout when fed up to 30% of the diet. Omnivorous and herbivorous fish, such as tilapia and carp, are able to tolerate higher levels of carbohydrate in the diet than carnivorous fish, such as salmonids. Some studies have reported no negative effects on the growth of herbivorous and omnivorous fish when the dietary concentrations of carbohydrate were as high as 300-500 g kg^{-1} (Panserat et al. 2000; Kumar et al., 2009; Tan et al. 2009). Herbivorous and omnivorous species can: 1) phosphorylate more glucose in muscle (Capilla et al., 2004); 2) inhibit gluconeogenesis in liver more effectively after feeding (Panserat et al., 2002) and 3) have higher insulin receptors number in white muscle tissue (Párrizas et al., 1994; 1995) compared to carnivorous fish species.

The results of studies on carbohydrate metabolism and glucose tolerance of finfish are confusing and often conflicting. This is compounded by the use of differing species, methods and experimental designs. It is therefore difficult to come to any overall conclusions about the effects of carbohydrate sources on the glucose metabolism in finfish. Thus, we performed a systematic review and meta-analysis on available published research on the dietary carbohydrates fed to finfish.

2.4 Hypothesis

Following the meta-analysis, we conducted three experiments for analysing digestibility and growth performance of modified corn, pea, lentil, and faba bean starch in Nile tilapia and compared the GI of these three pulse starches to pure cornstarch. For the digestibility trial, I hypothesize that the apparent digestibility of dry matter, crude protein, growth energy and starch of modified corn, pea, lentil, and faba bean starch will be significantly different in Nile tilapia. In growth trial, I fed diets containing 0, 150 or 300 g kg^{-1} of modified corn, pea, lentil or faba bean starch to Nile tilapia. I hypothesize that feeding up to 300g kg^{-1} of each pulse starch will have no negative effects on the growth performance of Nile tilapia. Finally, in the glycemic indices experiment, Nile tilapia were fed 0.5 g available carbohydrate per kg body weight of glucose, pure corn, pea, lentil or faba bean starch to determine the GI of each ingredient. I hypothesize that Nile tilapia fed cornstarch will have a significantly higher glycemic response than those fed pulse starch.

2.5 Organization of Thesis

A brief summary of the defendant’s contribution to the study, where the manuscript will be submitted for publication and how the chapter fits into the thesis as a whole is provided below the title of each manuscript chapter. The final chapter summarizes and integrates the findings from all chapters of the thesis.

3 COMPARING THE GROWTH PERFORMANCE AND DIFFERENT DIETARY AMYLOSE-AMYLOPECTIN RATIOS OF FISH FED GLUCOSE, DEXTRIN AND STARCH AS A CARBOHYDRATE SOURCE USING META-ANALYSIS.

3.1 Publication Fate and Contribution

This chapter is a meta-analysis of the existing literature on the effects of starch on fish growth that was needed to better synthesize and understand the potential utility of greater starch inclusion in aquafeeds before proceeding to test diets with high carbohydrate content in Nile tilapia in the subsequent chapter. Mr. Guo contributed to 100% of the literature search, meta-analyses and chapter writing. The defendant's supervisor, laboratory colleagues and thesis committee provided help with study design and editing of the manuscript and chapter.

3.2 Introduction

Feed is the major cost in aquaculture production comprising up to 70% of the total cost of production and the price of feed ingredients is rising rapidly. This is due primarily to the increase in the price of fishmeal and fish oil, which have increased in price by a factor of three from 2003 to 2013 (FAO 2014). The major strategy to decrease feed costs has been to replace marine protein and oil sources with plant ingredients. However, most plant ingredients are high in starch, which may not be utilized efficiently by many fish species.

The utilization of carbohydrate sources by finfish depends on the type of carbohydrate (glucose, starch, and dextrin), carbohydrate processing (raw starch versus hydrothermally processed starch), amount of carbohydrate in the diet, and fish species, age and size. While most aquaculture feeds generally contain low carbohydrate (< 10% by weight), starch is the main carbohydrate source when present. Starch consists of two different compounds: amylose and amylopectin. Amylose consists of a single strand of glucose residues joined by an α (1,4) linkage. The single strand is wound in a tightly bound helical structure that is slowly digested by intestinal amylases. In contrast, amylopectin has a branched structure with glucose residues joined by α (1,4) linkages and α (1,6) linkages at the branching points. Amylopectin is digested more rapidly compared with amylose because of this open, branched structure (Schafer and Drew, 2007). Thus, the amylose and amylopectin content of dietary starch may influence the growth of fish. Lastly, raw starch is difficult to dissolve in water owing to its complex structure and is thought to contribute to poor utilization of raw starch by fish (Polakof et al., 2012a). To overcome this, hydrothermal process can be used to swell and gelatinize starch, which disintegrates the structure of starch granules, making it more digestible (Stone et al., 2003).

Research comparing the utilization of starch between other carbohydrate sources reports conflicting results. The utilization of starch is considered to be more efficient than simple carbohydrates such as glucose for marine species including starry flounder (*Platichthys stellatus*) and European sea bass (*Dicentrarchus labrax*; Lee and Lee, 2004; Enes et al., 2006), and freshwater species such as white sturgeon (*Acipenser transmontanus*), southern catfish (*Silurus meridionalis*), gibel carp (*Carassius auratus gibelio*) and Chinese longsnout catfish (*Leiocassis longirostris* Günther*)* (Lin et al., 1997; Fu, 2005; Tan et al., 2006). However, the growth performance of Chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout (*Oncorhynchus mykiss*), gilthead sea bream (*Sparus aurata*), and grass carp (*Ctenopharyngodon idella*) fed glucose as a simple carbohydrate source increased compared with feeding starch (Buhler and Halver, 1961; Bergot, 1979; Tian et al., 2004; Enes et al., 2008). Furuichi and Yone (1982) reported that the starch utilization was more efficient than dextrin (short glucose polymers) or glucose for the common carp (*Cyprinus carpio*) and red sea bream (*Sparidae*). However, some studies indicated that the utilization of dextrin was more efficient than starch for channel catfish (*Ictalurus punctatus*), rainbow trout, and blunt snout bream (*Megalobrama amblycephala*; Wilson and Poe, 1987; Hung and Storebakken, 1994; Ren et al., 2015). Further confusing this research was the large differences in experimental design, carbohydrate source, processing and fish species and size, which make it difficult to come to any overall conclusion about the feeding value of varying carbohydrate sources. To help clarify this body of research, we performed a systematic literature review and 5 meta-analyses. We hypothesized that 1) fish fed raw starch diets will have growth rates that were significantly greater than fish fed glucose diet, but no significant difference with dextrin diet; 2) fish fed hydrothermally processed starch diet will have higher growth performance than raw starch diet; 3) fish will have higher growth performance when fed starch source with higher amylopectin content as carbohydrate source than higher amylose content starch.

3.3 Materials and Methods

3.3.1 Search key words and papers selection

The scientific databases Web of Science and Google Scholar were searched during in February 2016. The search terms used and Boolean operators were "(Starch OR Glucose) AND (Starch OR Dextrin) AND Fish AND Growth" in Web of Science, and "(Starch Dextrin Growth Aquaculture) OR (Starch Glucose Growth Aquaculture)" in Google Scholar for the comparison of starch (raw starch or hydrothermally processed starch) versus glucose or dextrin; and "(Amylopectin and Amylose) AND (Fish OR Aquaculture)" in Web of Science, "(Amylose Amylopectin Fish Growth) OR (Amylose Amylopectin Aquaculture Growth)" in Google Scholar for the comparison of starch sources with different amylose and amylopectin ratio. The paper selection was based on title and abstract (Figure 3.4.1.1 and Figure 3.4.1.2) and excluded papers that had no fish species, were not growth studies and were not published in English. Papers were also excluded based on the experimental design. Papers with no relevant carbohydrate source, or with no measure of growth or growth variance were excluded. Studies using diets that were not iso-nitrogenous and iso-caloric, and did not have proper control treatment were also excluded.

3.3.2 Data extraction

The growth indicator, standard deviation (SD), and sample size (N) in each study were extracted for meta-analysis (See Appendix Tables 7.1 to Table 7.5). Specific growth rate (SGR), weight gain (WG) or final weight (FW) were used as growth rate parameters for the meta-analyses and in the case that more than one of these growth parameters was reported, SGR was used if available, then WG followed by FW.

Four random effects models were conducted to compare the growth performance of fish fed different carbohydrate sources in diets 1) raw starch versus glucose; 2) raw starch versus dextrin; 3) hydrothermally processed starch versus glucose; 4) hydrothermally processed starch versus dextrin. Both raw starch and hydrothermally processed starch were considered as a control group, while glucose and dextrin were considered as experimental groups. Studies in meta-analysis 1 and 2 were further divided into 2 groups based on fish species: 1) carnivorous fish species, 2) herbivorous and omnivorous fish species. However, studies in meta-analysis 3 and 4 were not divided into subgroups owing to the limited studies.

The starch sources with less than 10% amylose (more than 90% amylopectin) were considered as a control group and other diets with different amylose and amylopectin content were considered as an experimental group (meta-analysis 5).

Standard deviation, when not provided in the studies examined, was calculated based on equation: SD= Standard error*√n, (n = sample size).

3.3.3 Statistical analysis

Review manager 5 (RevMan5) is professional software that uses meta-analyses to compare study protocols, then provides full reviews and graphically shows results. The output of I^2 (heterogeneity) in RevMan5 illustrates the degree of heterogeneity amongst findings, which is considered as the % of variance owing to real difference (Higgins and Thompson, 2002). The linear regression model in SPSS Statistics 22 was used for analysing the relationship between effect size and amylose content (%) with effect size as dependent variable, amylose content and $(\text{amylose content})^2$ as independent variables, and the weight value in the meta-analysis as Weighted Least Squares.

The effect size was indicated by the standard mean difference (SMD) between the control and experimental means was calculated using Hedges' g in following equation (Hedges, 1981), with a 95% confidence interval (CI). A $P \leq 0.05$ of overall effect size was considered to be a significant difference between the means of control and experimental effects.

$$\text{Hedges}' \quad \text{g} = \frac{\bar{\text{x}}_1 - \bar{\text{x}}_2}{s_p}$$

$$s_p = \sqrt{\frac{(\text{n}_1 - 1)\text{s}_1^2 + (\text{n}_2 - 1)\text{s}_2^2}{\text{n}_1 + \text{n}_2 - 2}}$$

Where:

Hedges' g = the effect size = SMD

$\bar{x}_1$ and $\bar{x}_2$ = the mean of the treatment and control, respectively

n_1 and n_2 = the sample size of treatment and control, respectively

s_p = the pooled standard deviation.

s_1 and s_2 = the standard deviation of treatment and control, respectively.

The overall SMDs were compared using T-test in the following pairwise comparisons:

1) The overall SMD of raw starch versus glucose in carnivorous studies was compared to the overall SMD of raw starch versus glucose in omnivorous and herbivorous studies;

2) Uniform SMD of raw starch versus dextrin in carnivorous studies was compared to omnivorous and herbivorous studies;

3) The overall SMD of raw starch versus glucose was compared to the overall SMD of hydrothermally processed starch versus glucose and,

4) The overall SMD of raw starch versus dextrin was compared to the overall SMD of hydrothermally processed starch versus dextrin.

3.4 Results and Discussion

3.4.1 Paper selection

Using Web of Science and Google Scholar, 1356 and 1200 papers were found, respectively for starch, glucose and dextrin use in finfish feeds (Figure 3.4.1.1 and Figure 3.4.1.2). After excluding the inappropriate studies based on the aforementioned criteria, twenty-one papers were left for full review, which includes one paper using different calculations (Lee and Lee, 2004) for WG and one paper using fish at larvae size (Shiau and Chung, 1995). After all exclusions, five papers used hydrothermally processed starch, thirteen papers used raw starch, and one paper used both hydrothermally processed and raw starch were the remaining papers used for data extraction (Figure 3.4.1.1). Searching through the Web of Science and Google Scholar, 28 and 374 papers were found, respectively, for comparing the effect size under different amylose and amylopectin content. After further exclusion of inappropriate studies, seven papers were used for full review (Figure 3.4.1.2).

In some cases, a given study was divided into several subgroups due to different experimental conditions such as fish species, feeding frequencies, protein (%) in diets, carbohydrate (%) in diets, different temperature, different starch or dextrin type (See Appendix Table 7.1 and Table 7.3 for specifics), and different feed processing methods (See Appendix Table 7.2 and Table 7.4).

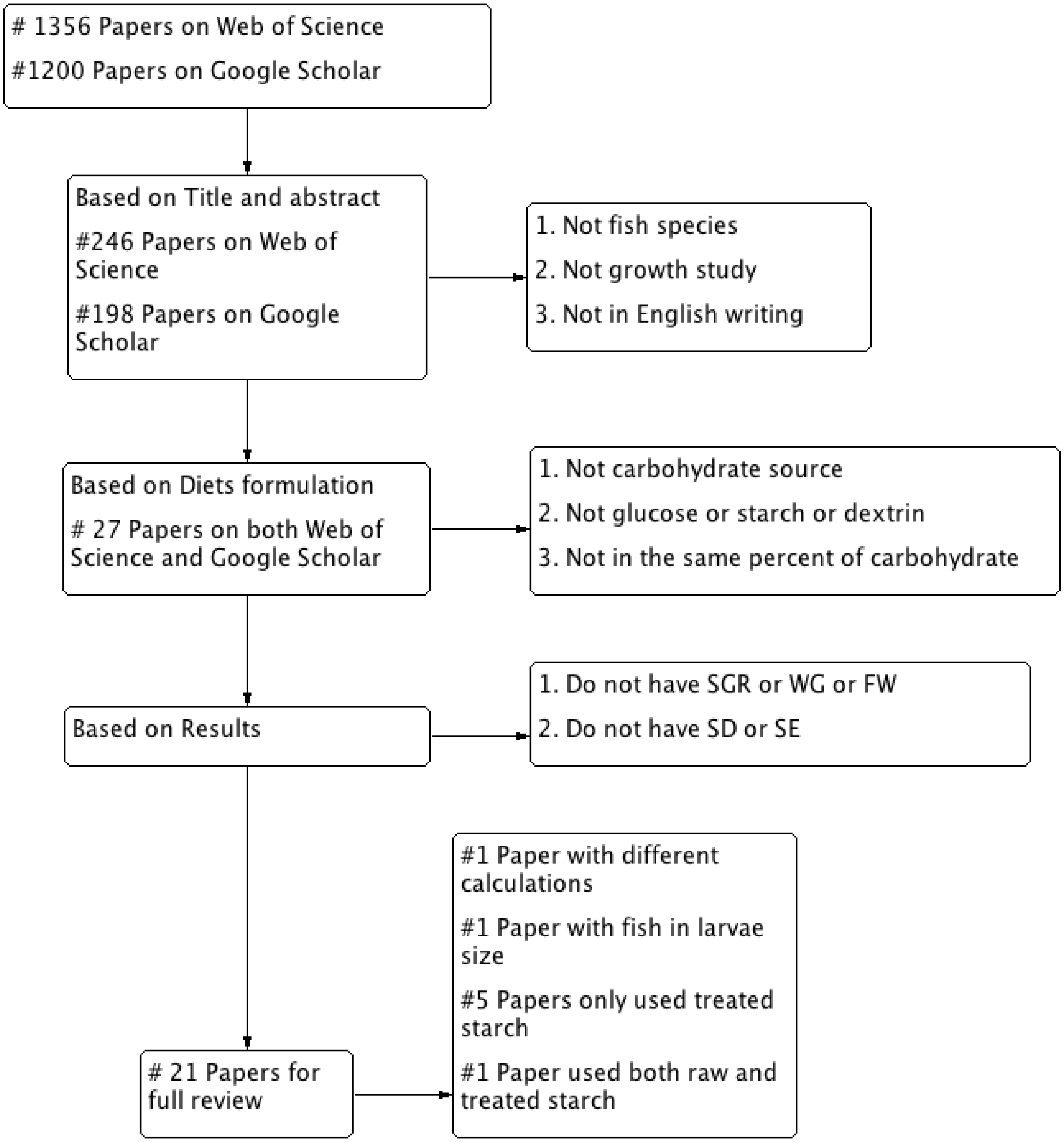


Figure 3.4.1.1 Papers selection for comparing the growth performance of starch versus glucose, and starch versus dextrin as carbohydrate sources in fish diets.

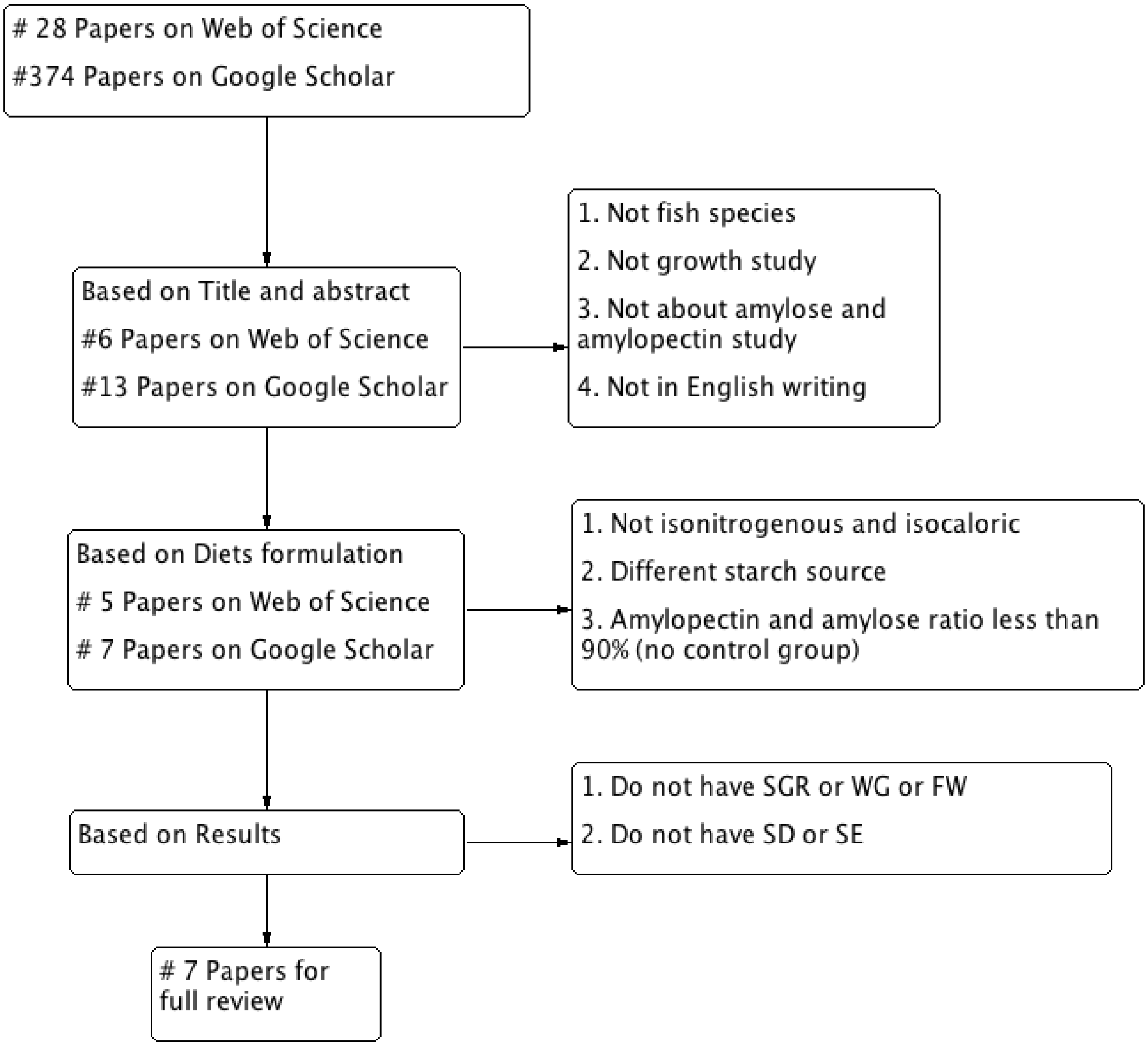


Figure 3.4.1.2 Papers selection for comparing the growth performance of fish by feeding different amylose and amylopectin content diets.

3.4.2 Growth indicators

All the selected papers, which reported FW as a growth indicator had SGR or WG. SGR indicates the rising tendency of fish growth using a natural log (ln) function, which was considered as the first choice of growth indicator for fish growth rate. WG indicates the growth of fish by comparing FW to initial weight, which was used as the second choice for data extraction. Fish are randomly assigned to each tank initially for the fish growth trials, which eliminated the difference among fish initial weight in each tank. The experimental time for each tank is the same. According to the WG and SGR calculations, both indicator values are based on the FW. Therefore, both SGR and WG can be considered as the same type of indicator for measuring the growth performance of fish.

3.4.3 Fixed model and random effect model

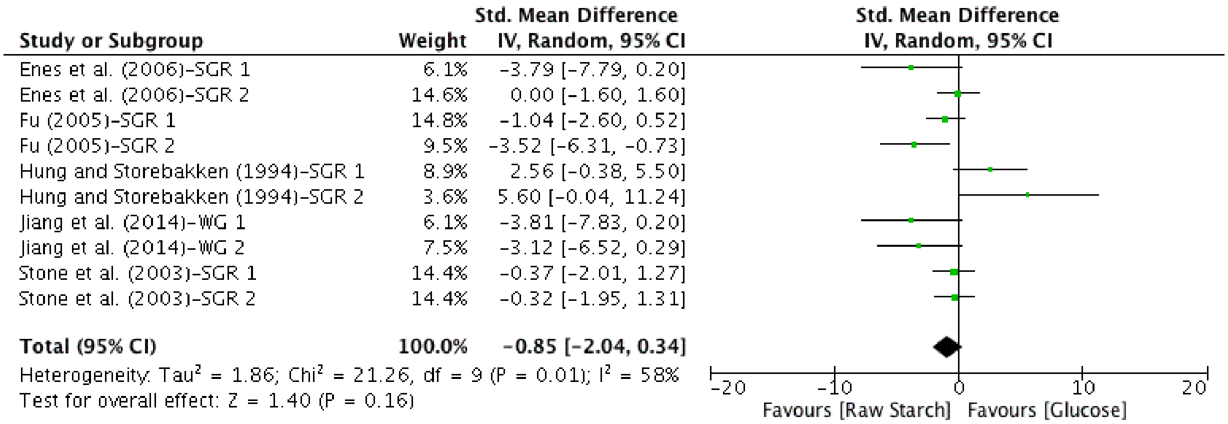
Using the fixed model in a meta-analysis assumes a common (“true”) effect size in all included studies. However, the random effect model considers both variability between studies (heterogeneity) and variability within a study (sampling error; Hedges and Vevea 1998, Sales and Glencross, 2011).

The I^2 can be considered as indicator for heterogeneity. Huedo-Medina et al. (2006) indicated that heterogeneity could be considered as low, medium and high when I^2 value at 25%, 50%, and 75%, respectively. I^2 states low statistical power when a limited number of studies (n<20) were included in a meta-analysis (Huedo-Medina et al. 2006). The I^2 of raw starch and glucose comparison for all studies interpreted medium heterogeneity (I^2=51%, P< 0.01; Figure 3.4.4.3), which means the random effect model was appropriate for the meta-analysis. The I^2 of raw starch and dextrin comparison for all studies illustrated low heterogeneity (Figure 3.4.4.6), which means both the fixed and random effect models would be appropriate. The number of studies in other comparisons was all less than 20, which should then be interpreted with caution based on high I^2. Therefore, the random effect model was considered as an appropriate model for analysing the data in our study.

3.4.4 Comparison of starch with glucose and dextrin

The growth performance of fish fed raw starch as a carbohydrate source showed no significant difference between fish fed glucose in the carnivorous studies (P= 0.16; Figure 3.4.4.1) and the omnivorous and herbivorous studies, (P= 0.13; Figure 3.4.4.2) respectively. The t-test between SMD of carnivores (SMD= -0.85, CI[-2.04, 0.34], N=10) and omnivores and herbivores (SMD= -0.55, CI[-1.26, 0.15], N=19) showed no significant difference for the effect size between the two groups (P= 0.62; Table 3.4.4.1). However, there was a significant difference in the growth performance of fish fed raw starch versus glucose as a carbohydrate source (SMD= -0.64, CI[-1.24, -0.04], N= 29) when all fish studies were included for calculation (P= 0.04; Figure 3.4.4.3). The ability to detect an effect only when all studies were included may be due to the resulting higher sample size (N=29 for all studies). In comparison, splitting studies according to feeding preference lowered sample size to N=10 for carnivorous studies and N=19 herbivorous and omnivorous studies, resulting in a decrease in statistical power (Table 3.4.4.1). Based on the findings of all studies combined, utilization of glucose was lower than raw starch for fish species in our study. Moreover, our comparison indicated that the effect size was not significantly different between fish fed by raw starch and glucose in carnivorous species. This differs from several studies indicating that salmonids could use glucose more efficiently than starch (Buhler and Halver, 1961; Bergot, 1979). Based on this observation, we then excluded the only salmonid (rainbow trout) study (Hung and Storebakken, 1994) among the carnivorous studies used for this meta-analysis. When trout were excluded, we found that carnivorous fish fed raw starch versus glucose as carbohydrate sources also showed a significant difference in growth (SMD= -1.25, CI[-2.23, -0.27], *P*= 0.01).

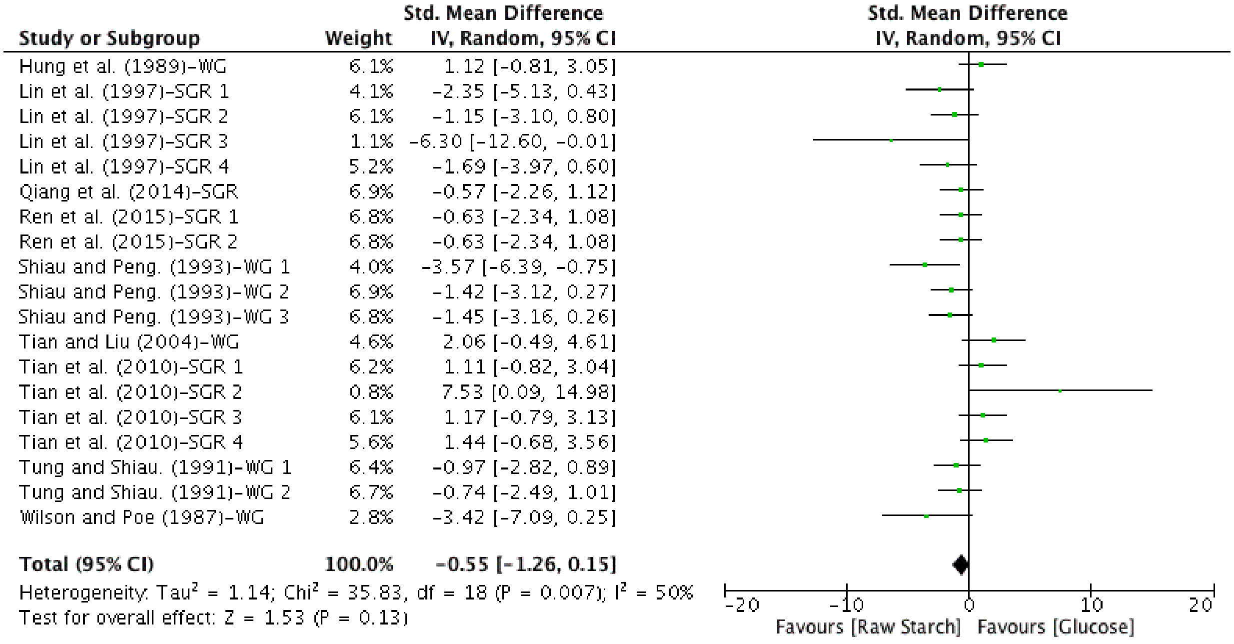
Next, a comparison was made between effects on growth of fish diets that included either raw starch or dextrin. Fish fed dextrin, as a carbohydrate source, grew significantly better than fish fed diets with raw starch. This was true whether only carnivorous studies were examined (SMD= 0.83, CI[0.27, 1.38], *P*< 0.01; Figure 3.4.4.4) or whether all studies were included (SMD= 0.61, CI[0.17, 1.06], *P*< 0.01; Figure 3.4.4.6). However, there was no significant difference between diets that included raw starch versus dextrin when only studies of omnivores and herbivores were examined (*P*= 0.36; Figure 3.4.4.5). There was no significant difference between the SMD of carnivorous studies (SMD= 0.83, CI[0.27, 1.38], N= 14) versus herbivores and omnivores studies (SMD= 0.33, CI[-0.38, 1.04], N= 9) when raw starch- and dextrin-containing diets were compared (*P*= 0.23; Table 3.4.4.1).



Study or Subgroup	Weight	Std. Mean Difference IV, Random, 95% CI
Enes et al. (2006)-SGR 1	6.1%	-3.79 [-7.79, 0.20]
Enes et al. (2006)-SGR 2	14.6%	0.00 [-1.60, 1.60]
Fu (2005)-SGR 1	14.8%	-1.04 [-2.60, 0.52]
Fu (2005)-SGR 2	9.5%	-3.52 [-6.31, -0.73]
Hung and Storebakken (1994)-SGR 1	8.9%	2.56 [-0.38, 5.50]
Hung and Storebakken (1994)-SGR 2	3.6%	5.60 [-0.04, 11.24]
Jiang et al. (2014)-WG 1	6.1%	-3.81 [-7.83, 0.20]
Jiang et al. (2014)-WG 2	7.5%	-3.12 [-6.52, 0.29]
Stone et al. (2003)-SGR 1	14.4%	-0.37 [-2.01, 1.27]
Stone et al. (2003)-SGR 2	14.4%	-0.32 [-1.95, 1.31]
Total (95% CI)	**100.0%**	**-0.85 [-2.04, 0.34]**

Heterogeneity: Tau^2 = 1.86; Chi^2 = 21.26, df = 9 (P = 0.01); I^2 = 58%
Test for overall effect: Z = 1.40 (P = 0.16)

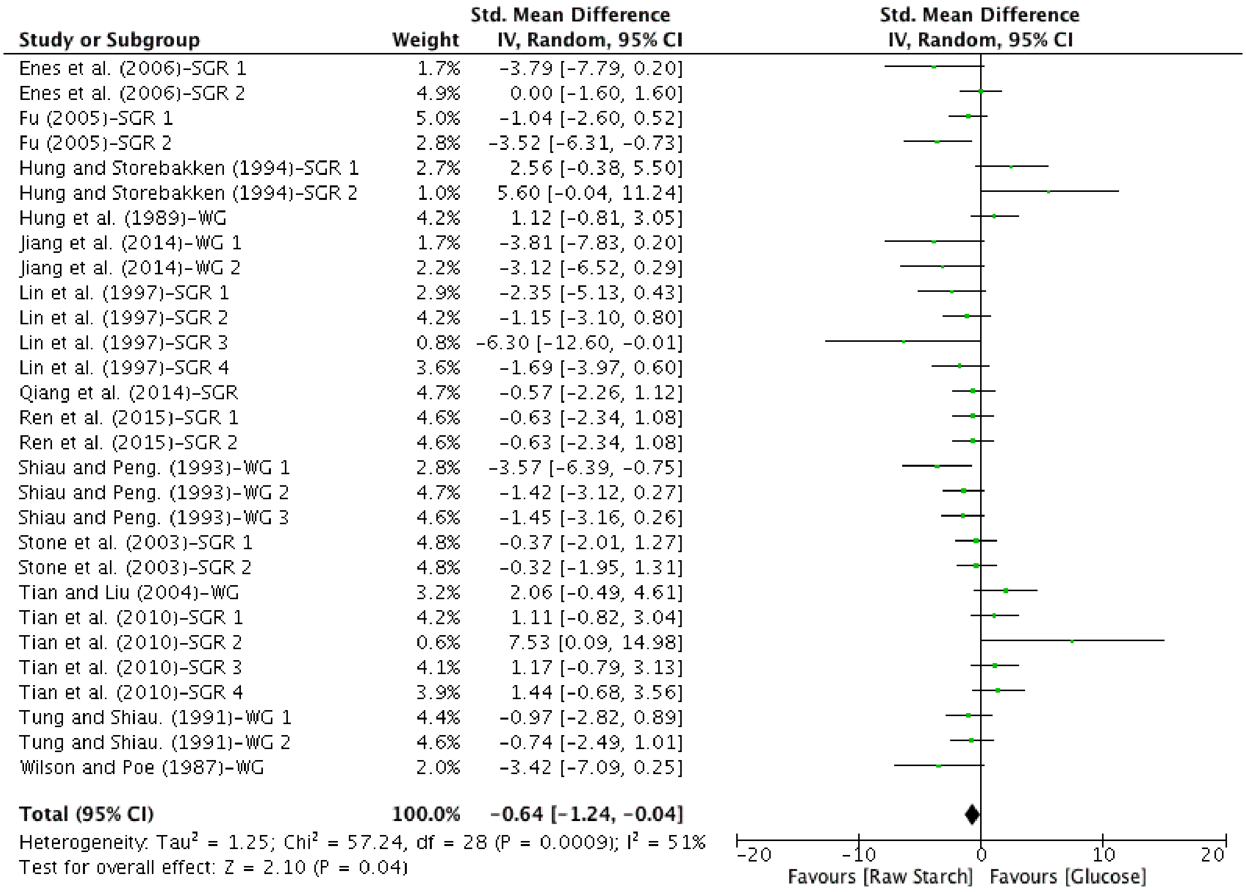
Figure 3.4.4.1 The weight and SMD between raw starch versus glucose for each study that examined carnivores with corresponding forest plot showing magnitude and direction of effect (SGR: specific growth rate; WG: weight gain; IV: inverse variation; CI: confidence interval; df: degree of freedom).



Study or Subgroup	Weight	Std. Mean Difference IV, Random, 95% CI
Hung et al. (1989)-WG	6.1%	1.12 [-0.81, 3.05]
Lin et al. (1997)-SGR 1	4.1%	-2.35 [-5.13, 0.43]
Lin et al. (1997)-SGR 2	6.1%	-1.15 [-3.10, 0.80]
Lin et al. (1997)-SGR 3	1.1%	-6.30 [-12.60, -0.01]
Lin et al. (1997)-SGR 4	5.2%	-1.69 [-3.97, 0.60]
Qiang et al. (2014)-SGR	6.9%	-0.57 [-2.26, 1.12]
Ren et al. (2015)-SGR 1	6.8%	-0.63 [-2.34, 1.08]
Ren et al. (2015)-SGR 2	6.8%	-0.63 [-2.34, 1.08]
Shiau and Peng. (1993)-WG 1	4.0%	-3.57 [-6.39, -0.75]
Shiau and Peng. (1993)-WG 2	6.9%	-1.42 [-3.12, 0.27]
Shiau and Peng. (1993)-WG 3	6.8%	-1.45 [-3.16, 0.26]
Tian and Liu (2004)-WG	4.6%	2.06 [-0.49, 4.61]
Tian et al. (2010)-SGR 1	6.2%	1.11 [-0.82, 3.04]
Tian et al. (2010)-SGR 2	0.8%	7.53 [0.09, 14.98]
Tian et al. (2010)-SGR 3	6.1%	1.17 [-0.79, 3.13]
Tian et al. (2010)-SGR 4	5.6%	1.44 [-0.68, 3.56]
Tung and Shiau. (1991)-WG 1	6.4%	-0.97 [-2.82, 0.89]
Tung and Shiau. (1991)-WG 2	6.7%	-0.74 [-2.49, 1.01]
Wilson and Poe (1987)-WG	2.8%	-3.42 [-7.09, 0.25]
Total (95% CI)	**100.0%**	**-0.55 [-1.26, 0.15]**

Heterogeneity: Tau^2 = 1.14; Chi^2 = 35.83, df = 18 (P = 0.007); I^2 = 50%
Test for overall effect: Z = 1.53 (P = 0.13)

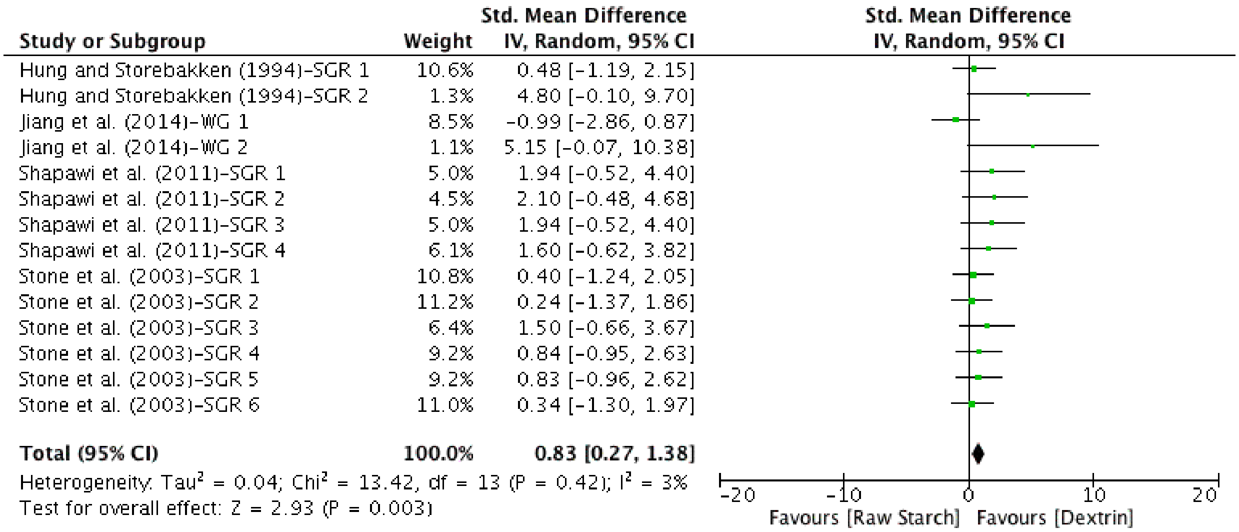
Figure 3.4.4.2 The weight and SMD for raw starch versus glucose comparison for each study that examined herbivores and omnivores with corresponding forest plot showing magnitude and direction of effect with forest plot (SGR: specific growth rate; WG: weight gain; IV: inverse variation; CI: confidence interval; df: degree of freedom).



Study or Subgroup	Weight	Std. Mean Difference IV, Random, 95% CI
Enes et al. (2006)-SGR 1	1.7%	-3.79 [-7.79, 0.20]
Enes et al. (2006)-SGR 2	4.9%	0.00 [-1.60, 1.60]
Fu (2005)-SGR 1	5.0%	-1.04 [-2.60, 0.52]
Fu (2005)-SGR 2	2.8%	-3.52 [-6.31, -0.73]
Hung and Storebakken (1994)-SGR 1	2.7%	2.56 [-0.38, 5.50]
Hung and Storebakken (1994)-SGR 2	1.0%	5.60 [-0.04, 11.24]
Hung et al. (1989)-WG	4.2%	1.12 [-0.81, 3.05]
Jiang et al. (2014)-WG 1	1.7%	-3.81 [-7.83, 0.20]
Jiang et al. (2014)-WG 2	2.2%	-3.12 [-6.52, 0.29]
Lin et al. (1997)-SGR 1	2.9%	-2.35 [-5.13, 0.43]
Lin et al. (1997)-SGR 2	4.2%	-1.15 [-3.10, 0.80]
Lin et al. (1997)-SGR 3	0.8%	-6.30 [-12.60, -0.01]
Lin et al. (1997)-SGR 4	3.6%	-1.69 [-3.97, 0.60]
Qiang et al. (2014)-SGR	4.7%	-0.57 [-2.26, 1.12]
Ren et al. (2015)-SGR 1	4.6%	-0.63 [-2.34, 1.08]
Ren et al. (2015)-SGR 2	4.6%	-0.63 [-2.34, 1.08]
Shiau and Peng. (1993)-WG 1	2.8%	-3.57 [-6.39, -0.75]
Shiau and Peng. (1993)-WG 2	4.7%	-1.42 [-3.12, 0.27]
Shiau and Peng. (1993)-WG 3	4.6%	-1.45 [-3.16, 0.26]
Stone et al. (2003)-SGR 1	4.8%	-0.37 [-2.01, 1.27]
Stone et al. (2003)-SGR 2	4.8%	-0.32 [-1.95, 1.31]
Tian and Liu (2004)-WG	3.2%	2.06 [-0.49, 4.61]
Tian et al. (2010)-SGR 1	4.2%	1.11 [-0.82, 3.04]
Tian et al. (2010)-SGR 2	0.6%	7.53 [0.09, 14.98]
Tian et al. (2010)-SGR 3	4.1%	1.17 [-0.79, 3.13]
Tian et al. (2010)-SGR 4	3.9%	1.44 [-0.68, 3.56]
Tung and Shiau. (1991)-WG 1	4.4%	-0.97 [-2.82, 0.89]
Tung and Shiau. (1991)-WG 2	4.6%	-0.74 [-2.49, 1.01]
Wilson and Poe (1987)-WG	2.0%	-3.42 [-7.09, 0.25]
Total (95% CI)	**100.0%**	**-0.64 [-1.24, -0.04]**

Heterogeneity: $Tau^2 = 1.25$; $Chi^2 = 57.24$, df = 28 (P = 0.0009); $I^2 = 51\%$
Test for overall effect: Z = 2.10 (P = 0.04)

Figure 3.4.4.3 The weight and SMD for raw starch versus glucose comparison for all fish studies with corresponding forest plot showing magnitude and direction of effect (SGR: specific growth rate; WG: weight gain; IV: inverse variation; CI: confidence interval; df: degree of freedom).

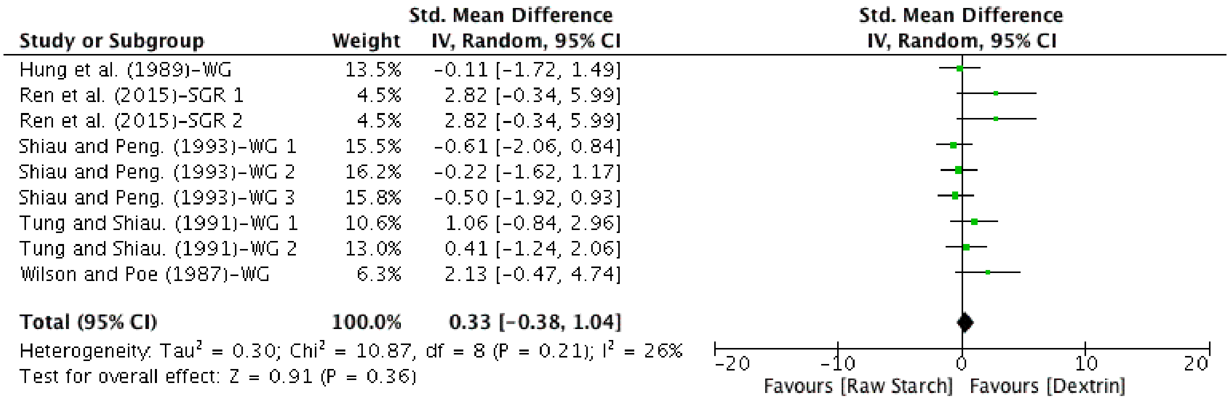


Study or Subgroup	Weight	Std. Mean Difference IV, Random, 95% CI
Hung and Storebakken (1994)-SGR 1	10.6%	0.48 [-1.19, 2.15]
Hung and Storebakken (1994)-SGR 2	1.3%	4.80 [-0.10, 9.70]
Jiang et al. (2014)-WG 1	8.5%	-0.99 [-2.86, 0.87]
Jiang et al. (2014)-WG 2	1.1%	5.15 [-0.07, 10.38]
Shapawi et al. (2011)-SGR 1	5.0%	1.94 [-0.52, 4.40]
Shapawi et al. (2011)-SGR 2	4.5%	2.10 [-0.48, 4.68]
Shapawi et al. (2011)-SGR 3	5.0%	1.94 [-0.52, 4.40]
Shapawi et al. (2011)-SGR 4	6.1%	1.60 [-0.62, 3.82]
Stone et al. (2003)-SGR 1	10.8%	0.40 [-1.24, 2.05]
Stone et al. (2003)-SGR 2	11.2%	0.24 [-1.37, 1.86]
Stone et al. (2003)-SGR 3	6.4%	1.50 [-0.66, 3.67]
Stone et al. (2003)-SGR 4	9.2%	0.84 [-0.95, 2.63]
Stone et al. (2003)-SGR 5	9.2%	0.83 [-0.96, 2.62]
Stone et al. (2003)-SGR 6	11.0%	0.34 [-1.30, 1.97]
Total (95% CI)	**100.0%**	**0.83 [0.27, 1.38]**

Heterogeneity: Tau^2 = 0.04; Chi^2 = 13.42, df = 13 (P = 0.42); I^2 = 3%

Test for overall effect: Z = 2.93 (P = 0.003)

Figure 3.4.4.4 The weight and SMD for raw starch versus dextrin comparison each study that examined carnivores with corresponding forest plot showing magnitude and direction of effect (SGR: specific growth rate; WG: weight gain; IV: inverse variation; CI: confidence interval; df: degree of freedom).

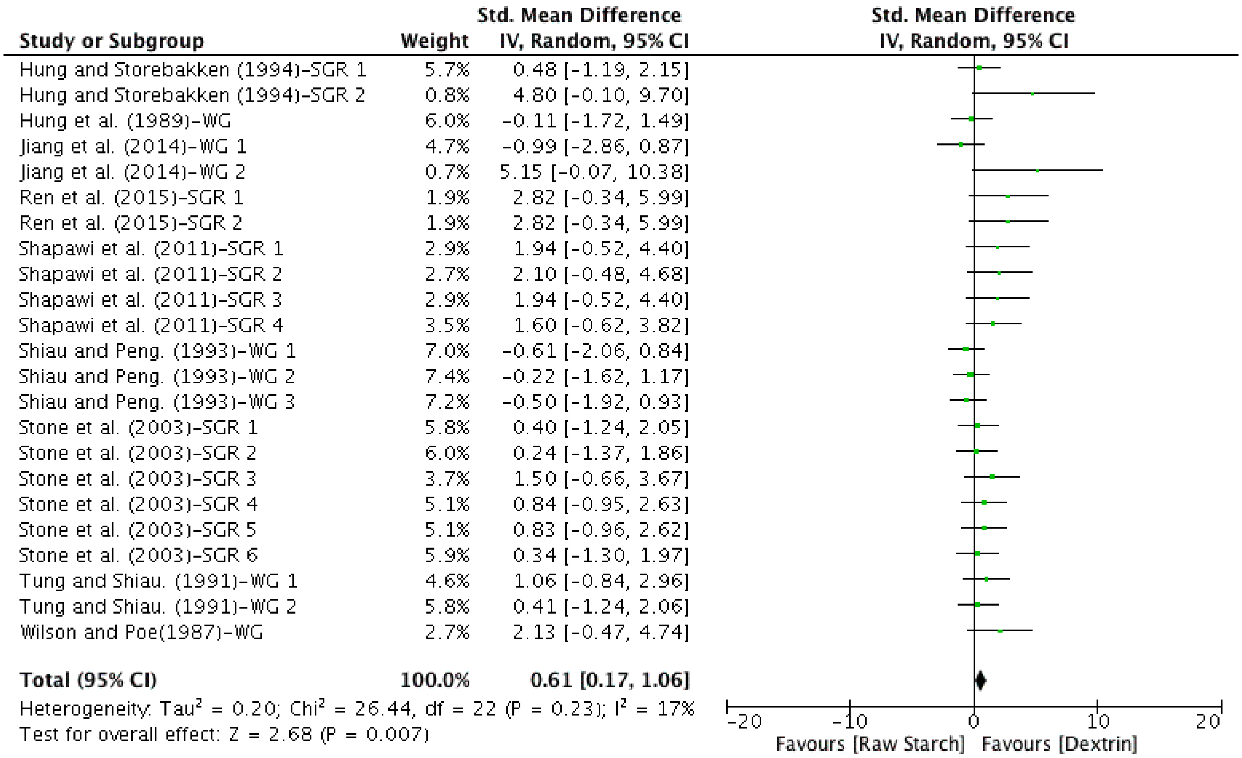


Study or Subgroup	Weight	Std. Mean Difference IV, Random, 95% CI
Hung et al. (1989)-WG	13.5%	-0.11 [-1.72, 1.49]
Ren et al. (2015)-SGR 1	4.5%	2.82 [-0.34, 5.99]
Ren et al. (2015)-SGR 2	4.5%	2.82 [-0.34, 5.99]
Shiau and Peng. (1993)-WG 1	15.5%	-0.61 [-2.06, 0.84]
Shiau and Peng. (1993)-WG 2	16.2%	-0.22 [-1.62, 1.17]
Shiau and Peng. (1993)-WG 3	15.8%	-0.50 [-1.92, 0.93]
Tung and Shiau. (1991)-WG 1	10.6%	1.06 [-0.84, 2.96]
Tung and Shiau. (1991)-WG 2	13.0%	0.41 [-1.24, 2.06]
Wilson and Poe (1987)-WG	6.3%	2.13 [-0.47, 4.74]
Total (95% CI)	**100.0%**	**0.33 [-0.38, 1.04]**

Heterogeneity: Tau^2 = 0.30; Chi^2 = 10.87, df = 8 (P = 0.21); I^2 = 26%

Test for overall effect: Z = 0.91 (P = 0.36)

Figure 3.4.4.5 The weight and SMD for raw starch versus dextrin comparison for each study that examined herbivores and omnivores with corresponding forest plot showing magnitude and direction of effect (SGR: specific growth rate; WG: weight gain; IV: inverse variation; CI: confidence interval; df: degree of freedom).



Study or Subgroup	Weight	Std. Mean Difference IV, Random, 95% CI
Hung and Storebakken (1994)-SGR 1	5.7%	0.48 [-1.19, 2.15]
Hung and Storebakken (1994)-SGR 2	0.8%	4.80 [-0.10, 9.70]
Hung et al. (1989)-WG	6.0%	-0.11 [-1.72, 1.49]
Jiang et al. (2014)-WG 1	4.7%	-0.99 [-2.86, 0.87]
Jiang et al. (2014)-WG 2	0.7%	5.15 [-0.07, 10.38]
Ren et al. (2015)-SGR 1	1.9%	2.82 [-0.34, 5.99]
Ren et al. (2015)-SGR 2	1.9%	2.82 [-0.34, 5.99]
Shapawi et al. (2011)-SGR 1	2.9%	1.94 [-0.52, 4.40]
Shapawi et al. (2011)-SGR 2	2.7%	2.10 [-0.48, 4.68]
Shapawi et al. (2011)-SGR 3	2.9%	1.94 [-0.52, 4.40]
Shapawi et al. (2011)-SGR 4	3.5%	1.60 [-0.62, 3.82]
Shiau and Peng. (1993)-WG 1	7.0%	-0.61 [-2.06, 0.84]
Shiau and Peng. (1993)-WG 2	7.4%	-0.22 [-1.62, 1.17]
Shiau and Peng. (1993)-WG 3	7.2%	-0.50 [-1.92, 0.93]
Stone et al. (2003)-SGR 1	5.8%	0.40 [-1.24, 2.05]
Stone et al. (2003)-SGR 2	6.0%	0.24 [-1.37, 1.86]
Stone et al. (2003)-SGR 3	3.7%	1.50 [-0.66, 3.67]
Stone et al. (2003)-SGR 4	5.1%	0.84 [-0.95, 2.63]
Stone et al. (2003)-SGR 5	5.1%	0.83 [-0.96, 2.62]
Stone et al. (2003)-SGR 6	5.9%	0.34 [-1.30, 1.97]
Tung and Shiau. (1991)-WG 1	4.6%	1.06 [-0.84, 2.96]
Tung and Shiau. (1991)-WG 2	5.8%	0.41 [-1.24, 2.06]
Wilson and Poe(1987)-WG	2.7%	2.13 [-0.47, 4.74]
Total (95% CI)	**100.0%**	**0.61 [0.17, 1.06]**

Heterogeneity: $\text{Tau}^2 = 0.20$; $\text{Chi}^2 = 26.44$, df = 22 ($P = 0.23$); $I^2 = 17\%$
Test for overall effect: $Z = 2.68$ ($P = 0.007$)

Figure 3.4.4.6 The weight and SMD for raw starch versus dextrin comparison for all fish studies with corresponding forest plot showing magnitude and direction of effect (SGR: specific growth rate; WG: weight gain; IV: inverse variation; CI: confidence interval; df: degree of freedom).

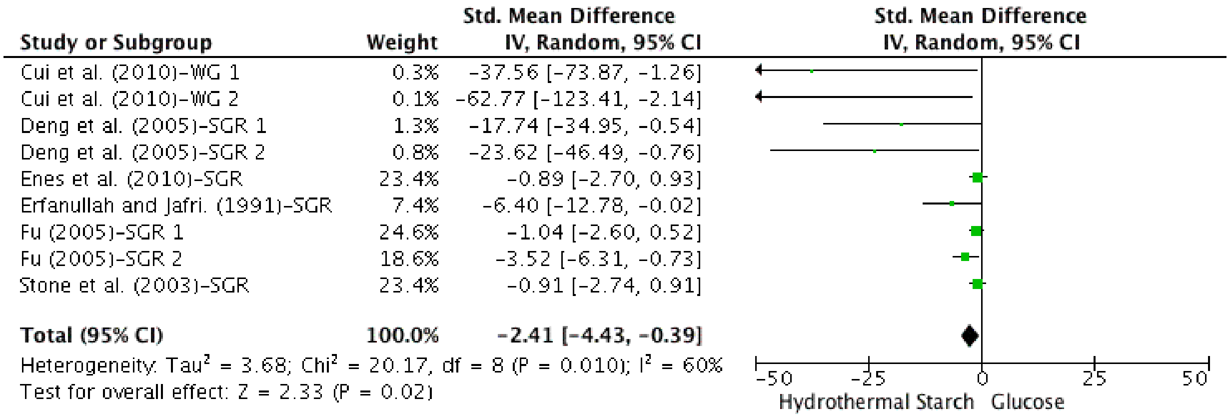
Table 3.4.4.1 The overall effect size between raw starch versus glucose, raw starch versus dextrin in carnivorous studies, omnivorous and herbivorous studies and all species included studies.

	Overall effect size (SMD)	95% CI	N	*P*-value	T-test *P*-value
Raw starch versus glucose comparison					
Carnivores	-0.85	[-2.04, 0.34]	10	0.16	0.62
Omnivore and Herbivore	-0.55	[-1.26, 0.15]	19	0.13	
All species	-0.64	[-1.24, -0.04]	29	0.04	
Raw starch versus dextrin comparison					
Carnivores	0.83	[0.27, 1.38]	14	< 0.01	0.23
Omnivore and Herbivore	0.33	[-0.38, 1.04]	9	0.36	
All species	0.61	[0.17, 1.06]	23	< 0.01	

CI = confidence interval; N= sample size; SMD = standard mean difference

Six studies (with nine subgroups) and four studies (with seven subgroups) were selected for hydrothermally processed starch versus glucose comparison, and hydrothermally processed starch versus dextrin comparison, respectively. Due to the small sample size if separated in dietary preference, the studies on hydrothermally processed starch were not divided into carnivores and herbivores and omnivores as subgroups. Erfanullah and Jafri (1999), Deng et al. (2005), and Cui et al. (2010) indicated that the utilization of pre-cooked cornstarch, hydrolyzed potato starch, and extruded feed (corn and wheat starch) were more efficient than glucose for stinging catfish *(Heteropneustes fossilis)*, white sturgeon (Acipenser transmontanus), and cobia *(Rachycentron canadum Linnaeus)*, respectively. However, Stone et al. (2003), Fu (2005), and Enes et al. (2010) fed gelatinized wheat starch, precooked cornstarch, and pre-gelatinized maize starch to juvenile silver perch, southern catfish, and gilthead sea bream juveniles, respectively, found that the growth performance of fish fed these hydrothermally processed starches showed no significant difference compared to fish fed glucose as a carbohydrate source.

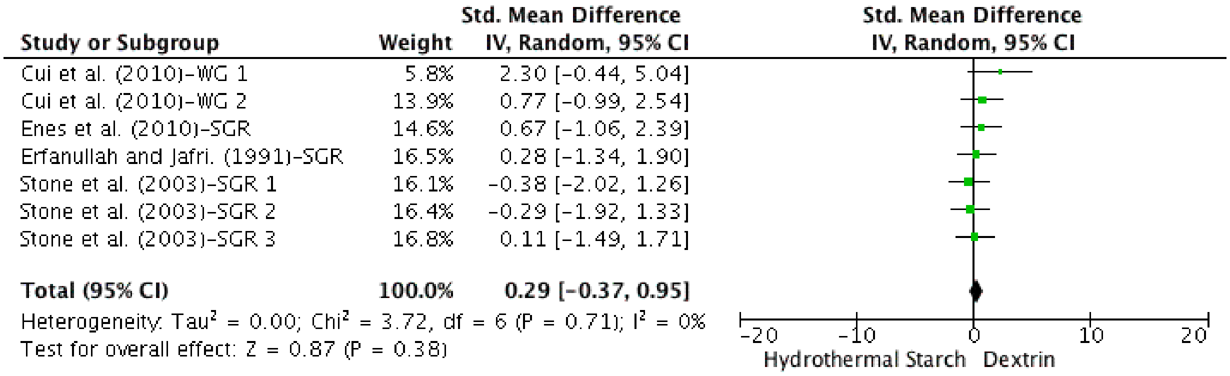
In the current meta-analysis study, the growth performance of fish fed diets containing glucose was again significantly poorer than fish fed hydrothermally processed starch ($P= 0.04$ and 0.02, respectively; Figure 3.4.4.3 and Figure 3.4.4.7). Although both starches were better than glucose, hydrothermally processed starch was more effective than raw starch in promoting fish growth, as indicated by a t-test between SMD of raw starch versus glucose, and hydrothermally processed starch versus glucose ($P= 0.02$; Table 3.4.4.2), the effect size between raw starch versus dextrin was calculated to be 0.61, CI[0.17, 1.06] ($P< 0.01$; Figure 3.4.4.6), which means dextrin was more effective as a carbohydrate source compared to raw starch in fish diets. In contrast, there was no significant difference between dextrin and hydrothermally processed starch ($P= 0.38$; Figure 3.4.4.8). The SMD between raw starch versus dextrin and hydrothermally processed starch versus dextrin showed no significant difference with $P= 0.44$ using a t-test (Table 3.4.4.2).



Study or Subgroup	Weight	Std. Mean Difference IV, Random, 95% CI
Cui et al. (2010)-WG 1	0.3%	-37.56 [-73.87, -1.26]
Cui et al. (2010)-WG 2	0.1%	-62.77 [-123.41, -2.14]
Deng et al. (2005)-SGR 1	1.3%	-17.74 [-34.95, -0.54]
Deng et al. (2005)-SGR 2	0.8%	-23.62 [-46.49, -0.76]
Enes et al. (2010)-SGR	23.4%	-0.89 [-2.70, 0.93]
Erfanullah and Jafri. (1991)-SGR	7.4%	-6.40 [-12.78, -0.02]
Fu (2005)-SGR 1	24.6%	-1.04 [-2.60, 0.52]
Fu (2005)-SGR 2	18.6%	-3.52 [-6.31, -0.73]
Stone et al. (2003)-SGR	23.4%	-0.91 [-2.74, 0.91]
Total (95% CI)	**100.0%**	**-2.41 [-4.43, -0.39]**

Heterogeneity: $Tau^2 = 3.68$; $Chi^2 = 20.17$, df = 8 (P = 0.010); $I^2 = 60\%$
Test for overall effect: Z = 2.33 (P = 0.02)

Figure 3.4.4.7 The weight and SMD for hydrothermally processed starch versus glucose comparison for all fish studies with corresponding forest plot showing magnitude and direction of effect (SGR: specific growth rate; WG: weight gain; IV: inverse variation; CI: confidence interval; df: degree of freedom).



Study or Subgroup	Weight	Std. Mean Difference IV, Random, 95% CI
Cui et al. (2010)-WG 1	5.8%	2.30 [-0.44, 5.04]
Cui et al. (2010)-WG 2	13.9%	0.77 [-0.99, 2.54]
Enes et al. (2010)-SGR	14.6%	0.67 [-1.06, 2.39]
Erfanullah and Jafri. (1991)-SGR	16.5%	0.28 [-1.34, 1.90]
Stone et al. (2003)-SGR 1	16.1%	-0.38 [-2.02, 1.26]
Stone et al. (2003)-SGR 2	16.4%	-0.29 [-1.92, 1.33]
Stone et al. (2003)-SGR 3	16.8%	0.11 [-1.49, 1.71]
Total (95% CI)	**100.0%**	**0.29 [-0.37, 0.95]**

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 3.72$, df = 6 (P = 0.71); $I^2 = 0\%$
Test for overall effect: Z = 0.87 (P = 0.38)

Figure 3.4.4.8 The weight and SMD for hydrothermally processed starch versus dextrin comparison for fish studies with corresponding forest plot showing magnitude and direction of effect (SGR: specific growth rate; WG: weight gain; IV: inverse variation; CI: confidence interval; df: degree of freedom).

Table 3.4.4.2 The comparison of overall effect size between hydrothermally processed or raw starch versus glucose or dextrin.

	Overall effect size (SMD)	95% CI	N	*P*-value	T-test *P*-value
	Starch versus glucose comparison				
Raw starch	-0.64	[-1.24, -0.04]	29	0.04	0.02
Hydrothermal starch	-2.41	[-4.43, -0.39]	9	0.02	
	Starch versus dextrin comparison				
Raw starch	0.61	[0.17, 1.06]	23	< 0.01	0.44
Hydrothermal starch	0.29	[-0.37, 0.95]	7	0.38	

CI = confidence interval; N= sample size; SMD = standard mean difference.

3.4.5 The impacts of carbohydrate level in the diet and fish size

The amount of carbohydrate in diets can influence the tolerance of starch and glucose. Shiau and Peng (1993) indicated that the growth performance of hybrid tilapia (*Oreochromis niloticus X Oreochromis aureus*) fed raw starch as a carbohydrate source was significantly higher than glucose when the carbohydrate amount was 33% by weight in diets. However, this benefit to growth performance declined when 37%, 41% and 44% carbohydrate was included in fish diets (Table 3.4.5). Although raw starch can be utilized more efficiently than glucose, this may because efficiency of utilization occurs only at lower levels in the diet. Higher levels of raw starch may show declines due to saturation of digestive processes leading to decreased digestibility, saturation of intestinal absorption or some other alteration in post-absorptive metabolic processes. Thus, when the amount of carbohydrate increases beyond a certain point (approximately at 30% inclusion), growth decreases. The rate at which growth of tilapia fed increasing levels of raw starch as a carbohydrate source starts to decline appears to be slower compared to the more rapid decline observed with increasing glucose inclusion (Table 3.4.5).

Fish size can be considered as another factor to evaluate tolerance to increasing inclusion of carbohydrate in fish diets. This is especially critical at the larval stage, which generally requires greater nutrient density due to naturally high growth rates than fish in other life stages. There was no significant difference between fed glucose and raw starch at 41% in the diet when fish were 2.9g, and between fed glucose and starch at 44% in the diet when fish were 7.8g (Tung and Shiau, 1991; Shiau and Peng, 1993; Table 3.4.5). However, the growth performance of fish fed raw starch diet was significantly higher than fish fed glucose in the larval stage (0.8g; Shiau and Chuang, 1995; Table 3.4.5). This confirms an exaggerated benefit to utilization of raw starch in tilapia larvae compared to glucose in diets compared to juvenile or adult fish.

Table 3.4.5 Weight gain of hybrid tilapia fed diets with differing glucose and raw starch inclusion levels

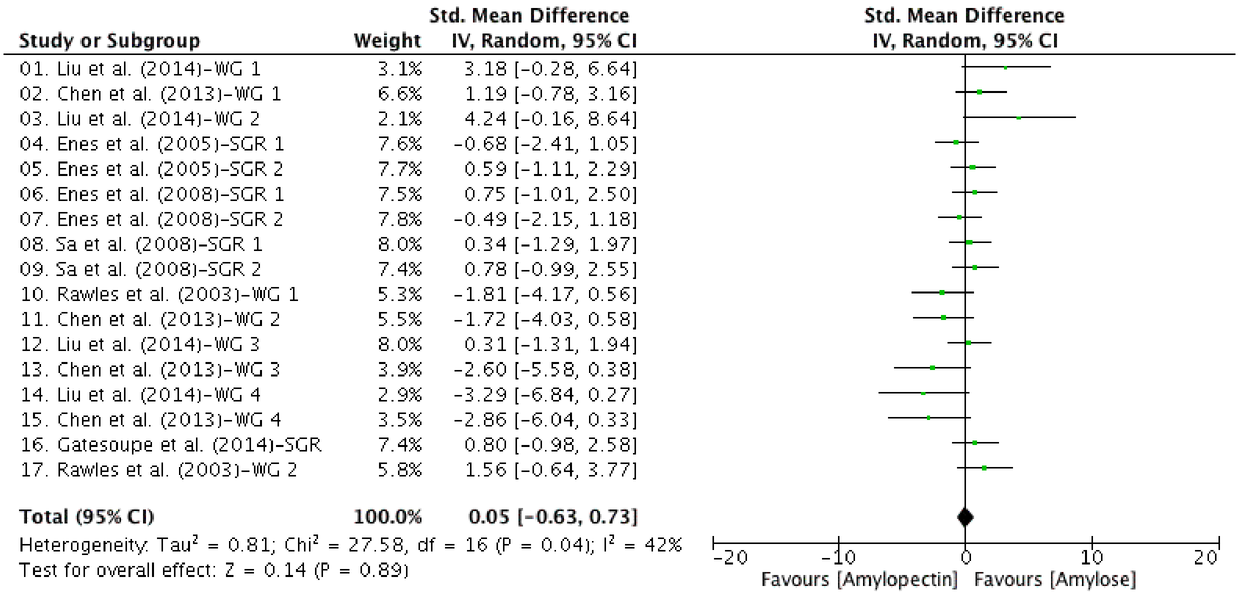
Author	Fish species (Initial weight)	Carbohydrate conc.	Glucose WG (%)[1]	Starch WG (%)[1]	*P*-value
Shiau and Peng, (1993)	Hybrid tilapia (2.9g)	33%	353 ± 30.1	480 ± 31.4	< 0.01
Shiau and Peng, (1993)	Hybrid tilapia (2.9g)	37%	339 ± 21.1	392 ± 41.2	> 0.05
Shiau and Peng, (1993)	Hybrid tilapia (2.9g)	41%	313 ± 28.2	365 ± 34.2	> 0.05
Tung and Shiau, (1991)	Hybrid tilapia (7.8g)	44%	225 ± 19.2	246 ± 15.7	> 0.05
Tung and Shiau, (1991)	Hybrid tilapia (7.8g)	44%	270 ± 26.0	288 ± 7.6	> 0.05
Shiau and Chuang, (1995)	Hybrid tilapia (0.8g)	44%	100 ± 11.2	426 ± 11.2	< 0.01

[1] Mean WG ± SD

3.4.6 Amylose and amylopectin comparison

There was no significant difference in fish growth when fish were fed diets with different amylose to amylopectin ratios (SMD= 0.05, CI[-0.63, 0.73], *P*=0.89; Figure 3.4.6.1). The value of I^2 was 42%, meaning there was moderate variability between studies. However, the relationship between effect size (Y-axis) and amylose content (X-axis) is shown in Figure 3.4.6.2 and can be interpreted using a quadratic regression: $Y=0.005X^2-0.442X+8.637$ (*P*=0.004, $R^2=0.545$). Based on this regression, the effect size reached its lowest value i.e. growth was poorest when amylose content is 44.2%, (Figure 3.4.6.2). Conversely, the biggest positive effect size i.e. greatest growth was observed when amylose content was the lowest.

In the current meta-analysis, increasing amylose content from 12% to 44% decreased the effect size i.e. decreased growth rate in fish (Figure 3.4.6.2). Liu et al. (2014) indicated a similar tendency using regression analyses. Specifically, the growth performance increased between 0 and 24% amylose, but decreased between 24 and 48% amylose (Liu et al. 2014). Chen et al. (2013) also indicated that fish fed 14.9% amylose starch in diets had higher growth performance compared with 32%, 43.2% and 49.5% amylose starch, but had no significant difference with 9.9% amylose starch in diets. In the current meta-analysis, the effect size appeared to show a U-shaped relationship with increasing amylose since effect size tended to increase again from the lowest effect when amylose content ranged from 44.2% to 70% (Figure 3.4.6.2). However, this potential U-shape should be interpreted with caution since the two points at 70% amylose may be exerting excess influence on the regression. Without these two points, the relationship would likely be best fit by a negative linear relationship. Therefore, more studies that include more diets with higher amylose content are required to prove the accuracy of the currently proposed quadratic regression model.



Study or Subgroup	Weight	Std. Mean Difference IV, Random, 95% CI
01. Liu et al. (2014)-WG 1	3.1%	3.18 [-0.28, 6.64]
02. Chen et al. (2013)-WG 1	6.6%	1.19 [-0.78, 3.16]
03. Liu et al. (2014)-WG 2	2.1%	4.24 [-0.16, 8.64]
04. Enes et al. (2005)-SGR 1	7.6%	-0.68 [-2.41, 1.05]
05. Enes et al. (2005)-SGR 2	7.7%	0.59 [-1.11, 2.29]
06. Enes et al. (2008)-SGR 1	7.5%	0.75 [-1.01, 2.50]
07. Enes et al. (2008)-SGR 2	7.8%	-0.49 [-2.15, 1.18]
08. Sa et al. (2008)-SGR 1	8.0%	0.34 [-1.29, 1.97]
09. Sa et al. (2008)-SGR 2	7.4%	0.78 [-0.99, 2.55]
10. Rawles et al. (2003)-WG 1	5.3%	-1.81 [-4.17, 0.56]
11. Chen et al. (2013)-WG 2	5.5%	-1.72 [-4.03, 0.58]
12. Liu et al. (2014)-WG 3	8.0%	0.31 [-1.31, 1.94]
13. Chen et al. (2013)-WG 3	3.9%	-2.60 [-5.58, 0.38]
14. Liu et al. (2014)-WG 4	2.9%	-3.29 [-6.84, 0.27]
15. Chen et al. (2013)-WG 4	3.5%	-2.86 [-6.04, 0.33]
16. Gatesoupe et al. (2014)-SGR	7.4%	0.80 [-0.98, 2.58]
17. Rawles et al. (2003)-WG 2	5.8%	1.56 [-0.64, 3.77]
Total (95% CI)	**100.0%**	**0.05 [-0.63, 0.73]**

Heterogeneity: $\text{Tau}^2 = 0.81$; $\text{Chi}^2 = 27.58$, df = 16 ($P = 0.04$); $I^2 = 42\%$
Test for overall effect: $Z = 0.14$ ($P = 0.89$)

Figure 3.4.6.1 The weight and SMD of starch sources with different amylose and amylopectin comparison for all fish studies with corresponding forest plot showing magnitude and direction of effect (SGR: specific growth rate; WG: weight gain; IV: inverse variation; CI: confidence interval; df: degree of freedom).

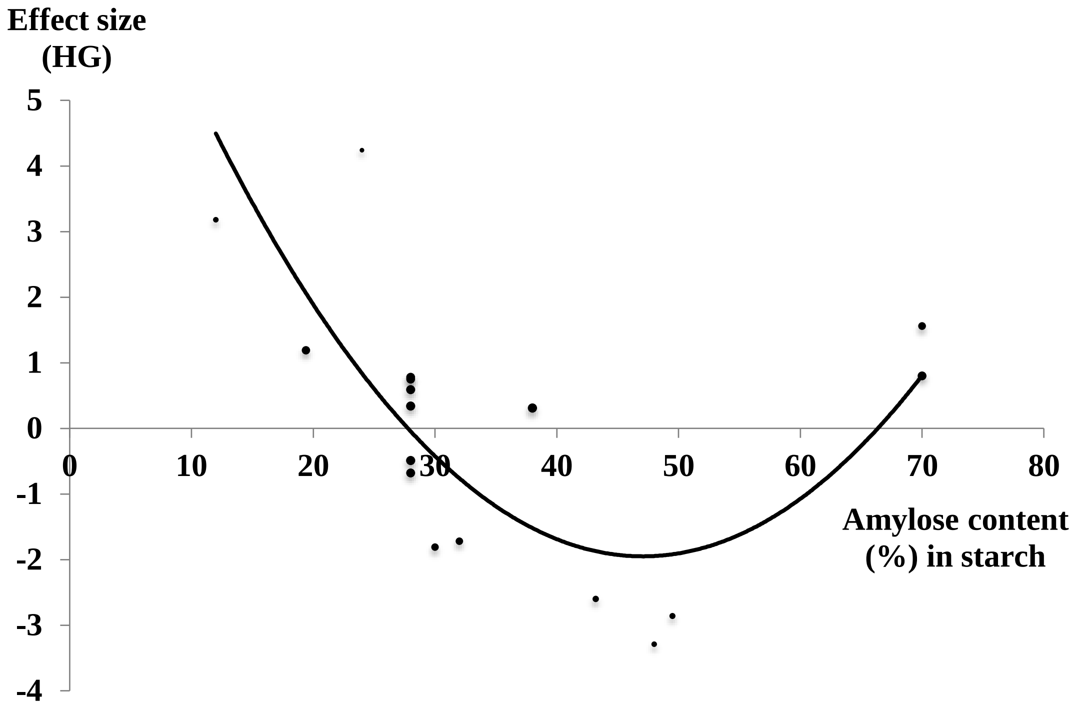


Figure 3.4.6.2 The effect of feeding diets with different amylose versus amylopectin content in fish. The quadratic relationship between the effect size (growth of fish (HG) shown on the y-axis) and amylose content (% amylose shown on the x-axis) of the starch source in the diet is shown.

3.5 Conclusion

In conclusion, our meta-analysis indicated that fish fed raw starch as a carbohydrate source had significantly higher growth performance than fish fed glucose, however no significant difference was found when fish were fed dextrin. This suggests that raw starch can be used as an efficient carbohydrate source in the diet of omnivorous fish such as Nile tilapia however there may be some limitations with use in the diets in salmonids. Moreover, fish fed hydrothermally processed starch diet had higher growth performance than those fed raw starch diet. Therefore, hydrothermal process of starch should be encouraged in feed production for the aquaculture industry.

The comparison of different amylose and amylopectin ratios in the meta-analysis indicated that fish fed starch with 44% amylose as a carbohydrate source had the lowest growth performance. However, since the number of studies of amylose from 0 to 12% and from 44 to 100% was insufficient, more studies are required to improve the accuracy of the regression model. Moreover, there is a need to test if fish fed corn or faba bean starch with lower amylose content between 20-30% show higher growth performance than fish fed lentil or pea starch with higher amylose content between 34.9-39%.

4 DETERMINATION OF THE EFFECTS OF CORN, PEA, LENTIL AND FABA BEAN STARCHES ON THE DIGESTIBILITY, GROWTH PERFORMANCE AND GLYCEMIC INDICES IN NILE TILAPIA *(OREOCHROMIS NILOTICUS)*.

4.1 Publication Fate and Contribution

This chapter is follows from the meta-analysis to test the effects of increasing starch on fish growth performance using Nile tilapia as a model species. This chapter provides information that will be valuable to the field of aquaculture and aquafeed formulation. Mr. Guo contributed to 100% of the feed formulation, feed production, animal testing, data handling and chapter writing. The defendant’s supervisor, laboratory colleagues and thesis committee provided help with study design and editing of the manuscript and chapter.

4.2 Introduction

Carbohydrates are an inexpensive and therefore commonly used ingredient in fish feed for energy supply. Maximizing carbohydrate content in fish diets can reduce the overall cost of the feed and maintain the sustainable development of the aquaculture industry. Starch is the primary low-cost carbohydrate source that can be found in most animal feeds, although most aquafeeds currently content <10% carbohydrate. The appropriate carbohydrate level in the diet can decrease ammonia excretion, promote protein sparing and can contribute to positive growth performance in various fish species (Peres and Oliva-Teles, 2002, Mohanta et al., 2007; Xiong et al., 2014; Azaza et al., 2015).

However, fish are considered to have a low tolerance for carbohydrates and are considered to be sensitive to becoming hyperglycemic (Polakof et al., 2012a). Moreover, many carbohydrate sources also contain anti-nutritional factors, which can result in negative effects on fish health and growth (Drew et al., 2007). Anti-nutritional factors can be broken down into two categories: heat-labile and heat stable. The secondary compounds from heat-labile anti-nutritional factors can be eliminated by heat treatment such as extrusion. However, removal of heat stable secondary compounds requires the fractionation of crops. Fractionation technologies of crops range from low effort methods such as dehulling, medium effort methods such as air classification and high effort technologies such as aqueous or solvent protein purification (Drew et al., 2007). Moreover, different intrinsic amylose and amylopectin ratios of starches used, starch granule types, or processing methods can result in different rates of digestion and digestibility of starches, which may in turn lead to differences in starch utilization and overall growth performance in fish. Therefore, studies on the nutritional values and appropriate levels of various starch sources in fish diets are necessary for the evaluation of the ingredient efficiency. Understanding the knowledge of digestibility, ingredient palatability and nutrient utilization of ingredients is beneficial to optimize feed formulation in the future (Glencross et al., 2007).

The utilization of carbohydrates varies among fish species, but is generally considered to be greater in omnivorous or herbivorous species such as Nile tilapia (*Oreochromus niloticus*; Polakof et al., 2012a). Wilson (1994) indicated that warm water fish species could use dietary carbohydrates as an energy source more efficiently than cold water and marine fish species. Wang et al. (2005) indicated that juvenile tilapia fed greater than 22 and up to 46% starch in the diet had significantly higher growth performance than fish fed 6% and 14% starch inclusion. Anderson and colleagues (1984) indicated that the growth performance of Nile tilapia fed different carbohydrate diets increased with the increasing level of glucose, sucrose, dextrin or starch from 0% to 40%. Furthermore, Shiau and Chuang (1995) stated that juvenile tilapia fed starch as a carbohydrate-based diet had significantly higher WG than other carbohydrate sources including glucose, maltose, sucrose and lactose. Shiau and Lin (1993) reported that when hybrid tilapia was fed a 40% cornstarch diet over an 8-week period, tilapia had a high WG of 896.8%. Therefore, starch can be used as an efficient carbohydrate source and promote positive growth performance in tilapia. However, to the best of our knowledge, all previous studies have examined grain or root-derived starches and no study so far has examined the growth performance of tilapia fed pulse and/or modified cereal starch relative to different amylose and amylopectin ratios or starch granule types.

A GI value is a single value that represents the magnitude and duration of blood glucose change after consuming a particular food. This is quantitated by calculating area under the curve (AUC) of the post-prandial glucose response after ingesting a defined amount of available carbohydrate and normalized to that of a control food (pure glucose or white bread; Wolever et al., 1992). Therefore, consuming high GI foods can cause prolonged hyperglycemia and increase oxidative stress in humans and mammals (Dhar et al., 2008, 2010). Consuming a low GI diet can reduce blood glucose, insulin, and triglyceride levels, which in turn is known to control diabetes and hypertriglyceridemia in humans (Wolever et al., 1992) compared to eating a high GI diet, which can cause or at least worsen both of these diseases. Xiong et al. (2014) reported that diets containing excess digestible carbohydrate can lead to negative effects on fish health, including persistent hyperglycemia (Hatlen et al., 2005), oxidative stress and increased histopathologic problems in the liver (Russell et al., 2001, Azaza et al., 2013), excess fat deposition in the liver and whole body (Hemre et al., 2002), decreased red blood cell and hemoglobin levels (Abdel-Tawwab et al., 2010) and negative influences on bone development (Tan et al., 2009). Since these previous fish studies all utilized high GI starch sources, it is not clear whether high inclusion rates of all starches produce these adverse health effects or whether utilizing low GI starches such as pulses could mitigate the adverse effects while at the same time reducing aquafeed costs. Moreover, the relationship, if any, of post-prandial glucose responses and GI to digestibility or starch utilization in a growth trial is unclear. The aim of this study is to examine these relationships in Nile tilapia, with a focus on comparing different types of starch (pulses compared to a cereal starch).

Nile tilapia is an important fish species for the aquaculture industry as it ranks second for the largest production levels in the world (FAO 2014). Tilapia can be considered as an omnivorous or herbivorous fish species, which can tolerate higher dietary carbohydrate compared with carnivores (Hemre et al., 2002). Pulses are a major industry in Canada with the second largest production in the world (Hoover et al., 2010). Pulse starch is digested more slowly than cereals starch, which benefits the regulation of blood glucose in human and animal species (Hoover and Sosulski, 1985; Hoover et al., 2010).

Since high GI starches are hypothesized to be detrimental to the health and growth of fish at high inclusion rates, a comparison of pulse starches (high amylose content) to a cereal starch (low amylose content) was wanted, but all starches needed to have similar glycemic responses i.e. low GI values, to eliminate confounding effects from different GI values. However, since native, low amylose cereal starches such as cornstarch tend to have very high GI values (~100 or higher) in humans and dogs (Wolever et al., 1994; Adolphe et al., 2012a), we instead used a cornstarch with a covalent hydroxypropyl modification that was shown to have a low GI value of ~55 comparable to that of pulse starches in preliminary studies in dogs in our lab (Briens and Weber, unpublished data). Thus, we tested the GI of three pulse starches and compare it to a modified cornstarch. Then, experiments were conducted to study the digestibility and growth of these three dry processed starches (pea, lentil and faba bean) to modified cornstarch in Nile tilapia. This research is aimed at promoting the development of pulses as a new market for aquaculture feed as well as reducing economic and ecological impacts by reducing fishmeal content in aquafeeds without compromising fish growth rates or production.

4.3 Materials and Methods

4.3.1 Starch sources

Three dry processed pulses starches (pea, lentil and faba bean) were obtained from Alliance Grain Traders (Saskatoon, SK). Cornstarch (Thin-N-Thik 99; hydroxypropyl and cross-linked modification) was obtained from Tate-Lyle (Saskatoon, SK), for use as a control starch source.

Table 4.3.1 indicates the nutrient composition of the four starch ingredients, which was analyzed by Central Testing Laboratory Ltd. The dry matter, gross energy and starch ranged from 89.94-91.75%, 4133-4412 kcal kg^{-1}, and 68.51-81.11%, respectively. Modified cornstarch had the highest value for dry matter and starch content at 91.75 and 81.11%, respectively, but the lowest value for crude protein and gross energy at 0.53 and 4133 kcal kg^{-1}, respectively. The crude protein content of modified cornstarch was negligible compared with three pulse starches, which ranged from 15.15 to 21.76%. Faba bean starch had highest value in crude protein and gross energy content at 21.76% and 4412 kcal kg^{-1}, respectively, but lowest value in starch content at 68.51%.

Table 4.3.1 The chemical composition of the four starch ingredients

	Starch source			
	Modified corn	Lentil	Pea	Faba bean
Nutrient content (%)				
Dry matter	91.75	89.94	89.85	90.52
Crude protein (N*6.25)	0.53	16.80	15.15	21.76
Gross energy (kcal kg^{-1})	4133	4354	4326	4412
Fat	0	0.61	0.61	0.94
Starch (Acid hydrolysis)	81.11	73.49	71.50	68.51
Crude fibre	0	0.15	0.07	0.22
No-Fibre carbohydrate	98.15	79.83	81.50	73.67
Ash	0.41	1.72	1.78	2.51

4.3.2 Fish husbandry

Mixed sex Nile tilapia (*Oreochromis niloticus*, mean initial weight 336 g, N=405) were obtained from AmeriCulture Inc. (Animas, NM, USA) and housed in 360 L tanks at the Prairie Aquaculture Research Centre (PARC; University of Saskatchewan, Saskatoon, SK) fitted with a recirculating system using biological filtration. Water temperature was maintained at 25ºC ± 2. Temperature, pH, and dissolved oxygen were monitored daily, while nitrate, nitrite, ammonia and chlorine levels were tested weekly. Photoperiod was maintained on a 14h light: 10h dark cycle. Fish were randomly assigned to fifteen experimental units (25 fish per tank), with three replicates per treatment. The fish were fed by hand to visual satiation twice a day. The husbandry of fish followed the guideline of the Canadian Council on Animal Care (CCAC, 2005).

4.3.3 Experiment 1: Digestibility of modified cornstarch and pulse starches in Nile Tilapia

4.3.3.1 Digestibility diets

The reference diet (Table 4.3.3.1.1) was prepared according to Bureau and Cho (1994), and the test diets were formulated by 700 g kg^{-1} of the reference diet and 300 g kg^{-1} of the experimental ingredient (modified cornstarch, pea, lentil or faba bean starch). Celite (Celite 545, Celite Co., World Minerals Co., Lompoc, CA, USA) was added to the reference diet at 10 g kg^{-1}

as an inert non-absorbable marker for the determination of digestibility. The nutrients of each diet satisfied the nutrient requirement of Nile tilapia (Table 4.3.3.1.2; NRC 2011). The nutrient values in Table 4.3.3.1.2 were based on dry matter basis. All diets were prepared at the University of Saskatchewan (Saskatoon, Saskatchewan), where dry ingredients were weighed and mixed for 15 minutes in a Hobart Legacy Floor Mixer (Hobart Corporation, Troy, OH). Canola oil and cold water were added to the dry mix to achieve a dough and mixed for an additional 15 minutes. The dough was then cold extruded using a 3 mm 4822 Hobart Food Grinder (Hobart Corporation, Troy, OH). Lastly, the diets were dried in a forced air oven (55°C, 12 h), chopped and screened to obtain the appropriate pellet size. Representative samples of feed were taken for chemical analysis.

Table 4.3.3.1.1 Diet formulation of the reference diet for the digestibility trial (g kg^{-1})

Ingredient	Reference Diet
Poultry by-product meal	300
Wheat flour	280
Soybean protein concentrate	170
Corn gluten meal	130
Canola oil	100
Vitamin mineral premix[1]	10
Celite	10
Starch source	0

[1] Vitamin-mineral premix (mg/kg unless other wise stated), vitamin A (as acetate), 7500IUkg^{-1}; vitamin D3 (as cholecalcipherol), 6000IUkg^{-1}; vitamin E (as DL- α -tocopheryl-acetate), 150IUkg^{-1}; vitamin K (as menadione Na-bisulfate), 3; vitamin B12 (as cyanocobalamin), 0.06; ascorbic acid (as ascorbyl polyphosphate), 650; D-biotin, 42; choline (as chloride), 3000; folic acid, 3; niacin (as nicotinic acid), 30; pantothenic acid, 60; pyridoxine, 15; riboflavin, 18; thiamin, 3; NaCl, 6.12; ferrous sulphate, 0.13; copper sulphate, 0.06 ; manganese sulphate, 0.18; potassium iodide, 0.02; zinc sulphate, 0.3; and carrier (starch); EWOS Canada Ltd.

Table 4.3.3.1.2 The nutrient composition of digestibility trial diets

	Diets				
	Control	Modified cornstarch	Lentil starch	Pea starch	Faba bean starch
Nutrient content (%)					
Dry matter	96.10	94.80	95.66	95.25	93.02
Crude protein	43.11	29.65	36.29	33.89	36.69
Gross energy (kcal kg^{-1})	5055	4831	4845	4854	4917
Starch	22.87	31.19	34.71	35.35	34.02

4.3.3.2 Sample collection

Faeces were collected via a settling column installed at the bottom of each tank. Following feeding, each tank was siphoned in order to remove any uneaten feed. After collection, faeces were centrifuged (3500rpm, 15 min), frozen and freeze dried for future analysis.

4.3.3.3 Analytical methods

All experimental diets and faecal samples were ground to 1 mm to determine dry matter, acid insoluble ash, protein, and energy content and to 0.5 mm for starch analysis using a ZM 100 Retsch Mill (Retsch GmbH, Haan, Germany). All samples were analysed in duplicate. Dry matter was determined by drying in a 135ºC oven for 2 hours (AOAC, 1990, method, 934.01). Acid insoluble ash was determined by 4N hydrochloric acid washing after ashing (AOAC, 1990; method no. 924.05). Crude protein was determined via 6.25*total nitrogen via Leco analyser (model FP-528, Leco Corporation, St. Joseph, MI) and gross energy was determined using a bomb calorimeter (Parr Adiabatic Calorimeter, Model 1200, Moline, Illinois). Finally, starch was analyzed using an AOAC approved assay (996.11) Megazyme Assay Kit KTSTA (Megazyme, Bray Co., Wicklow, Ireland).

4.3.3.4 Calculations

The apparent digestibility coefficient (ADC; %) for the diets were calculated using the following equation:

ADC = 100 – (nutrient in the faeces/nutrient in the diet*indicator in the diet/indicator in the faeces)*100.

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

ADC of the test ingredient = ADCT + ((1-s) DR/s DI) (ADCT – ADCR))

Where:

ADCT = Apparent digestibility coefficient of test diet
ADCR = Apparent digestibility coefficient of the control diet
DR = % nutrient (or kcal kg^{-1} gross energy) of the control diet mash
DI = % nutrient (or kcal kg^{-1} gross energy) of the test ingredient
s = Proportion of test ingredient in test diet mash

Digestible nutrient content of ingredient = Nutrient content of test ingredient × Nutrient ADC of test ingredient.

4.3.4 Experiment 2: Effects of feeding modified cornstarch vs. pulse starches to Nile tilapia for 8 weeks

4.3.4.1 Experimental diets

The diet formulation for the growth trial is shown in Table 4.3.4.1. Nine diets were formulated to be iso-energetic and iso-proteinoic and contain either 0 g kg^{-1} starch (control diet), 150 or 300 g kg^{-1} of modified cornstarch, pea, lentil or faba bean starch (150 corn, 300 corn, 150 pea, 300 pea, 150 lentil, 300 lentil, 150 faba bean and 300 faba bean, respectively). The chemical composition of each diet was analyzed by Central Testing Laboratory Ltd. All diets met or exceeded the dietary requirements for Nile tilapia. Diets were extruded at the Saskatchewan Food Industry Development Centre (Saskatoon, SK) and extrusion parameters were maintained as follows: 5 mm die size; temperature, 135 °C; moisture level, 20%; pressure, 24 bar and a screw speed, 396 rpm.

4.3.4.2 Experimental design

An 8-week growth trial was conducted to determine the effects of feeding modified cornstarch versus pulse starch on growth performance. Nile tilapia (mean initial weight 531 g; N=378) were randomly assigned to one of twenty-seven 360 L tanks with three replicates per treatment (14 fish per tank). Diets were randomly assigned to each tank. Fish were fed twice daily by hand to apparent satiation (09:00AM and 04:00PM). Fish were batch weighted on day 0 and a 7-day adaptation period was provided. After adaption, diets consumption was recorded for 8 weeks. Finally, fish were batch weighed on day 63. Fish husbandry was the same as described in Experiment 1.

Table 4.3.4.1 Growth trial diet formulation and calculated nutrient composition

	Diets (g kg^{-1})								
	Control	150 Corn	300 Corn	150 Pea	300 Pea	150 Lentil	300 Lentil	150 Faba	300 Faba
Ingredients									
Fishmeal	100	100	100	100	100	100	100	100	100
Modified cornstarch	-	150	300	-	-	-	-	-	-
Pea starch	-	-	-	150	300	-	-	-	-
Lentil starch		-	-	-	-	150	300	-	-
Faba bean starch	-	-	-	-	-	-	-	150	300
Soy protein concentrate	-	100	274.6	98.6	194.6	83.8	149.3	50	100
Soybean meal	250	50	20	50	27.4	50	61.1	50	111.8
Chicken meal	138.6	100	20	100	50	107.7	70.9	127.1	60.7
Corn gluten meal	25	181.3	25	150	41.9	150	25	167.4	25
Canola oil	132.4	98.5	115.7	105.7	106.2	103.8	103.2	102.5	111.6
Cellulose	100	80	10	100	45	100	45	100	45
WDDGS	97.1	10	10	10	10	10	10	10	10
Wheat flour	50	50	50	50	50	50	50	50	50
DiCalcium phosphate	39.7	20	20	25.1	20	30	30	30	30
Calcium carbonate	32.1	25.9	20	25.9	20	30	20	28.3	20
Wheat gluten	20	20	20	20	20	20	20	20	20
Vitamin/Mineral[1]	10	10	10	10	10	10	10	10	10

DL-Methionine	3.1	2.3	2.8	2.7	2.9	2.7	3.6	2.7	3.9
Choline chloride	2	2	2	2	2	2	2	2	2
Nutrient composition (%)									
Dry matter	95.81	95.41	95.31	95.20	95.01	95.55	95.10	95.15	95.55
Gross energy (kcal kg^{-1})	4993	4803	4837	4820	4854	4784	4771	4723	4832
Crude protein	39.50	40.67	35.54	40.48	37.76	39.77	37.37	38.56	37.11
Fat	15.02	8.79	9.84	9.24	9.46	9.78	9.01	9.31	10.34
Crude Fibre	11.81	9.93	4.76	10.06	7.06	10.16	6.57	11.56	6.94
Ash	13.26	10.04	8.87	10.17	8.77	11.45	9.93	11.80	10.15
Non fibre carbohydrate	19.46	29.63	40.04	29.09	36.00	27.88	36.18	27.82	34.50
Calculated nutrient composition (%)									
Digestible energy (kcal kg^{-1})	3625	3610	3610	3610	3610	3610	3610	3610	3610
Digestible protein	30.8	30.6	30.5	30.6	30.5	30.6	30.5	30.6	30.5
Digestible fat	17.39	13.1	13.3	13.84	13	13.75	13	14	13.9
Calcium	2	1.6	1	1.59	1.13	1.79	1.22	1.81	1.16
Phosphorus	1.2	0.85	0.68	0.93	0.71	1.04	0.93	1.08	0.87
Methionine	0.8	0.8	0.77	0.8	0.75	0.8	0.8	0.8	0.8
Cysteine	0.37	0.43	0.37	0.41	0.34	0.4	0.33	0.4	0.31
Lysine	1.91	1.75	1.95	1.68	1.71	1.63	1.65	1.56	1.61
Tryptophan	0.42	0.38	0.32	0.37	0.32	0.37	0.33	0.39	0.3

Arginine	1.82	1.74	2.03	1.68	1.76	1.62	1.67	1.54	1.54

[1] Vitamin-mineral premix (mg kg^{-1} unless other wise stated), vitamin A (as acetate), 7500 IU kg^{-1}; vitamin D3 (as cholecalcipherol), 6000 IU kg^{-1}; vitamin E (as DL-α-tocopheryl-acetate), 150 IU kg^{-1}; vitamin K (as menadione Na-bisulfate), 3; vitamin B12 (as cyanocobalamin), 0.06; ascorbic acid (as ascorbyl polyphosphate), 650; D-biotin, 42; choline (as chloride), 3000; folic acid, 3; niacin (as nicotinic acid), 30; pantothenic acid, 60; pyridoxine, 15; riboflavin, 18; thiamin, 3; NaCl, 6.12; ferrous sulphate, 0.13; copper sulphate, 0.06 ; manganese sulphate, 0.18; potassium iodide, 0.02; zinc sulphate, 0.3; and carrier (starch); EWOS Canada Ltd.

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4.3.4.3 Growth indicators calculations

Average daily gain (ADG), specific growth rate (SGR), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated by 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 index of cornstarch, pea, lentil or faba bean starch in Nile tilapia

4.3.5.1 Preliminary experiment

Fish were anesthetized [10mg L^{-1} Aquacalm (metomidate hydrochloride, Western Chemical, Ferndale, WA)] and force-fed a solution containing 1g of carbohydrate per kg of fish weight and either glucose (N=2), modified cornstarch (N=2) or grocery store cornstarch (N=1) (Table 4.3.5.1). The available carbohydrate content was tested using the Megazyme Available Carbohydrate and Dietary Fibre assay kit (K-ACHDF, Megazyme International, Wicklow, Ireland). Then a 1 ml syringe (BD, Franklin Lakes, NJ 07417 USA) was rinsed with 0.035 ml of heparin prior to collecting a 0.35 ml blood sample from the caudal vein at 0, 3, 6, 12, and 24 to determine the appropriate feeding method, feeding amount and time period for the experiment.

Table 4.3.5.1 The available carbohydrate content (g kg^{-1}) of two corn starch sources versus glucose tested in preliminary experiments and the amount required to feed 1.0 g available carbohydrate to fish.

Starch sources	Total available carbohydrate (g kg^{-1})	Amount needed to feed 1g available carbohydrate (g)
Glucose	1000.0	1.00
Modified cornstarch	659.5	1.52
Grocery store cornstarch	1000.0	1.00

4.3.5.2 Experimental design

The GI of tilapia fed pea, lentil, faba bean or modified cornstarch was determined by comparing to a control feeding of pure glucose. Due to unexpected postprandial glycemic responses to the modified cornstarch (See Results Table 4.4.3.1) that was used in the digestibility and growth trials, a comparison was made for glycemic testing only to grocery store cornstarch (wet processed, highly pure, but otherwise unmodified starch, Fleischmann's, ACH Food Companies, Mississauga, ON). Due to the solution leaking out through the gills, the glucose or starch source was packed into a starch free capsule (Double "00" Vcaps; Bloomingdale, IL 60108) and administered via an oral gavage. However, the amount of 1 g available carbohydrate per kg of fish would not fit into one capsule, so the amount of glucose or starch fed was calculated according to Table 4.3.5.2 so that a 0.5 g bolus of available carbohydrate was used for all carbohydrate sources. Fish that were being maintained on commercial diets and that were naïve to the test carbohydrate diets were fasted for 48 h prior to glycemic testing. A 0.5 g sample of available carbohydrate per kg body weight of glucose or starch source was weighed into the starch free capsule and force-fed to an anaesthetized fish (average weight 651g; N=6 fish at each time point per ingredient). Blood samples were taken from the caudal vein at 0, 3, 6, 12, 24, and 48 h then centrifuged (3500 rpm, 10 min, 4°C) for serum separation. Serum samples were stored at -80°C for future analysis. Blood glucose was analyzed using a colorimetric enzyme-based kit according to methods previously used by this research group (Adolphe et al., 2012a,b).

Table 4.3.5.2 The available carbohydrate content (g kg^{-1}) of the four starch sources and the amount required to feed 0.5g available carbohydrate to fish.

Starch sources	Total available carbohydrate (g kg^{-1})	Amount needed to feed 0.5g available carbohydrate (g)
Grocery store cornstarch	1000.0	0.50
Pea	752.8	0.67
Lentil	758.9	0.66
Faba bean	712.3	0.71

4.3.5.3 Glycemic indices calculation

The blood glucose of 0 h was considered as 100%, other points were standardized to 0 h as a percent. The AUC for glucose and the four starch sources was calculated using polynomial (order= 3) models. The GI of each starch source was calculated by the AUC of the fish fed starch divided by the AUC of fish fed the glucose control.

4.3.6 Statistical analysis

All three experiments were analysed using SPSS 22 (SPSS Inc., Chicago, IL, USA). The results in Experiment 1 were analysed as a completely randomized design using the General Linear Model procedure with tank as the experimental unit. In Experiment 2, tank was also the experimental unit and both linear and quadratic regression models were used to analyse the results. The appropriated model was selected based on the lower *P*-value of two models. In experiment 3, the individual fish was the experimental unit and results were analysed using the General linear model. The Ryan-Einot-Gabriel-Welsh F test was used to differentiate the means of results ($P<0.05$).

4.4 Results

4.4.1 Experiment 1 – Digestibility Trial

The apparent digestibility coefficient (ADC) of dry matter for lentil starch was not significantly different than that of pea starch (69.1 vs. 61.0; $P> 0.05$), but it was significantly higher than faba bean starch at 60.8 and modified cornstarch at 53.2 (Table 4.4.1). We were unable to calculate an ADC for protein for the modified cornstarch due to the crude protein content being close to zero (Table 4.3.1). Therefore, statistical analysis of ADC of protein is based on three pulse starches rather than all four-starch ingredients. The ADCs of protein in lentil and faba bean starch were significantly higher than pea starch (87.4 vs. 66.5; $P < 0.05$). The ADC of gross energy in lentil, pea and faba bean starch showed no significant differences, but all were significantly higher than modified cornstarch. There was no significant difference for starch ADC between modified cornstarch, lentil or pea starch. However, the ADC for starch in lentil was significantly higher than for faba bean (60.9 vs. 50.5; $P < 0.05$). Overall, lentil starch had the highest apparent digestibility for all parameters (Table 4.4.1).

Table 4.4.1 The apparent digestibility coefficients of for each starch source.

	Starch source			
	Modified corn	Lentil	Pea	Faba bean
Dry matter	53.2^{c}	69.1^{a}	61.0^{ab}	60.8^{bc}
Crude protein	nd	87.4^{a}	66.5^{b}	87.4^{a}
Gross energy	48.9^{b}	66.6^{a}	60.0^{a}	64.0^{a}
Starch	54.9^{ab}	60.9^{a}	57.2^{ab}	50.5^{b}

nd= not determined; ^{abc} means within each row with different superscripts are statistically different ($P < 0.05$).

4.4.2 Experiment 2 – Growth Trial

4.4.2.1 Average daily gain

The regression models of ADG for the four starch sources were indicated by the following equations (Table 4.4.2 and Figure 4.4.2.1):

Pea starch: $Y=0.005X+1.167$, $R^2= 0.543$, $P= 0.023$

Lentil starch: $Y=0.007X+1.069$, $R^2= 0.458$, $P= 0.045$

Faba bean starch: $Y=-2.756*10\wedge-5X^2+0.010X+1.023$ ($X= 181\text{g kg}^{-1}$), $R^2= 0.739$, $P= 0.018$

Modified cornstarch: $Y=0.006X+0.991$, $R^2= 0.908$, $P< 0.001$

There was a significantly positive linear relationship between starch inclusion level and the ADG for fish fed modified corn, pea or lentil starch diets ($P < 0.05$). However, there was a significant quadratic relationship between starch inclusion level and the ADG of fish fed the faba bean starch diets with a maximum ADG at 1.9g when faba bean starch inclusion reached 181 g kg^{-1} ($P < 0.05$).

4.4.2.2 Specific growth rate

The regression of SGR for four starch sources were indicated by following equations (Table 4.4.2 and Figure 4.4.2.2):

Pea starch: $Y=0.001X+0.203$, $R^2= 0.561$, $P= 0.020$

Lentil starch: $Y=0.001X+0.189$, $R^2= 0.474$, $P= 0.040$

Faba bean starch: $Y=-4.593*10^{\wedge}-6X^2+0.002X+0.180$ ($X= 218$ g kg^{-1}), $R^2= 0.757$, $P= 0.014$

Modified cornstarch: $Y=0.001X+0.177$, $R^2= 0.920$, $P< 0.001$

Similar regression models were indicated between ADG and SGR. There was a significantly positive linear relationship between starch inclusion level and SGR for fish fed modified corn, pea or lentil starch diets ($P< 0.05$). However, there was a significant quadratic relationship between starch inclusion level and the SGR of fish fed the faba bean starch diets with a maximum SGR of 0.4 at a starch inclusion of 218 g kg^{-1} ($P< 0.05$).

4.4.2.3 Average daily feed intake

The regression of average daily feed intake for four starch sources were indicated by following equations (Table 4.4.2 and Figure 4.4.2.3):

Pea starch: $Y=-3.111*10^{\wedge}-5X^2+0.012X+2.333$ ($X= 203$ g kg^{-1}), $R^2= 0.631$, $P= 0.050$

Lentil starch: $Y=0.004X+2.411$, $R^2= 0.578$, $P= 0.017$

Faba bean starch: $Y=-1.111*10^{\wedge}-5X^2+0.004X+2.333$, $R^2= 0.406$, $P= 0.209$

Modified cornstarch: $Y=0.003X+2.328$, $R^2= 0.712$, $P= 0.004$

There was a significantly positive linear relationship between starch inclusion level and ADFI for fish fed by modified corn and lentil starch diets ($P< 0.05$). ADFI had a significantly quadratic relationship with inclusion level for fish fed the pea starch diet ($P< 0.05$). When fish were fed 193 g kg^{-1} pea starch in diet, ADFI reach its lowest value at 3.5g. No significant difference between ADFI and starch inclusion level was indicated in fish fed the faba bean starch diet ($P> 0.05$).

4.4.2.4 Feed conversion ratio

The regression of feed conversion ratio for four starch sources were indicated by following equations (Table 4.4.2 & Figure 4.4.2.4):

Pea starch: $Y=-0.003X+2.241$, $R^2= 0.660$, $P=0.008$

Lentil starch: $Y=-0.003X+2.290$, $R^2= 0.432$, $P= 0.054$

Faba bean starch: $Y=2.437*10\text{^}-5X^2-0.010X+2.327$ ($X=205$ g kg^{-1}), $R^2= 0.770$, $P= 0.012$

Modified cornstarch: $Y=-0.004X+2.265$, $R^2= 0.834$, $P= 0.001$

A significant negative linear relationship between starch inclusion level and FCR was indicated in fish fed the pea and modified cornstarch diets ($P< 0.05$), but a significant quadratic relationships was also shown for the faba bean starch diet ($P< 0.05$) with the lowest FCR at 1.30 being at a starch inclusion level at 205 g kg^{-}1. Although not statistically significant, there may have also have been a relationship between FCR and lentil starch inclusion in the diet ($P= 0.054$, which is close to $P= 0.05$).

Table 4.4.2 Linear or quadratic regression parameters of average daily gain, specific growth rate, average daily feed intake and feed conversion ratio of Nile tilapia fed by 0, 150, and 300 g kg^{-1} pea, lentil, faba bean and modified cornstarch.

Regression Parameter	Inclusion^2	SEM	Inclusion	SEM	Constant	SEM	R-squared	*P*-value
Average daily gain								
Pea starch			0.005	0.002	1.167	0.342	0.543	0.023
Lentil starch			0.007	0.003	1.069	0.542	0.458	0.045
Faba bean starch	-2.756*10^-5	<0.001	0.010	0.003	1.023	0.168	0.739	0.018
Modified cornstarch			0.006	0.001	0.991	0.132	0.908	<0.001
Specific growth rate								
Pea starch			0.001	0	0.203	0.052	0.561	0.020
Lentil starch			0.001	<0.001	0.189	0.080	0.474	0.040
Faba bean starch	-4.593*10^-6	<0.001	0.002	<0.001	0.180	0.270	0.757	0.014
Modified cornstarch			0.001	<0.001	0.177	0.200	0.920	<0.001
Average daily feed intake								
Pea starch	-3.111*10^-5	<0.001	0.012	0.004	2.333	0.262	0.631	0.050
Lentil starch			0.004	0.001	2.411	0.278	0.578	0.017
Faba bean starch	-1.111*10^-5	<0.001	0.004	0.002	2.333	0.145	0.406	0.209
Modified cornstarch			0.003	0.010	2.328	0.150	0.712	0.004
Feed conversion ratio								
Pea starch			-0.003	0.001	2.241	0.170	0.660	0.008
Lentil starch			-0.003	0.001	2.290	0.276	0.432	0.054
Faba bean starch	2.437*10^-5	<0.001	-0.010	0.003	2.327	0.155	0.770	0.012
Modified cornstarch			-0.004	0.001	2.265	0.122	0.834	0.001

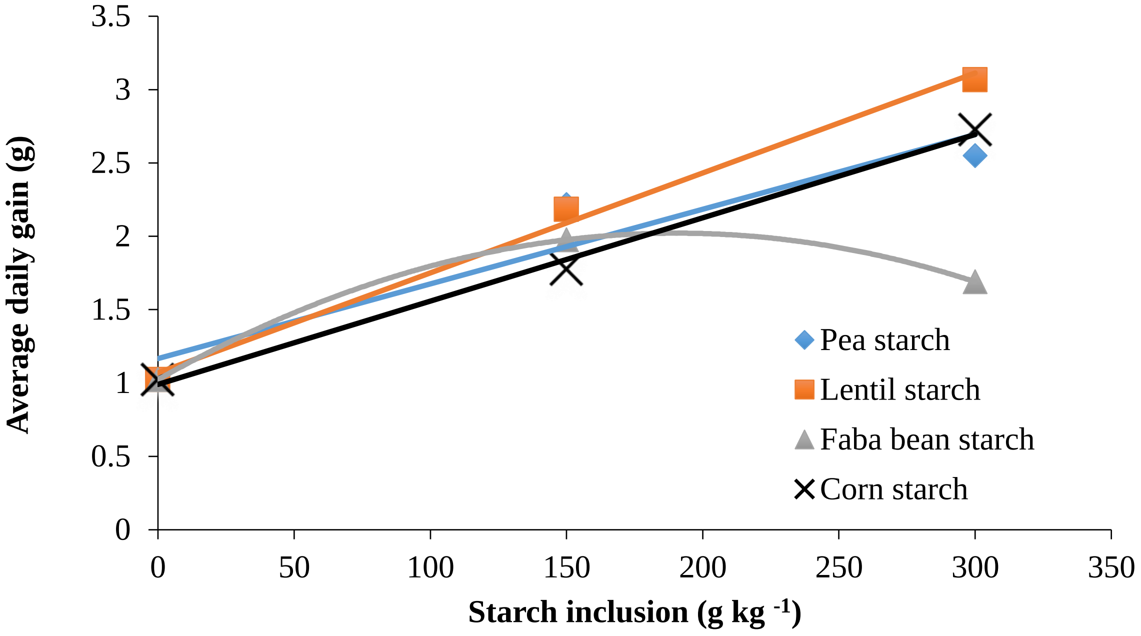

Figure 4.4.2.1 The regression models of average daily gain in Nile tilapia fed by 0, 150 and 300 g kg^{-1} four starch sources (each point shown is the mean of 3 replicates).

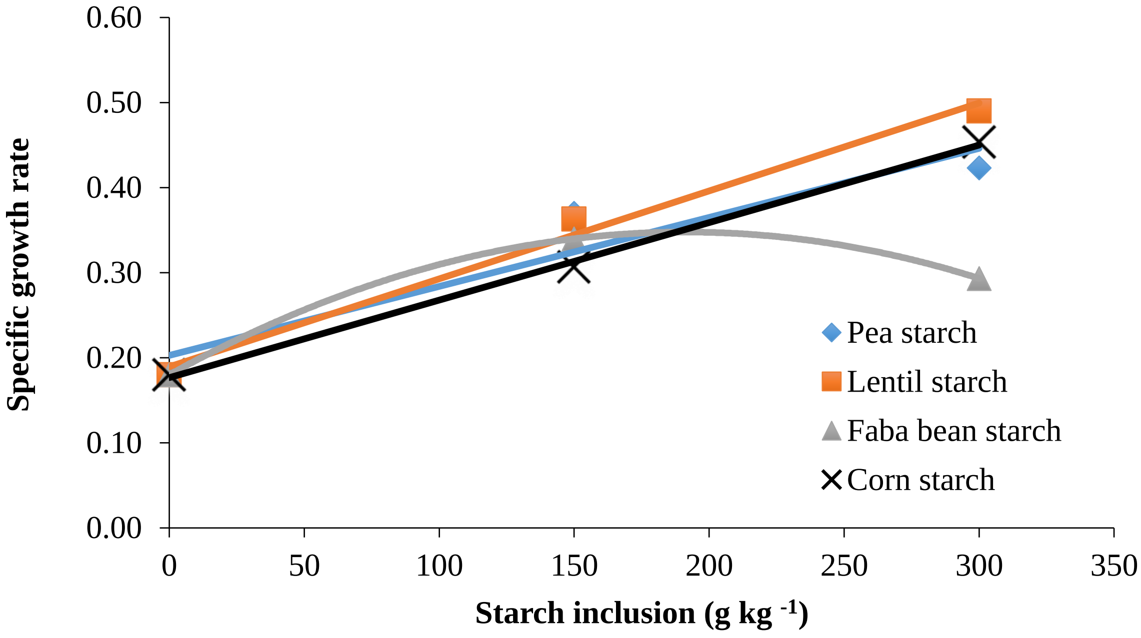

Figure 4.4.2.2 The regression models of specific growth rate in Nile tilapia fed by 0, 150 and 300g kg^{-1} four starch sources (each point shown is the mean of 3 replicates).

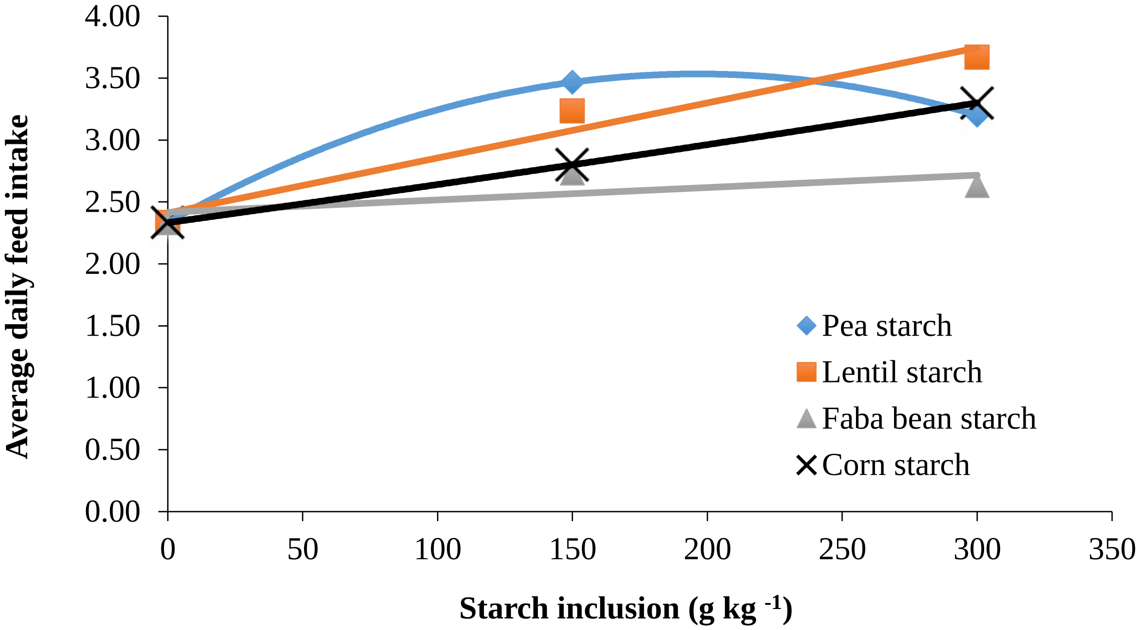

Figure 4.4.2.3 The regression models of average daily feed intake in Nile tilapia fed by 0, 150 and 300g kg^{-1} four starch sources (each point shown is the mean of 3 replicates).

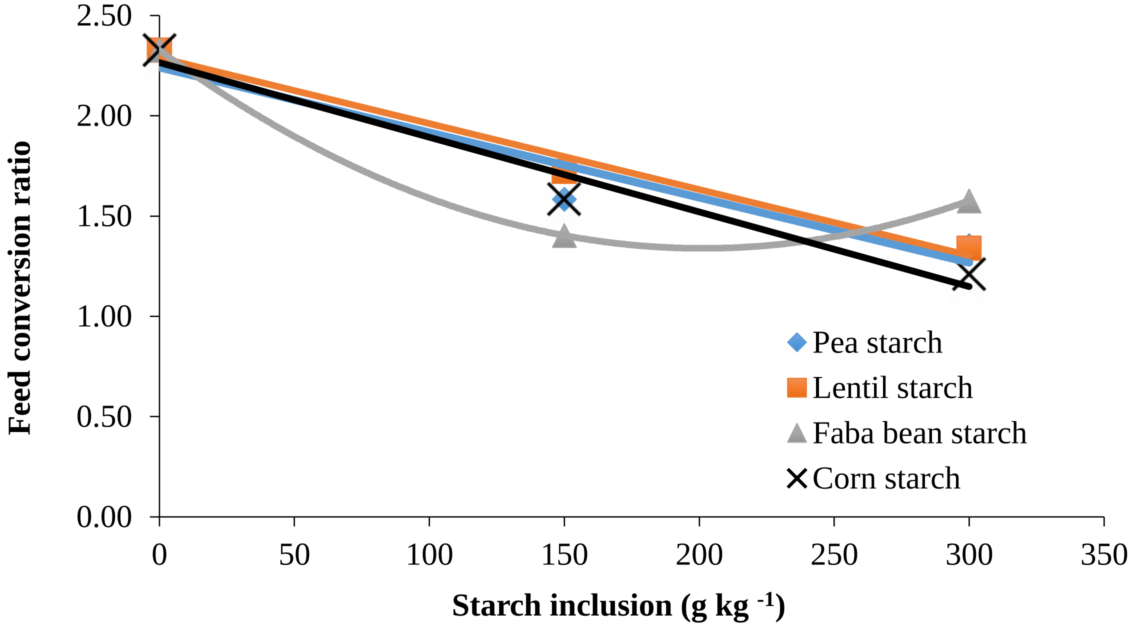

Figure 4.4.2.4 The regression models of feed conversion ratio in Nile tilapia fed by 0, 150 and 300g kg^{-1} four starch sources (each point shown is the mean of 3 replicates).

4.4.3 Experiment 3 – Glycemic index testing

Contrary to what was observed by our group in dogs, the hydroxypropyl cornstarch used in the digestibility and growth trials produced a GI at 453 in preliminary tilapia experiments. Moreover, at 24 hr, blood glucose remained significantly higher (3 times) than the baseline blood glucose value. Although an estimate of the AUC for the glucose response and GI of modified cornstarch are provided below in Table 4.4.3.1, the values shown seemed unreliable. Therefore, for comparison, we tested postprandial glucose responses to a grocery store cornstarch, a more common commercially available and highly pure cornstarch, and we extended the experimental time period to 48 hr. In this case, the blood glucose response did recover to baseline within 48 hr, and the AUC and GI values for the grocery store cornstarch fell within the range of the pulse starches tested in tilapia (Table 4.4.3.2). The GI of glucose, cornstarch, pea, lentil and faba bean starch was 100, 61, 54, 69 and 76 respectively. No significant differences were found between the carbohydrate sources for the AUC or GI. However high variability in glycemic responses, as reported previously for fish (Polakof et al., 2012a), is likely the cause for lack of statistical difference, especially for glucose and pea starch.

Table 4.4.3.1 The area under the curve for the postprandial blood glucose response and glycemic indices for glucose and two cornstarch sources in Nile tilapia fed by 1g available carbohydrate per kg fish weight in preliminary experiments.

	Area under the curve	N	SEM	Glycemic indices
Glucose	1822	2	485.4	100
Modified cornstarch*	8250	2	3616.0	453
Grocery store cornstarch	1360	1	nd	75

SEM = standard mean error; nd: not determined.

Table 4.4.3.2 The area under the curve for the postprandial blood glucose response and glycemic indices for glucose and starch sources in Nile tilapia fed by 0.5g available carbohydrate per kg fish weight.

	Area under the curve	SEM	Glycemic indices
Glucose	2858	857.8	100
Pea starch	1542	545.5	54
Lentil starch	1975	69.3	69
Faba bean starch	2186	300.5	76
Grocery store cornstarch	1743	260.3	61

SEM = standard mean error

4.5 Discussion

There are no previous studies on the digestibility of pulse starches in finfish, however there are a number of studies that have determined the digestibility of legume protein in finfish. The reported digestibility of protein in various pulse meals ranged from 77 to 88.5 (Fagbenro, 1998; Fontaínhas-Fernandes et al., 1999; Drew et al., 2007). The digestibility of the protein in the lentil and faba bean starches in the present studies falls in this range. However, the digestibility of protein in the pea starch is 66 and much lower than in previous studies. This suggests that either the processing method used to produce the pea starch significantly reduced the protein digestibility of the product or there was experimental error in the performance of the digestibility trial and/or in the analysis of feed or fecal samples.

The digestibilities of various plant starches, but not pulse starches, have been determined previously in Nile tilapia. Tilapia fed 310 g kg^{-1} and 470 g kg^{-1} raw cornstarch had a starch ADC value of 92 and 86, respectively (Shiau and Liang 1994). In another study by Kaushik et al. (1995) the ADC of starch decreased from 96 to 71 when the raw wheat starch inclusion increased from 280 to 660 g kg^{-1} in tilapia diets. These literature values are all much higher than those found in the present study (51-61) where we examined pulse starch digestibility with ~30% inclusion rate. Pulse starches have a higher amylose to amylopectin ratio than corn or wheat starches. Chen et al. (2013) indicated that the ADC of starch decreased from 73 to 62 when amylose to amylopectin ratios increased from 0.11 to 0.98. Since amylose is more resistant to digestion than amylopectin, our findings support the hypothesis that higher amylose produces lower digestibility for starches in fish. In contrast, the digestibility of the modified cornstarch used in the digestibility and growth trial experiments was 55, which was much lower than the values reported for cornstarch in previous studies. The cornstarch used in the present digestibility and growth trial studies was a hydroxypropyl substituted, cross-linked cornstarch. Crosslinking increases the resistance of starch to temperature, agitation and acids (Corn Starch, 2006), which likely also resulted in the modified cornstarch being more resistant to digestion compared to the highly processed, but unmodified cornstarch that is found in grocery stores and most often used in human foods. More importantly, the modified cornstarch produced a digestibility in tilapia within the range of those observed for the pulse starches tested in this study.

Test diets were formulated for a tilapia growth trial, taking the digestibilities into account, to produce increasing levels of available starch inclusion for pea, lentil, faba bean and modified cornstarch diets at 15 and 30% inclusion compared to a control diet with 0% inclusion of these starches. The ingredients except starch sources in each diet of growth experiment had different amounts, which may be considered as factor to influence growth performance. Therefore, both linear and quadratic regression models were performed between individual ingredient and growth performances (ADG, SGR, ADFI, and FCR). All *p*-values indicated other ingredients except starch did not significantly influence the growth performance of fish. Extrusion of diets is widely used in aqua-feed industry. However, extrusion resulted in the starch gelatinization in growth trial diets, which could affect the digestibility of starch in each diet. Since our diets were formulated based on digestible protein and digestible energy, gelatinization of starch may influence digestible energy. However, the starch in all diets would be gelatinized, which diminished the effects of extrusion to digestible energy among each diet.

Wang et al. (2005) reported that juvenile tilapia fed diets containing cornstarch from 22 to 46% had significantly higher grow rates than for diets containing 6 to 14% starch inclusion. Anderson et al. (1984) also indicated that the growth rates of Nile tilapia increased with increasing starch inclusion up to 400 g kg^{-1}. However, neither study fed diets formulated with equivalent digestible energy and digestible protein. The present study fed diets with equal digestible energy and protein, yet we were able to determine that the ADG and SGR of Nile tilapia still significantly increased when the pea, lentil or modified cornstarch inclusion rates increased from 0 to 300 g kg^{-1} in the diets. However, the growth performance of the Nile tilapia fed the faba bean diet maximized at approximately 200 g kg^{-1} of the diet, then decreased at the higher inclusion rate. This may be due to the well-known presence of antinutritional factors in faba beans. Faba beans differ from pea and lentils because they contain the antinutritional factors vicine and covicine, which can reduce the activity of glucose-6-phosphate dehydrogenase (Arese et al., 1981). Given that this enzyme is involved in carbohydrate metabolism, it is likely that the activity of these antinutritional factors reduced the efficiency of glucose metabolism and decreased the nutritional quality of faba bean starch, but only at high inclusion rates (> 20%).

Tilapia had FCR ranged from 1.4 to 1.8 (DeLong et al., 2009), However, fish fed by control diet had higher FCR at 2.3 than common range. Control diet contained higher amount of soybean meal, cellulose and WDDGS than other eight starch diets, and also had higher fat content at 15% than other starch diets with fat content between 8.79 and 10.34% (Table 4.3.4.1), which may be the reason why the growth performance of fish fed by control diet was lower than fish fed by starch diets (modified corn, pea and lentil) (Figure 4.4.2.1 to 4.4.2.4). Moreover, a study in rainbow trout indicated that fish had partial ability to regulate pancreatic metabolism when fed high carbohydrate (dextrin) inclusion diet (Polakof et al., 2012b), however, lipid metabolism can negatively impact glucose metabolism. According to this point of view, the growth control diet with high fat content in our study may lead to the improper regulation between glucose and lipid metabolism, which resulted in the relatively lower growth performance of fish.

Based on the growth studies, clearly Nile tilapia can utilize starches including pulse starches for growth and these can safely be used in aquafeeds. However, questions remain about how fish handle carbohydrates and glucose. There have been a limited number of studies that have examined glycemic responses of finfish. Rainbow trout fed diets containing 62 and 386 g kg^{-1} digestible carbohydrate had blood glucose peaks of 0.74 to 4.06 g L^{-1}, respectively, 7-hour post-feeding (Figueiredo-Silva et al., 2013). In the same study, Nile tilapia fed 24 and 453 g kg^{-1} digestible carbohydrate had blood glucose peaks of 0.57 and 1.15 g L^{-1}, respectively at 3-hour post feeding. This suggests that omnivorous fish species such as Nile tilapia have higher ability to regulate blood glucose than carnivores such as rainbow trout. Deng et al. (2001) indicated that there was no significant difference between the GIs of cornstarch (GI=40) and potato starch (GI=35) in white sturgeon when fed 1g carbohydrate per kg of fish body weight. The GI of hydroxypropyl modified cornstarch in Nile tilapia in present study was astonishingly high at 453 as already discussed above. However, the GI for the wet processed grocery store cornstarch produced a lower GI value in tilapia at 61 in this study. Not only was this GI value lower than the modified starch, but it was also much lower that values reported for similarly processed cornstarch in mammals. Wolever et al. (1994) reported that the GI of boiled corn meal and corn flakes were 97 and 105, respectively in humans. Similar results were obtained in pigs with a GI of 105 (Drew et al., 2012), but the GI of grocery store cornstarch in the present study was 61 in tilapia. In contrast, the GIs of lentil, pea and faba bean were 21-42, 51 and 63, respectively in human studies (Atkinson et al., 2008). However, it is hard to compare the human studies with animal studies, because the GIs of human studies were measured on cooked food, which resulted higher GI value than uncooked food. However, the GIs of the pulse starches in the current study in tilapia were even higher than the cooked food values reported in humans, since compared to the 69, 54 and 76 of lentil, pea and faba bean, respectively, found in fish. Moreover, the same starch sources tested in domestic cats by our research group, found GI values of 29, 7, 15 and 8 for corn, pea, lentil and faba bean starch, respectively (Briens and Weber, unpublished). In beagle dogs, the GI values for the same starches were 55, 49, 47 and 46, respectively (Briens and Weber, unpublished). Overall, the GI of the four starches in Nile tilapia had higher glycemic values observed for carnivorous cats and omnivorous dogs.

Native cornstarch has a lower amylose to amylopectin ratio than pulse starch, which should result in a higher GI than the high amylose to amylopectin ratio of pulse starches (Foster-Powell et al., 2002; Atkinson et al., 2008). However, the lentil and faba bean had higher, albeit not statistically significant, values for GI than cornstarch and much higher values than expected based on results for humans. The cross-linking modification of cornstarch in this study may have turned the low amylose, more readily digestible starch source into a starch that resembles a high amylose starch like the pulses. Our findings of reduced digestibility with modified cornstarch compared to reported values for normal cornstarch support this contention. The paradoxical finding is that Nile tilapia respond with higher than expected GI values for the high amylose pulses as well as the lower digestible, modified cornstarch. Thus, in tilapia, there is a difference from mammals where branched chain or cross-linked starches may preferentially lead to high postprandial glycemia. Whether this is due to increased intestinal absorption or alterations in post-absorptive metabolic processing is unclear and requires further study. However, this highlights a major difference between mammals and tilapia.

The stress of fish may result in large change of blood glucose. Therefore, the handling of fish in glycemic response trial was conducted cautiously and slowly. However, it may also cause the stress of and result in the change of blood glucose. A cannulation procedure can be conducted to diminish the stress of fish during taking blood samples. However, the procedure was unable to be conducted in Nile tilapia, but it is highly recommended in salmonids.

4.6 Conclusion

In conclusion, we accepted the hypothesis that our starch sources had different digestibilities, and Nile tilapia can tolerate 300g kg^{-1} lentil and pea starch inclusion without the negative effects on growth performance. However, fish fed more than 200g kg^{-1} faba bean starch in the diet resulted in negative effects on fish growth, which is possibly due to the presence of anti-nutritional factors: vicine and covicine. Therefore, more studies are needed to examine the anti-nutritional factors found in faba bean and their effects on fish growth performance. Lastly, pea and lentil starch supply ample protein in the diet and are recommended for use in diets for Nile tilapia based on the findings of the current study. However, the influence of high amylose content in pulse starch to fish growth requires additional research.

There was no significant difference between GI of each starch ingredient, which could possible due to the difference of physiological conditions in individual fish. However, faba bean starch had relatively higher GI and poor growth performance. It is not clear about the relationship between GI and growth performance. Therefore, more studies are needed to examine the differences between the GI, digestive enzymes and glucose transporters of pulse starches in finfish.

5 GENERAL CONCLUSION AND FUTURE PERSPECTIVES

The Canadian pulse industry has grown tremendously from 0.2 million hectares of total farm area in 1981 to 2.2 million hectares 2011, with Canada becoming the second largest producer and exporter of pulses in the world (Statistics Canada, 2011). Pulses contain both protein and carbohydrate. They have been an important food source in both human and animal diets, especially in areas with limited consumption of animal proteins owing to limited availability and religious and cultural beliefs. In terms of a carbohydrate source, pulses have a higher amylose to amylopectin ratio than standard cereals starch sources. Therefore, pulses are considered to be digested more slowly and have a low glycemic response, which benefits the management of blood glucose levels in diabetes patients, carnivores and fish species. The main pulses grown in Canada include dry peas, lentil, chicken peas and dry beans. Therefore, the research in pulse utilization benefits the development of the pulse industry in Canada.

Our meta-analysis indicated that using either raw or hydrothermally processed starch as a carbohydrate source in fish diets is more efficient than using glucose. Moreover, hydrothermal process is recommended to improve the utilization of starch in fish. However, the recommended starch inclusion in fish diets varies among fish species, fish size, and source of starch. Our meta-analysis also indicated that starch source with low amylose content may have higher growth performance than high amylose content with the interval between 12 and 44%, but since the number of studies involved in this amylose-amylopectin meta-analysis model was limited, more studies are required to test the influence of starch with different amylose content to fish growth.

The results of our digestibility experiment indicate that lentil starch had the highest ADC of dry matter, crude protein, gross energy and starch compared to all the other starch sources. Moreover, fish fed lentil starch as a carbohydrate source had relatively higher growth performance than those fed the other starch sources, even though no significant difference was found. The ADC of starch varied between different sources in our study, which was lower than raw cereals starch sources in other studies. Overall, growth performance of fish increased with the increasing starch inclusion in the diets up to 300 g kg^{-1}. Therefore, both our meta-analysis and growth trial indicated that starch could be used as an efficient carbohydrate source in fish diets. However, fish fed more than approximately 200g kg^{-1} faba bean starch in the diet showed reduced growth performance which may due to the influence of vicine and covicine. Therefore, more research is needed to study the influence of the anti-nutritional factors found in faba beans.

This could be accomplished by feeding vicine- and covicine- free faba bean starch diets to test whether tilapia can tolerate higher than 200g kg^{-1} starch inclusion. Furthermore, inclusion levels above 300 g kg^{-1} for pea and lentil starches also need to be tested, in addition to pure cereal starch to determine the maximum tolerable level of each starch source and to determine the influence of starch source with different amylose and amylopectin content on fish growth performance.

Our meta-analysis indicated that glucose is an inefficient ingredient compared to starch in fish diets. The GIs of faba bean, lentil and pea follows the rank from higher value to lower value 76, 69 and 54. The GI of pea starch is relatively, but not significantly lower than the other starch sources, which could be due to granule change during processing. Therefore, it will be interesting to investigate the granule size of our pea starch in future studies. Fish fed the faba bean starch diets also had reduced growth performance compared to those fed the pea and lentil starches. It is known that vicine and covicine can inhibit the activity of glucose-6-phosphate dehydrogenase (Arese et al., 1981), which may prevent the metabolism of glucose in fish, resulting in higher GI. Therefore, the vicine and covicine in faba bean diets may be the reason why fish had both high GI and poor growth performance. This could be tested in one of two ways 1) faba bean starch, with known levels of vicine and covicine, could be force fed to fish to determine whether fish had a lower GI or 2) a glucose-6-phosphate dehydrogenase inhibitor could be added to a lentil or pea starch which is then force fed to fish to determine if the deficiency of glucose-6-phosphate dehydrogenase will increase the GI or not. Moreover, the GI results study of pure cornstarch, pea, lentil and faba bean starch sources indicated that Nile tilapia fed by 0.5 g available carbohydrate per kg body weight had no significant difference of GI between starch sources. It would be interesting to see the effects of increasing the carbohydrate level from 0.5g to 1g of available carbohydrate per kg of fish body weight to see if there are any significant difference between the GI of the four tested starch sources. Lastly, since tilapia are an omnivorous fish species, which are said to have a higher tolerance for carbohydrate it would be beneficial to conduct a similar experiment in a carnivorous fish species such as rainbow trout to compare the differences of pulse starch utilization.

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7 APPENDICES

Table 7.1 Raw data of growth comparison between raw starch versus glucose in all fish species

Author	Growth Indicators	Fish Species	Glucose	Glucose SD	Starch	Starch SD	N	Notes
				Carnivores				
Enes et al. (2006)	SGR	European sea bass	1.5	0.08	1.8	0.04	6	Waxy starch/25℃
Enes et al. (2006)	SGR	European sea bass	0.8	0.1	0.8	0.09	6	Waxy starch/18℃
Fu (2005)	SGR	Southern catfish	3.14	0.44	3.6	0.32	8	-
Fu (2005)	SGR	Southern catfish	1.47	0.38	3.09	0.42	8	-
Hung and Storebakken (1994)	SGR	Rainbow trout	1.37	0.05	1.21	0.05	6	Continuous feeding
Hung and Storebakken (1994)	SGR	Rainbow trout	1.23	0.05	0.88	0.05	6	Meal feeding
Jiang et al. (2014)	WG	Amur sturgeon	540.2	4.1	782.9	71.9	6	α-starch
Jiang et al. (2014)	WG	Amur sturgeon	540.2	4.1	582.5	14.8	6	-
Stone et al. (2003)	SGR	Juvenile silver perch	1.4	0.21	1.47	0.05	6	Raw wheat starch
Stone et al. (2003)	SGR	Juvenile silver perch	1.4	0.21	1.5	0.29	6	Raw pea starch
				Herbivores				
Ren et al. (2015)	SGR	Blunt snout bream	2.35	0.02	2.37	0.03	6	Wheat starch
Ren et al. (2015)	SGR	Blunt snout bream	2.35	0.02	2.37	0.03	6	Cornstarch
Tian et al. (2004)	WG	Grass carp	495.7	45.4	368.9	52.8	6	-
Tian et al. (2010)	SGR	Grass carp	1.87	0.1	1.75	0.07	6	Feeding times 2
Tian et al. (2010)	SGR	Grass carp	2.23	0.03	1.99	0.02	6	Feeding times 6
Tian et al. (2010)	SGR	Grass carp	2.16	0.03	1.91	0.24	6	Feeding times12
Tian et al. (2010)	SGR	Grass carp	2.25	0.14	1.97	0.17	6	Feeding times 23[1]

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[1] Table continues next page

Author	Growth Indicators	Fish Species	Glucose	Glucose SD	Starch	Starch SD	N	Notes
			Omnivores					
Hung et al. (1989)	WG	White sturgeon	199.3	13.4	180.6	13.4	6	27.2% CHO
Lin et al. (1997)	SGR	White sturgeon	2.5	0.19	2.9	0.03	6	Continuous feeding
Lin et al. (1997)	SGR	White sturgeon	1.17	0.17	1.43	0.19	6	Feeding times 2
Lin et al. (1997)	SGR	Hybrid tilapia	1.75	0.05	2.05	0.02	6	Continuous feeding
Lin et al. (1997)	SGR	Hybrid tilapia	1.83	0.07	2	0.09	6	Feeding times 2
Qiang et al. (2014)	SGR	Nile tilapia	3.54	0.07	3.61	0.12	6	-
Shiau and Peng. (1993)	WG	Hybrid tilapia	353.85	30.1	480.12	31.42	8	32% protein
Shiau and Peng. (1993)	WG	Hybrid tilapia	339.39	21.11	392.89	41.21	8	28% protein
Shiau and Peng. (1993)	WG	Hybrid tilapia	313.31	28.2	365.6	34.23	8	24% protein
Tung and Shiau. (1991)	WG	Hybrid tilapia	225.15	19.21	246.29	15.66	6	Feeding times 2
Tung and Shiau. (1991)	WG	Hybrid tilapia	270.78	26.02	288.41	7.57	6	Feeding times 6
Wilson and Poe (1987)	WG	Channel catfish	206	35.16	324	16.97	6	-

SGR: specific growth rate; WG: weight gain; SD: standard deviation; N: sample size.

Table 7.2 Raw data of growth comparison between hydrothermally processed starch versus glucose in all fish species

Author	Growth indicator	Fish species	Glucose	Glucose SD	Starch	Starch SD	N	Notes
Cui et al. (2010)	WG	Cobia	109.30	9.70	820.50	19.10	6	Extrusion cornstarch
Cui et al. (2010)	WG	Cobia	109.30	9.70	854.90	9.30	6	Extrusion wheat starch
Deng et al (2005)	SGR	Juvenile white sturgen	0.22	0.10	2.33	0.09	6	Hydrolyzed potato starch /15% CHO
Deng et al (2005)	SGR	Juvenile white sturgen	0.02	0.04	2.86	0.13	6	Hydrolyzed potato starch /30% CHO
Enes et al. (2010)	SGR	Gilthead sea bream	0.72	0.05	0.76	0.01	6	Pre-maize starch
Erfanullah and Jafri. (1999)	SGR	Heteropneustes fossilis	1.66	0.07	2.22	0.07	6	Precooked cornstarch
Fu (2005)	SGR	Southern catfish	3.14	0.44	3.60	0.32	8	Precooked cornstarch 15% CHO
Fu (2005)	SGR	Southern catfish	1.47	0.38	3.09	0.42	8	Precooked cornstarch 30% CHO
Stone et al. (2003)	SGR	Juvenile silver perch	1.40	0.21	1.73	0.35	6	Gelatinized wheat starch

CHO: carbohydrate; SGR: specific growth rate; WG: weight gain; SD: standard deviation; N: sample size.

Table 7.3 Raw data of growth comparison between raw starch versus dextrin in all fish species

Author	Growth Indicators	Fish Species	Dextrin	Dextrin SD	Starch	Starch SD	N	Notes
Carnivores								
Hung and Storebakken (1994)	SGR	Rainbow trout	1.24	0.05	1.21	0.05	6	Continuous feeding
Hung and Storebakken (1994)	SGR	Rainbow trout	1.18	0.05	0.88	0.05	6	Meal feeding
Jiang et al. (2014)	WG	Amur sturgeon	716.00	25.30	782.90	71.90	6	α-starch
Jiang et al. (2014)	WG	Amur sturgeon	716.00	25.30	582.50	14.80	6	Cornstarch
Shapawi et al. (2011)	SGR	Humpback Grouper	1.30	0.03	1.20	0.05	6	Tapioca starch/10% CHO
Shapawi et al. (2011)	SGR	Humpback Grouper	1.40	0.05	1.30	0.02	6	Tapioca starch/20% CHO
Shapawi et al. (2011)	SGR	Humpback Grouper	1.30	0.03	1.20	0.05	6	Cornstarch/10% CHO
Shapawi et al. (2011)	SGR	Humpback Grouper	1.40	0.05	1.30	0.05	6	Cornstarch/20% CHO
Stone et al. (2003)	SGR	Juvenile silver perch	1.60	0.36	1.47	0.05	6	Raw wheat starch
Stone et al. (2003)	SGR	Juvenile silver perch	1.60	0.36	1.50	0.29	6	Raw pea starch
Stone et al. (2003)	SGR	Juvenile silver perch	1.77	0.22	1.47	0.05	6	Raw wheat starch
Stone et al. (2003)	SGR	Juvenile silver perch	1.77	0.22	1.50	0.29	6	Raw pea starch
Stone et al. (2003)	SGR	Juvenile silver perch	1.60	0.17	1.47	0.05	6	Raw wheat starch
Stone et al. (2003)	SGR	Juvenile silver perch	1.60	0.17	1.50	0.29	6	Raw pea starch
Herbivores								
Ren et al. (2015)	SGR	Blunt snout bream	2.46	0.02	2.37	0.03	6	Wheat starch
Ren et al. (2015)	SGR	Blunt snout bream	2.46	0.02	2.37	0.03	6	Cornstarch
Omnivores								
Hung et al (1989)	WG	White Sturgeon	178.70	13.40	180.60	13.40	6	27.2% CHO[2]

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[2] Table continues next page

Author	Growth indicators	Fish species	Dextrin	Dextrin SD	Starch	Starch SD	N	Notes
Shiau and Peng. (1993)	WG	Hybrid tilapia	461.50	20.12	480.12	31.42	8	32% Protein in diet/ 33% CHO
Shiau and Peng. (1993)	WG	Hybrid tilapia	383.29	32.60	392.89	41.21	8	28% Protein in diet/ 28% CHO
Shiau and Peng. (1993)	WG	Hybrid tilapia	346.95	31.13	365.60	34.23	8	24% Protein in diet/ 41% CHO
Tung and Shiau. (1991)	WG	Hybrid tilapia	262.39	7.18	246.29	15.66	6	Feeding times 2
Tung and Shiau. (1991)	WG	Hybrid tilapia	294.11	13.66	288.41	7.57	6	Feeding times 6
Wilson and Poe (1987)	WG	Channel catfish	358.00	6.06	324.00	16.97	6	-

CHO: carbohydrate; SGR: specific growth rate; WG: weight gain; SD: standard deviation; N: sample size

Table 7.4 Raw data of growth comparison between hydrothermally processed starch versus dextrin in all fish species

Author	Growth indicators	Fish species	Dextrin	Dextrin SD	Starch	Starch SD	N	Notes
Cui et al. (2010)	WG	Cobia	864.10	9.70	820.50	19.10	6	Extrusion cornstarch
Cui et al. (2010)	WG	Cobia	864.10	9.70	854.90	9.30	6	Extrusion wheat starch
Enes et al. (2010)	SGR	Gilthead sea bream	0.79	0.05	0.76	0.01	6	Pre-maize starch
Erfanullah and Jafri. (1999)	SGR	Heteropneustes fossilis	2.25	0.10	2.22	0.07	6	Precooked cornstarch
Stone et al. (2003)	SGR	Juvenile silver perch	1.77	0.22	1.73	0.35	6	Gelatinized wheat starch
Stone et al. (2003)	SGR	Juvenile silver perch	1.60	0.36	1.73	0.35	6	Gelatinized wheat starch
Stone et al. (2003)	SGR	Juvenile silver perch	1.60	0.17	1.73	0.35	6	Gelatinized wheat starch

SGR: specific growth rate; WG: weight gain; SD: standard deviation; N: sample size.

Table 7.5 Raw Data of diets with different amylose and amylopectin content

Author	Growth indicators	Fish species	Amylose (%)	Experiment	SD	Control	SD	N
Liu et al. (2014)	WG	Obscure puffer	12	217.00	5.00	199.00	4.00	6
Chen et al. (2013)	WG	Tilapia	19.4	659.70	35.58	606.70	35.58	6
Liu et al. (2014)	WG	Obscure puffer	24	223.00	5.00	199.00	4.00	6
Enes et al. (2005)	SGR	European sea bass	28	1.15	0.04	1.18	0.03	6
Enes et al. (2005)	SGR	European sea bass	28	1.23	0.03	1.19	0.07	6
Enes et al. (2008)	SGR	Gilthead sea bream	28	1.24	0.08	1.17	0.07	6
Enes et al. (2008)	SGR	Gilthead sea bream	28	1.25	0.10	1.30	0.06	6
Sa et al. (2008)	SGR	White sea bream	28	1.05	0.06	1.02	0.08	6
Sa et al. (2008)	SGR	White sea bream	28	1.00	0.04	0.95	0.06	6
Rawles et al. (2003)	WG	Sunshine Bass	30	205.20	7.26	221.60	7.26	6
Chen et al. (2013)	WG	Tilapia	32	530.00	35.58	606.70	35.58	6
Liu et al. (2014)	WG	Obscure puffer	38	201.00	6.00	199.00	4.00	6
Chen et al. (2013)	WG	Tilapia	43.2	490.90	35.58	606.70	35.58	6
Liu et al. (2014)	WG	Obscure puffer	48	186.00	2.00	199.00	4.00	6
Chen et al. (2013)	WG	Tilapia	49.5	479.70	35.58	606.70	35.58	6
Gatesoupe et al. (2014)	SGR	Sea bass	70	0.61	0.03	0.58	0.03	6
Rawles et al. (2003)	WG	Sunshine Bass	70	235.80	7.26	221.60	7.26	6

SGR: specific growth rate; WG: weight gain; SD: standard deviation; N: sample size.