Antinutritional factors in modeling plant-based rainbow trout diets

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By

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ABSTRACT

The effect of inclusion rate of pea meal (PM), pea protein concentrate (PPC), soybean meal (SBM), soy protein concentrate (SPC), canola meal (CM) and canola protein concentrate (CPC) in salmonid diets was determined through six corresponding meta-analyses of all data available in the literature for these six feed ingredients, which was followed by weighted regression analysis. Increasing dietary inclusion levels of SBM, SPC, CM and CPC reduced specific growth rate (SGR). Regression analysis determined all of these relationships to be linear declines in SGR ($P < 0.05$). Inclusion levels of PM or PPC did not influence salmonid SGR ($P > 0.05$). These results showed that the influence plant proteins have on salmonid SGR is dependent on ingredient type and inclusion level.

PM, PPC, SBM, SPC, CM an aqueous-extracted CPC and a high phytate CPC (PCPC) were analyzed for chemical nutrient (proximate, amino acid and phosphorus analysis) and antinutrient composition and total tract digestibility (two separate digestibility trials) in rainbow trout (*Oncorhynchus mykiss*). The digestibility of proximate chemical components and amino acids were significantly higher for the soy products than the pea products. These digestibilities were also significantly higher in protein concentrates than in plant meals. Dry matter and gross energy digestibility was higher in CPC than in CM ($P < 0.05$). Phosphorus digestibility was higher in CPC-fed fish than in CM-fed fish ($P < 0.05$), which is likely due to the fact that CPC did not contain phytic acid.

Six consecutive growth studies (one trial per test ingredient) were conducted over a 361-day period to determine the effects of feeding increasing inclusion rates of PM, PPC, SBM, SPC, CM and CPC on the growth performance of rainbow trout. Diets were formulated based on the digestible nutrient content of all ingredients as determined in the previously conducted digestibility trials, to contain 0, 75, 150, 225 or 300 g/kg of each test ingredient. All diets were nutritionally equal and contained 17.6 MJ/kg digestible energy, 386.2 g/kg digestible crude protein and were balanced for digestible essential amino acids to meet or exceed the requirements of rainbow trout. Linear and quadratic analysis was conducted on the experimental data. There were no significant regressions resulting from feeding PM, SPC or CPC at 0-300 g/kg for average daily feed intake (ADFI), specific growth rate (SGR), feed conversion ratio (FCR) or protein efficiency ratio (PER). A positive relationship was associated between PPC inclusion and ADFI ($P < 0.05$). There was a significantly negative quadratic equation associated with the inclusion level of SBM on SGR and FCR and significantly negative linear and quadratic equations for PER. There were significantly negative linear relationships between the inclusion rate of CM and the SGR, FCR and PER of rainbow trout ($P < 0.05$). Growth trial results suggest at inclusion levels up to 300 g/kg, PM, PPC, SPC and CPC are feasible plant-based fish meal replacements with predictable growth effects, provided the nutritional constraints set in this experiment are followed.

The results of these growth experiments were further analyzed using structural equation modeling to determine the relationship between ANF in the six ingredients and ADFI and SGR, which were transformed (tSGR and tADFI, respectively) to enable comparisons between experiments. All possible models between ingredient ANF (starch,

phytic acid, glucosinolates, tannins, isoflavones, total NSP, soluble NSP, insoluble NSP and saponins) and ADFI and SGR were calculated. The model with the highest likelihood, as determined by the Akaike Information Criteria$_{0,}$ contained 29 parameters and six degrees of freedom. tADFI positively influenced tSGR. Glucosinolates, saponins, and phytates had a significantly negative impact on tADFI, whereas tannins had a significantly positive impact. The presence of saponins in the diet resulted in a decrease in tSGR. This structural equation model had significant correlations between all ANF, with the exception of phytates and saponins. Future applications of this work will be to develop a nutritional model for optimal inclusion of plant-based feed ingredients in rainbow trout diets, based on their ANF content, which may improve the accuracy of diet formulation and growth prediction.

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DEDICATION

Dedication is a requirement for any graduate student hoping to make it through to the end of their study program. Because of that, I dedicate this thesis to all of my fellow graduate student friends and colleagues. People who know what it means to trade late nights studying for time with friends. People who have spent early mornings in the barn, weekends in the lab, and come up with inappropriate conversation topics in the lunch room. People I feel honoured and blessed to have shared this educational journey with.

I also dedicate it to my family, who has been more than flexible with holiday dates, helped me in the barn on weekends and didn't mind me sitting on their furniture when I smelled like fish feed. I'm glad you're mine.

So long, and thanks for all the fish.

-Douglas Adams

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
AEE	Acid ether extract
AIC	Akaike information criteria
AIC_0	Akaike information criteria of 0
BCE	Before current era
CD	Cohen's d
CM	Canola meal
CP	Crude protein
CPC	Canola protein concentrate
DM	Dry matter
GC	Gas chromatography
GE	Gross energy
HSI	Hepatosomatic index
HPLC	High performance liquid chromatography
NDF	Neutral detergent fibre
NSP	Non-starch polysaccharide
PCPC	High phytate canola protein concentrate
PM	Pea meal
PPC	Pea protein concentrate
SBM	Soybean meal
SEM	Standard error of the mean

SGR	Specific growth rate
SPC	Soy protein concentrate
TGC	Thermal growth coefficient

1. INTRODUCTION

Sustainability is a vital issue the aquaculture industry is confronting. The current research focus is on identifying potential feed ingredients that are readily available, nutritionally adequate and financially feasible fish meal replacements. Between 2006 and 2011, the world's total fish stocks rose from 137.3 to 154.0 million tonnes, and human fish consumption rose from 114.3 to 130.8 million tonnes. This growth can be attributed to an increase in aquaculture production, which rose from 47.3 to 63.6 million tonnes. In comparison, the contribution of capture fisheries to the world's fish supplies remained stable around 90 million tonnes (FAO, 2012). This is due to industry-supported fishery controls designed to manage and maintain wild fish stocks, although the sustainability of all fisheries has not yet been achieved (Shepherd et al., 2005; Pauly et al., 2002).

Traditional aquaculture feeds are high in fish meal (herring, anchovy, menhaden) supplied by capture fisheries (Ardura et al., 2012, Hevrøy et al., 2004; Li et al., 2004). Originally, Naylor et al. (2000) predicted the growth of aquaculture would lead to an increased demand for fish meal. However, due to the rising cost of fish meal and limits on capture fishery production, it is infeasible to increase production of aquafeeds and maintain these high levels of fish meal use. Tacon et al. (2008) predicted fish meal inclusion in aquafeeds would decrease and be used more strategically for fish at particular life stages, such as starter, finisher, and broodstock feeds. This forecast may be closer to reality as fish meal inclusion in aquaculture diets increased steadily until 2005, where it peaked at 4.23 million tonnes. It has since begun a steady decline and the FAO (2012) expect it will drop to 2.49 million tonnes by 2020.

As replacements for marine products in aquafeeds, a number of plant-based ingredients are currently in use while many others are undergoing research for their potential for future application. These include, but are not limited to, soybeans, peas, canola/rapeseed, lupins, flax/linseed and cottonseed meal (Francis et al., 2001). In carnivorous fish, particularly industrially significant fish species, such as salmonids, there are direct nutritional consequences seen when plant proteins are fed in place of marine ingredients. Some of these include decreased nutrient digestibility and growth performance and impairment of intestinal health, function and microbial populations (Desai et al., 2012; Mansfield et al., 2010; Barrows et al., 2007; Krogdahl et al., 2003; Bakke-McKellep et al., 2000; Baeverfjord and Krogdahl, 1996).

In terrestrial monogastric animals, such as swine and poultry, predictive growth modeling is developed to a level where it is possible to predict animal growth based on the dietary digestible nutrient composition. However, in salmonid fish, dietary formulation considering the same nutritional parameters with similar feed ingredients shows that additional considerations beyond digestible nutrient composition are required in the formulation process. A primary difference is that salmonids are carnivores, whereas pigs and chickens are omnivores. In carnivores and omnivores, a plant protein and its chemical components can have markedly different effects.

In salmonid diets, although its inclusion levels have decreased over the years, fish meal remains a dietarily essential feed ingredient. This is evidenced by the reduction in growth seen in studies where fish meal is fully or nearly all replaced by plant protein (Penn et al., 2011; Alami-Durant et al., 2010; de Francesco et al., 2004; Gomes et al., 1995). This is not true for all inclusion levels. Alami-Durant et al. (2010), for example,

observed a reduction in rainbow trout growth only when fish meal was replaced with plant meal at levels above 50%. In some studies (Carter and Hauler, 2000; Oliva-Teles et al., 1994), replacement of fish meal with plant protein led to improvements in fish growth, but this may be contingent on the quality of the fish meal that is replaced (Mundheim et al., 2004).

The difficulties associated with fully replacing fish meal with plant protein in salmonid diets may be due to a requirement for some of the low molecular weight compounds present in fish meal and animal protein sources but absent or present at low levels in plant proteins (Aksnes et al., 2006c). Some of these known low molecular weight compounds include nucleic acids, carnosine, anserine, free amino acids, and taurine, an amino acid that is also essential in the diet of cats, another obligate carnivore (Li et al., 2009; Aksnes et al., 2006c; Gaylord et al., 2006; Li and Gatlin, 2006; Burrells et al., 2001a, b; Morris et al., 1990). This additional requirement complicates dietary formulation beyond what is required in terrestrial animal nutrition as requirement levels and alternative sources have yet to be defined.

The plant sources that are most commonly used in aquafeeds are often selected based on their protein and amino acid content. Soybean meal (SBM), for example, is high in protein and has a similar amino acid balance to fish meal (El-Saidy and Gaber, 2002; Hardy, 1999). In addition to the absence of the low molecular weight compounds discussed above, a differing factor between fish meal and the plant proteins used to replace it are the abundance of additional compounds present in the plant that serve storage, metabolic and protective roles (Bennett and Wallsgrove, 1994). Although they are useful to the plants that produce them, in terms of aquaculture nutrition, these

additional compounds are antinutritional factors (ANF) due to the negative effect they have on fish production and health. The effects of these ANF can vary. Some effects include binding nutrients, altering metabolic processes, decreasing intestinal enzyme activity, causing intestinal damage and reducing nutrient absorption (Kraugerud et al., 2007; Francis et al., 2001). Processing treatments, such as air classification, solvent extraction, heat treatment or the addition of enzymes can be used to increase the nutritional value of plant proteins by reducing or deactivating these ANF, although not all methods target the same ANF (Penn et al., 2011; Barrows et al., 2007; Drew et al., 2007; Storebakken et al., 2000, 1998).

The type and quantity of ANF in a specific aquafeed varies, as it is dependent on the ingredients used and their dietary inclusion level. Because these ingredients, thus the diets they are included in, typically contain more than one ANF, it is difficult to ascertain whether the previously mentioned deleterious effects of feeding ANF to fish are due to a specific ANF or if they are due to additive effects of (or interactions between) a number of different ANF. Further research in this area is necessary to determine which ANF are specifically responsible.

Studies involving the effects of feeds and feed ingredients in aquaculture can be used to predict fish performance. These predictive studies are nutritional models based on how the fish respond to the dietary circumstances to which they have been subjected. Through the use of nutritional modeling, more accurate estimates with regards to feed requirements, feed utilization and waste output can be determined. In aquaculture nutrition studies, common models include regression analysis and dose-response models

to determine the effects of feed ingredients on growth and to determine nutrient requirements (Ytrestoyl and Bjerkeng, 2007; Krogdahl et al., 2003).

Provided enough nutritional information is available, nutritional modeling could be used to determine the complex relationships between the presence of multiple ANF in aquafeeds and their effect on fish growth and feed intake. This may be possible with the use of structural equation modeling, which can be used to describe interrelationships between observed data and unobserved, latent variables in the form of mathematical equations (Lamb et al., 2011; Schumacker and Lomax, 2010). With the current studies available in the literature, ingredient assessment studies vary in a number of different experimental methodologies. Due to differences in nutritional studies, primarily how experimental controls and design vary from study to study, researchers are limited in their ability to compare these results to develop predictive growth models for salmonid fish.

The objective of this thesis is to establish relationships between the dietary components of plant proteins and nutrient digestibility and growth in salmonids (with specific focus on rainbow trout).

2. LITERATURE REVIEW

2.1. Plant ingredients in salmonid aquaculture

Aquaculture has been used to cultivate fish since 2000-1000 BCE (Before current era), although the first known book on fish culture was not written until 475-473 BCE. Major advances in aquaculture occurred in 1700-1900, when advances in spawning technology allowed for the expansion of the aquaculture industry, and again in the 1970's, when aquaculture operations intensified their production while focusing on culturing profitable species (Rabanal, 1988). Another major advancement has been the development of aquafeeds that contain dietary components that deviate dramatically from the natural diets of fish. It has since evolved to the point where the nutritional needs of the fish can be met while feeding materials, such as land-based plant proteins, including soy, canola and peas, with the addition of amino acids, vitamins and minerals are used, even in carnivorous fish species, such as salmonids (NRC, 2011; Gatlin et al., 2007; NRC, 1993).

Downsides to replacing fish meal with plant proteins are the accompanying adverse growth and health effects. In numerous studies investigating the replacement of fish meal with alternative feed ingredients, it has been found that when large proportions of dietary fish meal are replaced with plant proteins, decreased growth performance is a consequence (Barrows et al., 2007; Lee et al., 2006; Drew et al., 2005; de Francesco et al., 2004; Glencross et al., 2004). In some cases, where fish meal is directly replaced with plant proteins without maintaining the same total digestible nutrient composition of all diets, these differences in growth may be partially attributed to differences in dietary nutrient availability. However, this does not account for all of the indicators of poor health also seen in fish.

SBM is particularly known for its adverse health effects when included in the diets of salmonid fish. One of the most commonly noted ailments is non-infectious subacute intestinal enteritis, which is a gastrointestinal disorder noted most markedly in the distal intestine (Krogdahl et al., 2003). Common features included shortening of the intestinal villi, rapid enterocyte turnover, and an infiltration of inflammatory cells in the lamina propria, whose presence is flagged by inflammatory markers such as proliferating cell nuclear antigen, immunoglobulin M, beta-actin and interleukin-1 beta. (Merrifield et al., 2009; Heikkinen et al., 2006; Krogdahl et al., 2003; Bakke-McKellep et al., 2000; Refstie et al., 2000; Burrells et al., 1999; Baeverfjord and Krogdahl, 1996). As non-infectious subacute intestinal enteritis is common in SBM-fed fish, it is also referred to as SBM-induced enteritis (Romarheim et al., 2013, 2011). This is not an entirely descriptive moniker as other plant proteins, such as PPC will also induce non-infectious subacute enteritis (Penn et al., 2011), which indicate these ingredients share a similar component not found in fish meal.

2.2. Antinutritional factors in plant-based feed ingredients

Plant-based feed ingredients often contain antinutritional factors (ANF), which can have detrimental effects when present in the diet (Francis et al., 2001). Antinutrients are primarily a result of storage, metabolic or protective mechanisms of the plant. Some of the consequences of feeding ANF to rainbow trout include reduced feed intake and growth, impaired nutrient metabolism, damage to the digestive tract and brush border enzyme activity, altered intestinal microflora and reproductive tract, organ (such as liver and thyroid) hypertrophy and increased mortality (Mansfield et al., 2010; Gontier-

Latonnelle et al., 2007; Tripathi and Mishra, 2007; Denstadli et al., 2006a; Ng et al., 2006; Krogdahl et al., 2003; Pereira et al., 2002; Bennetau-Pelissero et al., 2001; Francis et al., 2001).

It is not always clear which ANF are responsible for these deleterious effects. As plant proteins are used in aquaculture as fish meal replacements, any components differing from what is found in fish meal could potentially be considered an ANF. To identify which compounds are causative agents and which are nutritionally harmless, suspect ANF, such as phytic acid and saponins are fed to salmonids to determine whether a similar effect will be seen as when plant proteins containing these compounds are consumed (Sørensen et al., 2011; Denstadli et al., 2006a; Bureau et al., 1998).

Plant proteins can be processed into protein concentrates, which can have the benefit of removing or reducing some ANF, such as fibre (Forster et al., 1999; McCurdy and March, 1992; Thiessen et al., 2004). Other ANF require more ANF-specific targeting, such as the use of phytase to liberate phosphorus from phytic acid (Thiessen et al., 2004). Some ANF that are suspected to be problematic when included in salmonid diets include phytic acid, saponins, tannins, isoflavones, glucosinolates and dietary fibre.

2.2.1. Phytic acid

In plant tissues, phosphorus is stored as phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphate, or IP1, IP2, IP3, IP4, IP5 and IP6) or its salt form, phytate (myo-inositol-1,2,3,4,5,6-hexakis [dihydrogenphosphate]) (Raboy et al., 2001; Newkirk and Classen, 1998). Many of the plant meals used in aquaculture contain phytic acid, including soybeans, peas, canola, wheat, barley and oats (Lehrfeld, 1989).

In the form of phytic acid, phosphorus is not available for absorption by the gastrointestinal tract and passes undigested in the feces. It also interferes with gastric and intestinal proteases (Morales et al., 2011) and has a negative impact on amino acid digestibility (Kemme et al., 1999). At physiological pH, phytic acid chelates other minerals (other than phosphorus), such as calcium, magnesium, iron, copper and zinc, which can lead to deficiencies (Pallauf et al., 1998; Lehrfeld, 1989).

Through plant breeding strategies, it is possible to decrease the phytic acid of some crops while increasing their inorganic phosphorus levels, although these crop varieties have lower yields than their predecessors (Raboy, 2009). Some treatments include: heat treatment (Magoub and Elhag, 1998), soaking (Lestienne et al., 2005), sprouting/germination (Sokrab et al., 2012) and fermentation with lactobacilli, which is sometimes referred to as lactic acid fermentation (Palacios et al., 2008).

The most common and effective method to increase the available phosphorus in a plant-based animal feed is to treat it with phytase, a microbial enzyme. Supplementing organic acids, such as formic acid, in conjunction with phytase will further increase the available phosphorus in a diet (Jongbloed et al., 2000). Phytase is also present in some plant ingredients, such as wheat and barley, and improves phosphorus availability when included in pig diets. However, the dephosphorylation action of plant phytase takes place in the stomach (Kemme et al., 1998), which differs in temperature, size and retention time from rainbow trout. As phytase activity can be temperature-dependent, and the internal body temperature of fish such as rainbow trout is much lower than that of terrestrial animals, phytase activity may be impaired. Thus, it may more effective to

dephosphorylate phytic acid in an incubation step during feed processing, such as with the method used by Denstadli et al. (2006b).

2.2.2. Tannins

Tannins are phenolic compounds that complex with dietary components, such as proteins and starch (Mariscal-Landín et al., 2004; Francis et al., 2001; Chung et al., 1998), and minerals, such as iron, calcium, phosphorus, magnesium sodium, potassium and cobalt (Hassan et al., 2003, Al-Mamary et al., 2001, House and Van Campen, 1994) in the gastrointestinal lumen, decreasing their bioavailability. They also have an astringent flavor that negatively impacts diet palatability (Bravo, 1998; Kumar and Vaithiyanathan, 1990).

Tannins can be found in legumes such as cowpeas, groundbeans, field peas (yellow and green cotyledon) and soybeans, as well as in additional crops such as sorghum and canola (Khattab et al., 2010; Egounlety and Aworth, 2003; Wang et al., 1998; Fan et al., 1995; Chibber et al., 1978). As tannins are primarily found in the hull, dehulling is also effective at reducing tannins (Egounlety and Aworth, 2003; Chibber et al., 1978). Free tannins are solvent-extractable (Ping et al., 2011; Alipour and Rouzbehan, 2010; Chavan et al., 2001) and can be reduced during the production of protein concentrates, such as SPC and CPC, which employ solvent extraction processing methods. Tannins that are bound to protein and fibre require more extensive extraction procedures (Terrill et al., 1992).

2.2.3. Glucosinolates

Glucosinolates are secondary metabolites that play a protective role for the plants they occur in, acting as pesticides and defense against herbivores and pathogens (Bennett and Wallsgrove, 1994). There are approximately 120 known glucosinolates (Fahey et al., 2001). They can be found in cruciferous crops, particularly those in the *Brassica* genus (Tripathi and Mishra, 2007; Fenwick and Heaney, 1983).

Glucosinolates do not affect plant metabolism. They only cause active metabolic effects after cell breakdown and death, whereby the crushing of plant tissue activates the myrosinase-glucosinolate system (Grub and Abel, 2006). When activation occurs, myrosinase in the plant comes into contact with the glucosinolates, hydrolyzing these molecules (Bennett and Wallsgrove, 1994) to yield bioactive molecules such as thiocyanates, isothiocyanates, oxazolidone-2-thiones, epithioalkanes and nitriles, which are responsible for the sharp, bitter taste of glucosinolate-containing plants (Grub and Abel, 2006).

High levels of glucosinolates in the diet can have a goitrogenic effect, interfering with iodine uptake, in addition to reducing feed acceptance and growth and impairing liver and kidney function (Tripathi and Mishra, 2007; Pereira et al., 2002). In rainbow trout, glucosinolates impair growth and cause colloid goiter (Lanno and Dixon, 1996). In poultry, glucosinolates in the diet reduce growth and egg production and cause off-flavored eggs, enlarged thyroid glands and increased mortality (Tripathi and Mishra, 2007; Fenwick and Curtis, 1980). In pigs, glucosinolates impair growth and lead to enlarged thyroid glands and livers (Tripathi and Mishra, 2007; Schöne et al., 1988).

Burel et al. (2001) show the effects of glucosinolates can be reversed or at least reduced via dietary supplementation of iodine and monoiodothyronine. They also suggest these effects may not be as marked in fish raised in a marine environment, rather than fresh water, due to iodine supplied by the fishes' habitat. Schöne et al. (1988) were also able to improve the thyroid status of pigs fed high glucosinolate rapeseed meal by supplementing iodine in the diet. As dietary additives can counteract the negative dietary impact of glucosinolates, this may also be taken into consideration during diet formulation if negative growth effects are resulting from this ANF.

2.2.4. Saponins

Saponins, a compound found in snake venom (da Silva et al., 2007) are also present in alfalfa, soybeans, chickpeas, lentils and oats (Sparg et al., 2004; Francis et al., 2002). Saponins exist in a diverse range of forms. They are biologically active molecules that consist of a non-sugar steroid (primarily in monocotyledonous angiosperms) or triterpene (primarily in dicotyledonous angiosperms) aglycone bound to one, two or three sugar side-chains (Oleszek and Bialy, 2006; Sparg, 2004, Oleszek, 2002, Lacaille-Dubois and Wagner, 1996). Saponins are natural surfactants that foam in an aqueous environment, precipitate cholesterol, cause hemolysis of red blood cells and are toxic when injected (Knudsen et al., 2008, Sparg et al., 2004; Lacaille-Dubois and Wagner, 1996; Milgate and Roberts, 1995).

When saponins are consumed, they are less toxic, but do cause damage to the intestinal lumen. For poikilothermic animals, such as fish, saponins are considered toxins. Their presence increases the permeability of the gut wall (Iwashita et al., 2009; Sparg et

al 2004; Francis et al., 2002; Gee et al., 1996; Önning et al., 1996; Johnson et al., 1986), leaving it vulnerable to pathogens. This increase in gut permeability, in addition to morphological changes in the intestinal tract, is referred to as non-infectious subacute intestinal enteritis. It is accompanied by a reduction of growth when saponins are fed to a number of fish species, including salmonids (Chen et al., 2011; Sørensen et al., 2011; Refstie et al., 2010; Knudsen et al., 2008; Urán et al., 2008; Bureau et al., 1998), as well as a decrease in feed intake when fed to Chinook salmon and rainbow trout (Bureau et al., 1998).

2.2.5. Non-starch polysaccharides

Non-starch polysaccharide (NSP) is a term used for dietary fibre, which refers to all polysaccharides except starches (alpha-glucans) (Sinha et al., 2011). NSPs include cellulose, non-cellulosic polymers (arabinoxylans, mannans, xyloglucans, mixed link beta-glucans) and pectic polysaccharides (arabinans, galactans, arabinogalactans) (Sinha, 2011; Meng and Slominski, 2005). NSP can also be classified based on whether or not they are soluble in water (Englyst et al., 1994). Plants tend to contain both, although generally in different proportions. Insoluble NSP fibre has a laxative effect and can be found in higher proportions in wheat, barley, rice, beans and peas. Soluble NSP fibre can be found at high levels in carrots, oranges and oats and has the effect of lowering plasma cholesterol (Englyst, 1989; Topping, 2007).

In monogastric animals, NSP increase gut viscosity, which slows the passage rate of intestinal contents, impairs gut function (physiology, morphology, microbial population) and reduces nutrient digestibility (Sinha et al., 2011; Glencross et al., 2009;

Owusu-Asiedu et al., 2006; Meng and Slominski, 2005; Iji et al., 2001; Refstie et al., 1999).

2.2.6. Isoflavones

Isoflavones are secondary metabolites that protect the plant against fungal infection and consumption by herbivores (Want et al., 2006). Isoflavones can be found in legumes such as alfalfa, pea and chickpea (Wang et al., 2006, Berhow, 2002), although the majority of the studies involving plant isoflavones that are available in the literature primarily focused on those found in soy. The three isoflavones of primary interest in soy are genistein (4′,5,7-trihydroxyisoflavone), daidzein (4′,7-dihydroxyisoflavone) and glycitein (4′,7-dihydroxy-6-methoxyisoflavone). These three isoflavones also occur as malonyl-glucoside conjugates (ie. 6”-O-malonyl-7-O-glucosyl-genestein) and glucoside conjugates - daidzin (7-O-glucosyl-daidzein), glycitin (7-O-glucosyl-glycitein) and genistin (7-O-glucosyl genistein) (Berhow, 2002; Barnes et al., 1994; Ng et al., 2006).

Isoflavones have antioxidative properties (Dixit et al., 2012, Rüfer and Kulling, 2006), anti-inflammatory capabilities (Droke et al., 2007) and modulate inflammatory signaling pathways (Dijsselbloem et al., 2004; Kim et al., 1998). The chemical structure of isoflavones is similar to that of estrogen. Depending on the isoflavone and the system they are acting on, they can elicit estrogenic or antiestrogenic effects (Federici et al., 2006; Hwang et al., 2006). In sturgeon, and less markedly, in salmonids, isoflavones increase gametogenesis (Gontier-Latonnelle et al., 2007, Bennetau-Pelissero et al., 2001). In salmonids, they have also been shown to block estrogen metabolism (Ng et al., 2006).

2.3. Nutritional modeling

Feeding rate, digestion, metabolism and accretion of energy and elements are all functions of aquaculture nutrition. Each accrued bit of information can increase the knowledge and understanding about the effects an individual ingredient and a complete diet have on specific fish and fish species. A deterring factor to replacing fish meal with plant proteins in salmonid diets while maintaining the same level of growth is the fact that these plant-based feed ingredients contain ANF that impair fish growth. Nutritional modeling may be a suitable method of introducing novel feed ingredients to salmonid feeds by including the additional challenges associated with ANF in the formulation process. The basis of nutritional models varies between studies.

2.3.1. Mass balance

Mass balance techniques used in nutritional modeling investigate matter entering and leaving a system and take into account the difference. The input-output model (an elemental mass balance model) can be used to model factors such as food conversion efficiency and waste outputs. The model compares which components (and their levels) of a given feed are utilized for growth and which are lost as waste (Johansson and Nordvarg, 2002).

Input-output modeling can be used to determine the nutrient quality of an ingredient on a digestible nutrient basis. Common digestibility studies calculate the digestibility of nutrients in each ingredient based on solid waste output from the fish, often with the use of an indigestible marker to determine apparent digestibility of the ingredient (Thiessen et al., 2003). A diet formulated using digestible nutrients, such as

digestible amino acid and energy values will more efficiently make use of the resources the feed ingredients provide, without over- or under-utilizing what is available.

It should be noted that the commonly used digestibility calculations used in aquaculture studies do not truly represent total waste output. Examination of total waste excretion includes both solid and soluble output. Determining soluble waste excretion includes measuring waste nitrogen passed through the urine and via gill transfer (Lauff and Wood, 1996).

2.3.2. Energy flow

Feed energy is needed to maintain life processes and is used for osmoregulation, respiration, circulation and swimming. Formation of wastes also costs nutrient and feed energy. This must also be accounted for, not just the deposition of body components necessary for growth. Bio-energetics can be used to illustrate how fish derive nutrients and energy from their diets, with nutrients as standardized values with a common factor and unit. This allows feeding and growth to be described in terms of energy flows. Bio-energetics approaches used in the study of fish nutrition include investigating energy and nutrient retention and respirometry. This can allow growth, feed utilization and oxygen consumption to be modeled (McDonald et al., 1996, Zhou et al., 2005).

As growth rates vary, so do feed requirements. For every set of information gathered in the nutritional modeling process, this information is garnered for a narrow set of conditions. It is specific to age, species, genetics, size, diet, feed composition, water temperature, lighting, day length, production system, stocking density. Changes in feeds,

fish numbers, environmental factors, such as temperature all can have an effect on production data with two separate sets resulting from these changes.

2.3.3. Dose response models

Statistical evaluations of performance of fish fed increasing levels of ingredients or nutrients are carried out using a number of statistical models, including analysis of variance (ANOVA), the broken line model (BLM), the broken saturated kinetics model (BSKM) and the broken convex curve model (BCCM) (Hernandez-Llamas, 2009). There is debate as to which of these models are most suitable in nutritional modeling for aquafeeds, and as such, all are found in the literature, although ANOVA and the broken line model are most commonly used.

These models all operate by forming a curve resulting from increasing levels of an ingredient or nutrient until changes as a result of these increasing levels reach a plateau. This plateau point shows the minimum inclusion level of an ingredient required to produce the maximum positive effects on performance as a result of this ingredient. Accuracy of such plateau points is sometimes argued as actual values must in some cases be assumed based on inferences (Hernandez-Llamas, 2009).

Such models are commonly utilized to determine nutrient requirements, such as for vitamins and minerals (Mohamed et al., 2003; Skonberg et al., 1997). These models are also used to illustrate the relationship between a feed ingredient and growth, which is commonly expressed as a regression analysis (Krogdahl et al., 2003). Additionally, Ytrestoyl and Bjerkeng (2007) used a dose response model to investigate uptake of astaxanthin in Atlantic salmon.

2.3.4. Structural equation modeling

Structural equation modeling, more specifically, latent variable modeling is useful for studies where it is difficult to obtain direct empirical results (Lamb et al., 2011; Yeh et al., 2010; Lamb and Cahill, 2008, Golob, 2003, Baumgartner and Homburg, 1996). With the use of structural equation modeling, the interrelationships (direct and indirect) between different variables can be identified and expressed in the form of mathematical equations (Lamb et al., 2011).

2.4. Meta-analysis

Meta-analyses are statistical studies commonly utilized in the medical field. They are all-encompassing searches that thoroughly explore a specific scientific topic by comparing and assimilating all available research in that subject area (DerSimonian and Laird, 1986). Results are converted to a numerical index, or effect size that assigns a single value to the difference between results seen in a treatment group and those seen in the control group for the study. In this way, results can be compared among studies (Hedges and Vevea, 1998). To ensure unbiased results, meta-analyses are often planned and performed in accordance with a number of predetermined guidelines and standards (Higgins and Green, 2008; Moher et al., 2007; Moher et al., 1999).

2.5. Hypothesis

High levels of ANF, as well as high dietary inclusion levels of plant proteins containing ANF, will negatively affect nutrient digestibility and fish growth, although these effects

will be dependent on the type and dose of the ANF fed, as well as the level of processing undergone by the feed ingredient to remove specific ANF. A study of these relationships will lead to the formation of nutritional models that explain fish performance when different levels of plant-based feed ingredients and ANF are fed, and can be used to predict rainbow trout growth and feed intake under certain dietary conditions.

2.6. Objectives

The objectives of this study were to determine the chemical composition of six model ingredients (PM, PPC, SBM, SPC, CM, CPC) and their nutrient digestibility in rainbow trout, the effect of these ingredients on the growth performance of rainbow trout at individual inclusion levels of 0, 75, 150, 225 and 300 g/kg and the relationships between the antinutrients in these ingredients and fish growth and produce a model that explains the growth response of fish as a result of the quantity of specific antinutrients in the diet.

3. EFFECT OF DIETARY PLANT PROTEIN INCLUSION ON GROWTH RATE IN SALMONIDS: META-ANALYSIS OF SOYBEAN, PEA AND CANOLA / RAPESEED MEALS AND PROTEIN CONCENTRATES

This chapter was published in Aquaculture and is cited as: Collins, S.A., Øverland, M., Skrede, A., Drew, M.D. 2013. Effect of plant protein sources on growth rate in salmonids: meta-analysis of dietary inclusion of soybean, pea and canola/rapeseed meals and protein concentrates. Aquaculture. 400-401: 85-100. It is included in this thesis with permission from Elsevier. The purpose of this study was to use advanced statistical techniques to examine and compare all growth data available in the literature for salmonid fish fed pea, soybean and canola / rapeseed meals and protein concentrates. Through the use of these six meta-analyses, results were standardized, which allowed direct comparisons among studies, and regression analysis was conducted to compare growth results of fish fed these feed ingredients at varying dietary inclusion levels. This study produces an initial set of growth models that allows prediction of salmonid growth when fed these plant proteins. Further work in this manuscript will incorporate information revealed in this chapter with additional data, such as ingredient ANF composition to determine further information on why these plant proteins may have had varying effects on salmonid growth performance.

3.1. Abstract

Six parallel meta-analyses were conducted to determine the effect of the dietary inclusion rate of pea meal (PM), pea protein concentrate (PPC), soybean meal (SBM), soy protein concentrate (SPC), canola/rapeseed meal (CM) and canola/rapeseed protein concentrate (CPC) on the specific growth rate (SGR) of salmonid fish. From 1794 growth studies involving the feeding of these six test ingredients to salmonid fish, 45 studies were selected for inclusion in the meta-analysis. The relationship between SGR and the dietary inclusion level of plant-based feed ingredients was calculated using Cohen's d (CD), which measures differences between control and experimental means. The results of these meta-analyses showed an increase in the dietary inclusion of SBM, SPC, CM and CPC (not PM or PPC) leads to a significant reduction in SGR. Weighted regressions of inclusion level for each test ingredient on effect size showed significant, negative linear relationships between SGR and dietary inclusions of SBM, SPC, CM and CPC. For PM and PPC, there was no significant relationship between SGR and inclusion rate. The results suggest that the effect of plant ingredients on growth performance of salmonids depends on the specific ingredients and their inclusion levels. The higher effect sizes observed when ingredients are fed at lower inclusion levels and lack of significant impact of feeding mixed diets suggest that feeding low levels of several ingredients might be beneficial.

3.2. Introduction

Depletion of wild fish stocks has led to the necessity of including plant-based ingredients in fish feeds. A wide variety of plant-based ingredients and their use in fish feeds have been investigated, including pulses, such as soybeans, peas, faba beans and lupins, as well as protein sources such as canola, rapeseed, flax and cottonseed meal. The general consensus of these studies is that replacing fish meal with plant products at high levels in salmonid diets will negatively impact growth (Barrows et al., 2007; Lee et al., 2006; Drew et al., 2005; de Francesco et al., 2004; Glencross et al., 2004; Leatherland and Hilton, 1998; de la Higuera et al., 1988). These studies, however, use different methodologies to assess ingredients. The use of controls and test ingredient inclusion levels vary, as do the ways in which growth is depicted, including average daily gain, specific growth rate (SGR) and thermal growth coefficient (TGC). SGR is a common growth criteria in fish studies, although the use of TGC is increasing. There are discrepancies among studies in the calculations of these parameters, as in some cases, when growth is reported as SGR, the correct logarithmic equation is not used. What remains constant, regardless of the calculation utilized, is the purpose of these growth reporting methods, which is to serve as indicators of the effect of the test treatment.

Several reviews have been conducted on the topic of feeding plant proteins to fish. In a meta-analysis, Sales (2009) investigated the effect of soybean meal (SBM) on different fish species and Enami (2011) reviewed the use of canola/rapeseed in fish feeds. These papers examine a single protein source, which makes comparisons between ingredients difficult. A review article by Francis *et al*. (2001) addresses this dilemma, although the focus is on the antinutritive properties of feed ingredients, rather than fish

growth. In a recent study, Hua and Bureau (2012) used meta-analysis and simulated data to examine the effect of plant proteins on TGC.

We investigated six plant-based fish feed ingredients by systematic review and meta-analysis, using a standardized methodology to determine the relationships between the dietary inclusion of these feed ingredients on growth in salmonids. The six ingredients chosen for this study are: pea meal (PM), pea protein concentrate (PPC), SBM, soy protein concentrate (SPC), canola/rapeseed meal (CM) and canola/rapeseed protein concentrate (CPC). These ingredients vary in their nutrient (Table 2.1) and antinutrient composition (Torstensen et al., 2008; Lee et al., 2006; Drew et al., 2005; Burel et al., 2000; Oliva-Teles et al., 1994; Hilton and Slinger, 1986). They were selected on the basis of available data and because they are recognized as commonly acceptable protein sources. All are used in practice and are included in many commercial salmonid diets. Protein concentrates from each of the three plant sources were selected to determine if feeding these ingredients affects salmonid growth differently from conventional meals.

The purpose of this meta-analysis is to: 1) Examine the completeness of the research related to the replacement of fish meal with PM, PPC, SBM, SPC, CM and CPC in salmonid diets and identify any information gaps. 2) Form a comprehensive illustration and comparison of all available data in the literature.

Table 3.2.1. Typical composition of plant protein sources included in this meta-analysis (values averaged from papers in each data set, when reported), dry matter basis.

	Pea meal	Pea protein concentrate	Soybean meal	Soy protein concentrate	Canola/rapeseed meal	Canola/rapeseed protein concentrate
Nutritional factors						
Gross energy (MJ/kg)			20		8	21
Dry matter (MJ/kg)	925	900	888	932	903	956
Crude protein (g/kg)	229	421	495	627	387	724
Lipid (g/kg)	15	33	21	14	38	
Ash (g/kg)	32	51	63	64	94	93
Phosphorus (g/kg)			6	8	16	
Source(s)	Thiessen et al. (2003)	Øverland et al. (2009); Thiessen et al. (2003)	Øverland et al. (2009); Refstie et al. (2005); Thiessen et al, (2003); Vielma et al. (2000); Leatherland and Hilton, (1998); Refstie et al. (1998); Davies et al. (1997); Oliva-Teles et al. (1994); Watanabe et al. (1993); Hardy and Sullivan (1983)	Vielma et al. (2000)	Shafaeipour et al. (2008); Satoh et al. (1998); Thiessen et al. (2003); Burel et al. (2000); Leatherland and Hilton (1998); Abdou Dade et al. (1990); Hardy and Sullivan (1983)	Thiessen et al. (2004)

* Values averaged from papers in each data set, when reported.

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Ash (g/kg)	32	51	63	64	94	93
Phosphorus (g/kg)			6	8	16	
Source(s)	Thiessen et al. (2003)	Øverland et al. (2009); Thiessen et al. (2003)	Øverland et al. (2009); Refstie et al. (2005); Thiessen et al, (2003); Vielma et al. (2000); Leatherland and Hilton, (1998); Refstie et al. (1998); Davies et al. (1997); Oliva-Teles et al. (1994); Watanabe et al. (1993); Hardy and Sullivan (1983)	Vielma et al. (2000)	Shafaeipour et al. (2008); Satoh et al. (1998); Thiessen et al. (2003); Burel et al. (2000); Leatherland and Hilton (1998); Abdou Dade et al. (1990); Hardy and Sullivan (1983)	Thiessen et al. (2004)

* Values averaged from papers in each data set, when reported.

3.3. Materials and Methods

3.3.1. Search strategy and inclusion criteria

Mix Version 2.0 (Bax, 2010) was used to conduct the meta-analyses following the guidelines provided by the Cochrane Handbook for Systematic Reviews of Interventions (Higgins and Green, 2008) and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (Moher et al., 2007). A review protocol was not registered. In January of 2012, study selection was conducted searching ISI WEB OF KNOWLEDGE (1899-2010) and SCIRUS (1800-2011) using the following search terms and Boolean operators: Topic = (canola OR pea OR peas OR rapeseed OR soy OR soya OR soybean) AND Topic = (char OR salmon OR trout) AND Topic = (growth OR SGR). These studies were separated based on ingredient type: PM, SBM, CM, PPC, SPC and CPC. Manual searches supplemented the database search strategy and unpublished data from our laboratories (Collins et al., 2012a,b; Drew, unpublished results) was included. To prevent selection bias, pre-specified inclusion criteria were: 1) random allocation of participants; 2) use of plant protein, not plant oil; 3) growth study; 4) use of salmonid fish species; 5) presence of a control group not fed the test ingredient; 6) written in English or French. Duplicate reports, reviews and conference proceedings were removed. Studies that included high glucosinolate, high euricic acid rapeseed meal were excluded. Only defatted SBM and CM were included in their respective meta-analyses. Studies investigating other main effects, such as the effect of adding phytase to plant-based diets were excluded, as were any studies where the test ingredient was included in the control diet. In cases where diets contained more than one test ingredient, results were analyzed separately for each individual test ingredient.

3.3.2. Data extraction

A standardized proforma was used to independently extract relevant data from each study. These data included information on: study design, sample size, species, test ingredient type and inclusion level. Additional requirements included the use of an appropriate control diet, specific growth rate (SGR) as the measure of growth or sufficient data to calculate SGR and a reported standard deviation (SD) or data sufficient to calculate SD.

3.3.3. Statistical analysis

SGR values reported are based on the following equation: $SGR = 100 * [(\ln W_1) - (\ln W_0)] / D$, where W_0 and W_1 represent initial and final weights (experimental unit means), respectively, and D represents the number of feeding days. Where trials reported growth as TGC, SGR was calculated by the authors using other growth information reported (Romarheim et al., 2006). In trials with factorial designs, only the growth data for the treatments fed were compared (Yamamoto et al., 2002). In cases where experimental diet formulation changed during the course of the experiment, data from the first experimental period (until the change in dietary formulation) was used in the analysis, as in following periods, start weights differed among treatments (Torstensen et al., 2008). If data were not separated, the entire experimental period was used to calculate SGR.

Standardized differences between control and experimental means were measured using Cohen's d (CD; Cohen, 1998), with a 95% confidence interval (CI) and an alpha level of $P < 0.05$. The following equation was used to calculate CD: $CD = (SGR_C - SGR_T) / SD_{pooled}$, where SGR_C and SGR_T represent the SGR of control diet-fed and test

diet-fed fish, respectively, and SD_{pooled} represents the pooled standard deviation for the two groups. Data were analyzed for normality according to z scores and normal quantile plot (all six data sets were normally distributed). *Q* index was used to assess heterogeneity, which was quantified by t^2. Heterogeneity and sampling error were taken into account by using a random-effects model to calculate summary statistics (Hedges and Vevea, 1998). Data were pooled and weighted according to DerSimonian and Laird (1986). Any study result found to have a weight within a meta-analysis less than 1% was identified as an outlier and removed from the data set, which was then subjected to a second meta-analysis.

Weighted linear and quadratic regression analysis of inclusion rate on CD was performed using PASW Statistics Standard Version 19.0. (Version 19.0.0, SPSS Inc., Chicago, IL.). Data for each ingredient was subjected to linear and quadratic regressions and the highest order model with the lowest *P* - value was reported.

3.3.4. Additional factors influencing specific growth rate

Other factors that could affect the results of a meta-analysis were analyzed, including: initial weight, fish species (Atlantic salmon or rainbow trout), water environment (salt or fresh), dietary processing conditions (cold-pressed, steam-pelleted or extruded), dietary nutrient balance (defined as control and test diets that were isonitrogenous or balanced for the same amino acids and isoenergetic with CP:GE ratios within 5% of one another - yes or no), the use of palatability enhancers (betaine, krill or soluble fish protein - yes or no), feeding regime, the use of blended diets (where one or more of the six ingredients investigated in this paper were present in the diets of a study used to obtain information

for another ingredient's data set - yes or no) and the use of mismatched diets (where the control and test diets did not contain the same ingredients as one another, excluding micronutrient supplementation - yes or no). Additionally, whether or not the test ingredient replaced fish meal in the control diet was explored. The following four possibilities were considered: 1) fish meal inclusion was the same in the control and test diet; 2) there was more fish meal in the control diet than in the test diet; 3) there was more fish meal in the test diet than in the control diet; 4) no fish meal was included in either diet. The aforementioned analyses were performed only where sufficient data were available. Data were divided into the defined groups and differences between CD were analyzed using the GLM procedure of PASW Statistics Standard Version 19.0. Differences were considered significant at an alpha level of $P < 0.05$.

3.4. Results

3.4.1. Study description

The trial selection process for this meta-analysis experiment is illustrated in Figure 2.6.1. Sixty-four randomized, controlled trials were identified, of which 19 were excluded for the following reasons: seven trials reported no measure of variation (Morris et al., 2005; Teskeredzic et al., 1995; Arndt et al., 1999; Gomes et al., 1995; Kaushik et al., 1995; Gomes et al., 1993; McCurdy and March, 1992), three trials had only one experimental unit per treatment (Dabrowski et al., 1989; Alexis et al., 1985; Hardy and Sullivan, 1983), one trial did not include growth information for fish fed the control diet (Brown et al., 2003), six trials did not provide enough information to calculate SGR (Pierce et al., 2008; Burrells et al., 1999; Sanden et al., 2006; Médale et al., 1998; Higgs et al., 1983, 1982), one trial changed ingredient inclusion levels in the individual test diets throughout the feeding period without recording weight between periods of diet alteration (Refstie et al., 2001) and one trial did not include ingredient inclusion rates in the diet specifications (Médale et al., 1998).

The inclusion criteria were met by 45 trials (Tables 2.6.2, 2.6.3, 2.6.4, 2.6.5, 2.6.6, 2.6.7). The sample sizes of these trials ranged from two to six experimental units / treatment (a total of 858 experimental units) and were reported between 1986 and 2011. Rainbow trout and Atlantic salmon were the species included. Inclusion rates ranged as follows: PM, 75 – 300 g/kg; SBM, 50 – 780 g/kg; CM, 47 – 500 g/kg; PPC, 75 – 350 g/kg, SPC, 75 – 637 g/kg; CPC, 60 – 527 g/kg.

There were no outliers identified in the studies involving PM, PPC and CPC. Seventeen of the results in the SBM data set (Table 2.6.4), two in the SPC data set (Table

2.6.5) and four in the CM data set (Table 2.6.6) were identified as outliers and removed from their respective data sets.

Of the 45 studies used for meta-analysis, 34 reported a decrease in SGR as the result of the dietary inclusion of the six plant-based feed ingredients, while 15 reported positive or neutral effects on SGR at some inclusion levels. Forest plots in Figures 2.6.2, 2.6.3, 2.6.4, 2.6.5, 2.6.6 and 2.6.7 show the pooled effect of individual ingredient inclusion on SGR.

Table 3.4.1. Data set for studies on the effect of dietary pea meal (PM) on the specific growth rate (SGR) of salmonid fish

Author	Species	PM (g/kg)	Treatment SGR	Treatment SD	Control SGR	Control SD	N
Collins et al. (2012)	rainbow trout	75	1.41	0.088	1.62	0.088	6
Alami-Durante et al. (2010)	rainbow trout	80	2.57	0.010	2.61	0.030	8
Alami-Durante et al. (2010)	rainbow trout	120	2.50	0.050	2.61	0.030	8
Drew et al. (2005)	rainbow trout	120	1.13	0.122	1.19	0.122	12
Drew et al. (2005)	rainbow trout	120	1.18	0.122	1.19	0.122	12
Collins et al. (2012)	rainbow trout	150	1.32	0.088	1.62	0.088	6
Alami-Durante et al. (2010)	rainbow trout	163	2.25	0.020	2.61	0.030	8
de Francesco et al. (2004)	rainbow trout	163	0.90	0.030	1.05	0.010	4
Collins et al. (2012)	rainbow trout	225	1.30	0.088	1.62	0.088	6
Thiessen *et al*. (2003)	rainbow trout	250	1.93	0.070	1.87	0.070	8
Collins et al. (2012)	rainbow trout	300	1.53	0.088	1.62	0.088	6

Table 3.4.2. Data set for studies on the effect of dietary pea protein concentrate (PPC) on the specific growth rate (SGR) of salmonid fish.

Author	Species	PPC (g/kg)	Treatment SGR	Treatment SD	Control SGR	Control SD	N
Collins et al. (2012)	rainbow trout	75	1.35	0.033	1.25	0.033	6
Gao et al. (2011)	rainbow trout	100	1.50	0.069	1.58	0.069	6
Gao et al. (2011)	rainbow trout	102	1.51	0.069	1.50	0.069	6
Gao et al. (2011)	rainbow trout	104	1.65	0.069	1.56	0.069	6
Penn et al. (2011)	Atlantic salmon	130	0.61	0.017	0.63	0.017	6
Collins et al. (2012)	rainbow trout	150	1.34	0.033	1.25	0.033	6
Øverland et al. (2009)	Atlantic salmon	200	1.21	0.087	1.18	0.087	6
Øverland et al. (2009)	Atlantic salmon	200	1.23	0.087	1.18	0.087	6
Thiessen et al. (2003)	rainbow trout	200	1.94	0.070	1.87	0.070	8
Carter and Hauler (2000)	Atlantic salmon	206	1.54	0.018	1.40	0.034	6
Moreno-Rojas et al. (2008)	rainbow trout	210	1.07	0.027	1.06	0.027	8
Collins et al. (2012)	rainbow trout	225	1.34	0.033	1.25	0.033	6
Carter and Hauler (2000)	Atlantic salmon	276	1.48	0.025	1.40	0.034	6
Collins et al. (2012)	rainbow trout	300	1.33	0.033	1.25	0.033	6
Penn et al. (2011)	Atlantic salmon	350	0.56	0.017	0.63	0.017	6

Table 3.4.3. Data set for studies on the effect of dietary soybean meal (SBM) on the specific growth rate (SGR) of salmonid fish.

Author	Species	SBM (g/kg)	Treatment SGR	Treatment SD	Control SGR	Control SD	N
Torstensen et al. (2008)	Atlantic salmon	50	0.99	0.030	1.00	0.030	6
Collins et al. (2012)	rainbow trout	75	0.62	0.068	0.55	0.068	6
Vielma et al. (2000)	rainbow trout	121	1.46	0.017	1.42	0.017	6
Torstensen et al. (2008)	Atlantic salmon	130	0.99	0.030	1.00	0.030	6
Torstensen et al. (2008)*	Atlantic salmon	130	0.88	0.020	1.00	0.030	6
Refstie et al. (2010)	Atlantic salmon	135	1.26	0.030	1.32	0.030	6
Selden et al. (2001)*	rainbow trout	141	2.92	0.001	2.85	0.001	6
Lee et al. (2002)*	rainbow trout	147	2.31	0.030	2.40	0.010	6
Lee et al. (2002)	rainbow trout	147	2.37	0.010	2.40	0.010	6
Lee et al. (2002)	rainbow trout	147	2.39	0.010	2.40	0.010	6
Collins et al. (2012)	rainbow trout	150	0.67	0.068	0.55	0.068	6
Refstie et al. (2005)	Atlantic salmon	153	1.31	0.035	1.34	0.035	6
Brinker and Reiter (2011)*	rainbow trout	175	1.16	0.006	1.23	0.005	6
Brinker and Reiter (2011)*	rainbow trout	175	1.17	0.009	1.24	0.009	6
Refstie et al. (2005)	Atlantic salmon	175	1.27	0.035	1.34	0.035	6
Barrows et al. (2007)	rainbow trout	190	1.83	0.119	2.13	0.128	6
Gao et al. (2011)	rainbow trout	192	1.51	0.069	1.50	0.069	6
Gao et al. (2011)	rainbow trout	195	1.65	0.069	1.56	0.069	6
Gao et al. (2011)	rainbow trout	195	1.50	0.069	1.58	0.069	6
Øverland et al. (2009)	Atlantic salmon	200	1.09	0.087	1.18	0.087	6
Satoh et al. (2003)	rainbow trout	200	1.49	0.060	1.53	0.030	6
Carter and Hauler (2000)*	Atlantic salmon	204	1.52	0.009	1.40	0.034	6
Oliva-Teles et al. (1994)*	rainbow trout	213	1.68	0.049	1.50	0.049	4
Collins et al. (2012)	rainbow trout	225	0.54	0.068	0.55	0.068	6
Barrows et al. (2007)	rainbow trout	231	1.94	0.115	2.06	0.110	6
Oliva-Teles et al. (1994)*	rainbow trout	236	1.63	0.049	1.50	0.049	4
Romarheim et al. (2006)*	rainbow trout	249	0.77	0.053	1.12	0.053	6
Watanabe et al. (1993)*	rainbow trout	250	2.95	0.039	2.78	0.039	4
Watanabe et al. (1993)*	rainbow trout	250	2.91	0.041	2.78	0.039	4
Watanabe et al. (1993)*	rainbow trout	250	2.88	0.039	2.78	0.039	4
Watanabe et al. (1993)	rainbow trout	250	2.82	0.041	2.78	0.039	4
Selden et al. (2001)*	rainbow trout	255	2.81	0.001	2.85	0.001	6
Carter and Hauler (2000)	Atlantic salmon	273	1.45	0.030	1.40	0.034	6
Refstie et al. (1998)	Atlantic salmon	281	1.39	0.007	1.43	0.029	6
Selden et al. (2001)	rainbow trout	287	2.81	0.001	2.85	0.001	6
Refstie et al. (2000)	Atlantic salmon	296	0.78	0.010	1.01	0.004	6
Refstie et al. (2000)	rainbow trout	296	1.04	0.013	1.06	0.011	6
Collins et al. (2012)	rainbow trout	300	0.32	0.068	0.55	0.068	6
Pongmaneerat and Watanabe (1993)*	rainbow trout	300	3.28	0.049	3.42	0.053	4
Pongmaneerat and Watanabe (1993)	rainbow trout	300	3.38	0.048	3.42	0.056	4
Pongmaneerat and Watanabe (1993)	rainbow trout	300	3.75	0.050	3.81	0.057	4
Watanabe et al. (1993)	rainbow trout	300	2.71	0.038	2.78	0.039	4
Refstie et al. (2005)	Atlantic salmon	308	1.22	0.052	1.34	0.035	6
Refstie et al. (2010)*	Atlantic salmon	320	0.97	0.030	1.32	0.035	6
Refstie et al. (1998)	Atlantic salmon	339	1.15	0.025	1.43	0.029	6
Selden et al. (2001)*	rainbow trout	428	2.81	0.001	2.85	0.001	6
Yamamoto et al. (2002)	rainbow trout	430	2.13	0.044	2.32	0.037	12
Yamamoto et al. (2002)	rainbow trout	430	2.26	0.035	2.32	0.037	12
Heikkinen et al. (2006)*	rainbow trout	450	1.60	0.033	2.99	0.066	10
Heikkinen et al. (2006)	rainbow trout	450	2.71	0.092	2.71	0.083	10

(*continued on next page*)

* Indicates any study results found to have a weight within the meta-analysis less than 1%, which were identified as outliers and removed from the data set.

Author	Species	SBM (g/kg)	Treatment SGR	Treatment SD	Control SGR	Control SD	N
(*continued from previous page*)							
Kraugerud et al. (2007)*	Atlantic salmon	463	0.52	0.002	0.95	0.002	4
Davies and Morris (1997)*	rainbow trout	600	1.34	0.028	1.57	0.028	4
Davies and Morris (1997)*	rainbow trout	600	1.28	0.028	1.57	0.028	4
Davies and Morris (1997)*	rainbow trout	600	1.30	0.028	1.57	0.028	4
Davies and Morris (1997)*	rainbow trout	600	1.36	0.028	1.57	0.028	4
Davies and Morris (1997)*	rainbow trout	600	1.46	0.028	1.57	0.028	4
Refstie et al. (1997)*	rainbow trout	600	1.49	0.033	1.77	0.025	6
Rumsey et al. (1994)*	rainbow trout	780	1.02	0.013	2.13	0.013	4

* Indicates any study results found to have a weight within the meta-analysis less than 1%, which were identified as outliers and removed from the data set.

Table 3.4.4. Data set for studies on the effect of dietary soy protein concentrate (SPC) on the specific growth rate (SGR) of salmonid fish.

Author	Species	SPC (g/kg)	Treatment SGR	Treatment SD	Control SGR	Control SD	N
Collins et al. (2012)	rainbow trout	75	0.72	0.059	0.74	0.059	6
Penn et al. (2011)	Atlantic salmon	105	0.61	0.039	0.63	0.039	6
Collins et al. (2012)	rainbow trout	150	0.71	0.059	0.74	0.059	6
Stickney et al. (1996)	rainbow trout	159	2.77	0.102	3.09	0.102	6
Collins et al. (2012)	rainbow trout	225	0.55	0.059	0.74	0.059	6
Barrows et al. (2007)	rainbow trout	242	1.90	0.116	2.13	0.128	6
Collins et al. (2012)	rainbow trout	300	0.60	0.059	0.74	0.059	6
Penn et al. (2011)	Atlantic salmon	300	0.58	0.039	0.63	0.039	6
Vielma et al. (2000)	rainbow trout	315	1.46	0.017	1.42	0.017	6
Stickney et al. (1996)	rainbow trout	318	2.77	0.102	3.09	0.102	6
Mambrini et al. (1999)	rainbow trout	320	1.61	0.025	1.59	0.025	6
Barrows et al. (2007)	rainbow trout	322	1.91	0.116	2.06	0.110	6
Brinker and Reiter (2011)*	rainbow trout	351	1.07	0.003	1.23	0.005	6
Brinker and Reiter (2011)	rainbow trout	351	1.10	0.026	1.24	0.009	6
Denstadli et al. (2007)	Atlantic salmon	426	0.90	0.060	1.00	0.049	6
Stickney et al. (1996)	rainbow trout	477	2.75	0.102	3.09	0.102	6
Storebakken et al. (1998)	Atlantic salmon	480	0.96	0.010	1.04	0.022	6
Mambrini et al. (1999)	rainbow trout	490	1.43	0.025	1.59	0.025	6
Storebakken et al. (2000)	Atlantic salmon	500	0.88	0.040	0.89	0.030	6
Rumsey et al. (1994)*	rainbow trout	570	1.57	0.013	2.13	0.013	4
Stickney et al. (1996)	rainbow trout	637	2.59	0.102	3.09	0.102	6

* Indicates any study results found to have a weight within the meta-analysis less than 1%, which were identified as outliers and removed from the data set.

Table 3.4.5. Data set for studies on the effect of dietary canola / rapeseed meal (CM) on the specific growth rate (SGR) of salmonid fish.

Author	Species	CM (g/kg)	Treatment SGR	Treatment SD	Control SGR	Control SD	N
Alami-Durante et al. (2010)	rainbow trout	47	2.57	0.010	2.61	0.030	8
Shafaeipour et al. (2008)	rainbow trout	50	2.00	0.100	2.00	0.200	6
Alami-Durante et al. (2010)	rainbow trout	75	2.50	0.050	2.61	0.030	8
Collins et al. (2012)	rainbow tout	75	0.63	0.070	0.73	0.070	6
Burel et al. (2001)	rainbow trout	100	1.86	0.253	1.76	0.077	6
Alami-Durante et al. (2010)*	rainbow trout	100	2.25	0.020	2.61	0.030	8
de Francesco et al. (2004)*	rainbow trout	100	0.90	0.030	1.05	0.010	4
Shafaeipour et al. (2008)	rainbow trout	100	1.90	0.100	2.00	0.200	6
Satoh et al. (1998)*	Chinook salmon	130	0.93	0.030	1.07	0.032	4
Hilton and Slinger (1986)	rainbow trout	135	2.61	0.056	2.70	0.059	8
Collins et al. (2012)	rainbow tout	150	0.63	0.070	0.73	0.070	6
Shafaeipour et al. (2008)	rainbow trout	150	2.00	0.200	2.00	0.200	6
Abdou Dade et al. (1990)	rainbow trout	200	1.67	0.014	1.67	0.014	4
Burel et al. (2001)	rainbow trout	200	1.77	0.049	1.76	0.077	6
Shafaeipour et al. (2008)	rainbow trout	200	2.00	0.200	2.00	0.200	6
Thiessen et al. (2003)	rainbow trout	200	1.87	0.070	1.87	0.070	8
Collins et al. (2012)	rainbow tout	225	0.49	0.070	0.73	0.070	6
Shafaeipour et al. (2008)	rainbow trout	250	1.90	0.200	2.00	0.200	6
Satoh et al. (1998)*	Chinook salmon	262	0.79	0.034	1.07	0.032	4
Hilton and Slinger (1986)	rainbow trout	269	2.49	0.061	2.70	0.059	8
Leatherland and Hilton (1998)	rainbow trout	269	2.56	0.050	2.66	0.052	6
Burel et al. (2000)	rainbow trout	300	2.21	0.129	2.36	0.121	6
Burel et al. (2001)	rainbow trout	300	1.79	0.085	1.76	0.077	6
Collins et al. (2012)	rainbow tout	300	0.48	0.070	0.73	0.070	6
Shafaeipour et al. (2008)	rainbow trout	300	2.00	0.200	2.00	0.200	6
Hilton and Slinger (1986)	rainbow trout	350	2.41	0.030	2.75	0.126	8
Hilton and Slinger (1986)	rainbow trout	350	2.41	0.103	2.75	0.126	8
Hilton and Slinger (1986)	rainbow trout	360	2.34	0.070	2.75	0.126	8
Hilton and Slinger (1986)	rainbow trout	404	2.38	0.033	2.70	0.059	8
Burel et al. (2001)	rainbow trout	500	2.00	0.140	2.36	0.121	6

* Indicates any study results found to have a weight within the meta-analysis less than 1%, which were identified as outliers and removed from the data set.

Table 3.4.6. Data set for studies on the effect of dietary canola / rapeseed protein concentrate (CPC) on the specific growth rate (SGR) of salmonid fish.

Author	Species	CPC (g/kg)	Treatment SGR	Treatment SD	Control SGR	Control SD	N
Thiessen et al. (2004)	rainbow trout	60	1.40	0.096	1.35	0.096	10
Collins et al. (2012)	rainbow trout	75	0.56	0.095	0.52	0.095	6
Thiessen et al. (2004)	rainbow trout	120	1.26	0.096	1.35	0.096	10
Collins et al. (2012)	rainbow trout	150	0.54	0.095	0.52	0.095	6
Thiessen et al. (2004)	rainbow trout	180	1.22	0.096	1.35	0.096	10
Thiessen et al. (2004)	rainbow trout	180	1.31	0.096	1.35	0.096	10
Drew et al. (2007)	rainbow trout	193	1.25	0.104	1.38	0.104	12
Collins et al. (2012)	rainbow trout	225	0.34	0.095	0.52	0.095	6
Stickney et al. (1996)	rainbow trout	259	2.96	0.102	3.09	0.102	6
Drew et al. (2007)	rainbow trout	290	1.24	0.104	1.38	0.104	12
Collins et al. (2012)	rainbow trout	300	0.34	0.095	0.52	0.095	6
Thiessen et al. (2004)	rainbow trout	330	2.17	0.213	2.21	0.213	8
Thiessen et al. (2004)	rainbow trout	330	2.15	0.213	2.21	0.213	8
Drew et al. (2007)	rainbow trout	386	1.19	0.104	1.38	0.104	12
Forster et al. (1999)	rainbow trout	416	1.86	0.072	1.94	0.072	6
Thiessen et al. (2004)	rainbow trout	490	1.98	0.213	2.21	0.213	8
Thiessen et al. (2004)	rainbow trout	490	2.18	0.213	2.21	0.213	8
Stickney et al. (1996)	rainbow trout	527	2.64	0.102	3.09	0.102	6

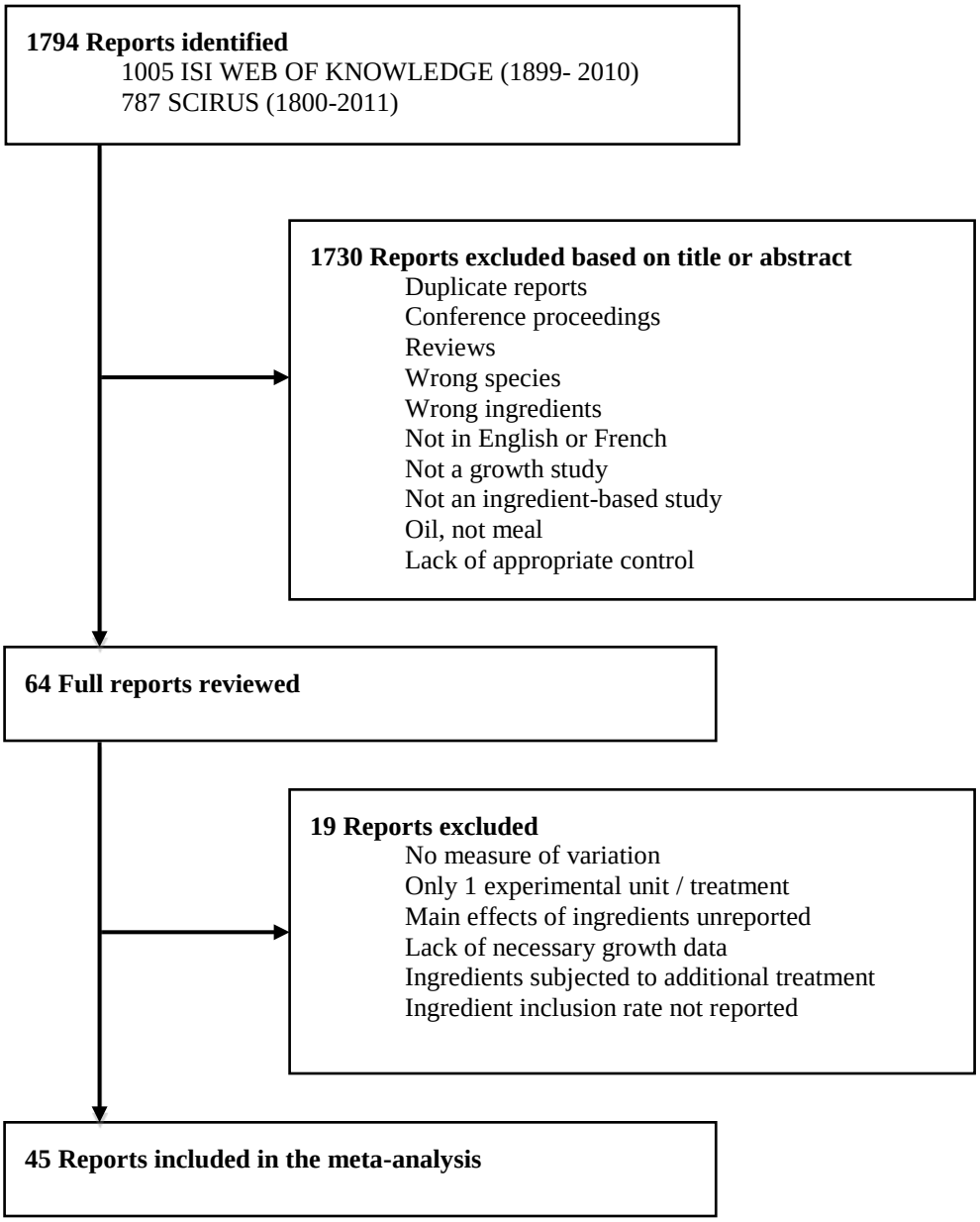

Figure 3.4.1. Flow diagram of study selection process for meta-analysis.

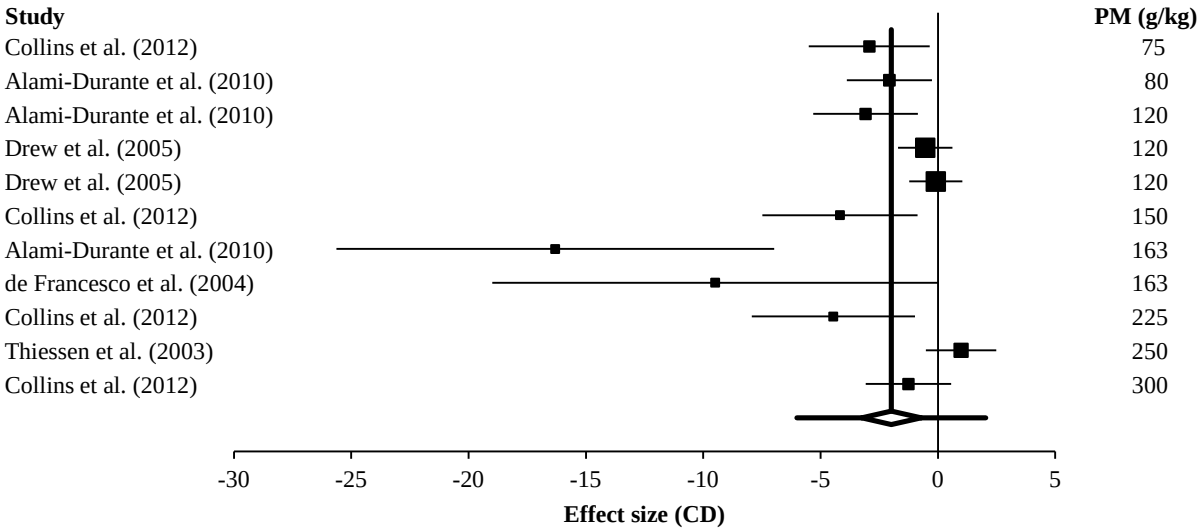

Figure 3.4.2. Forest plot of treatment effect sizes (CD) by pea meal (PM) dietary concentration.

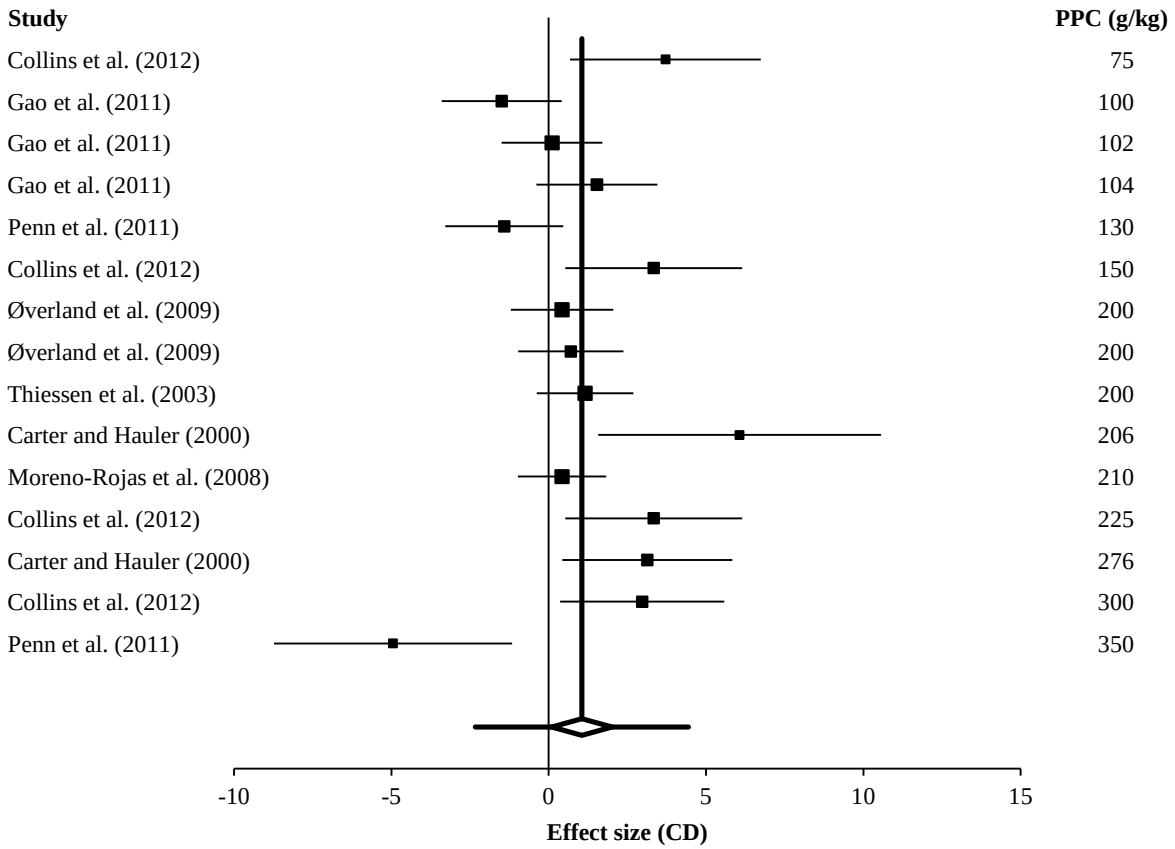

Figure 3.4.3. Forest plot of treatment effect sizes (CD) by pea protein concentrate (PPC) dietary concentration.

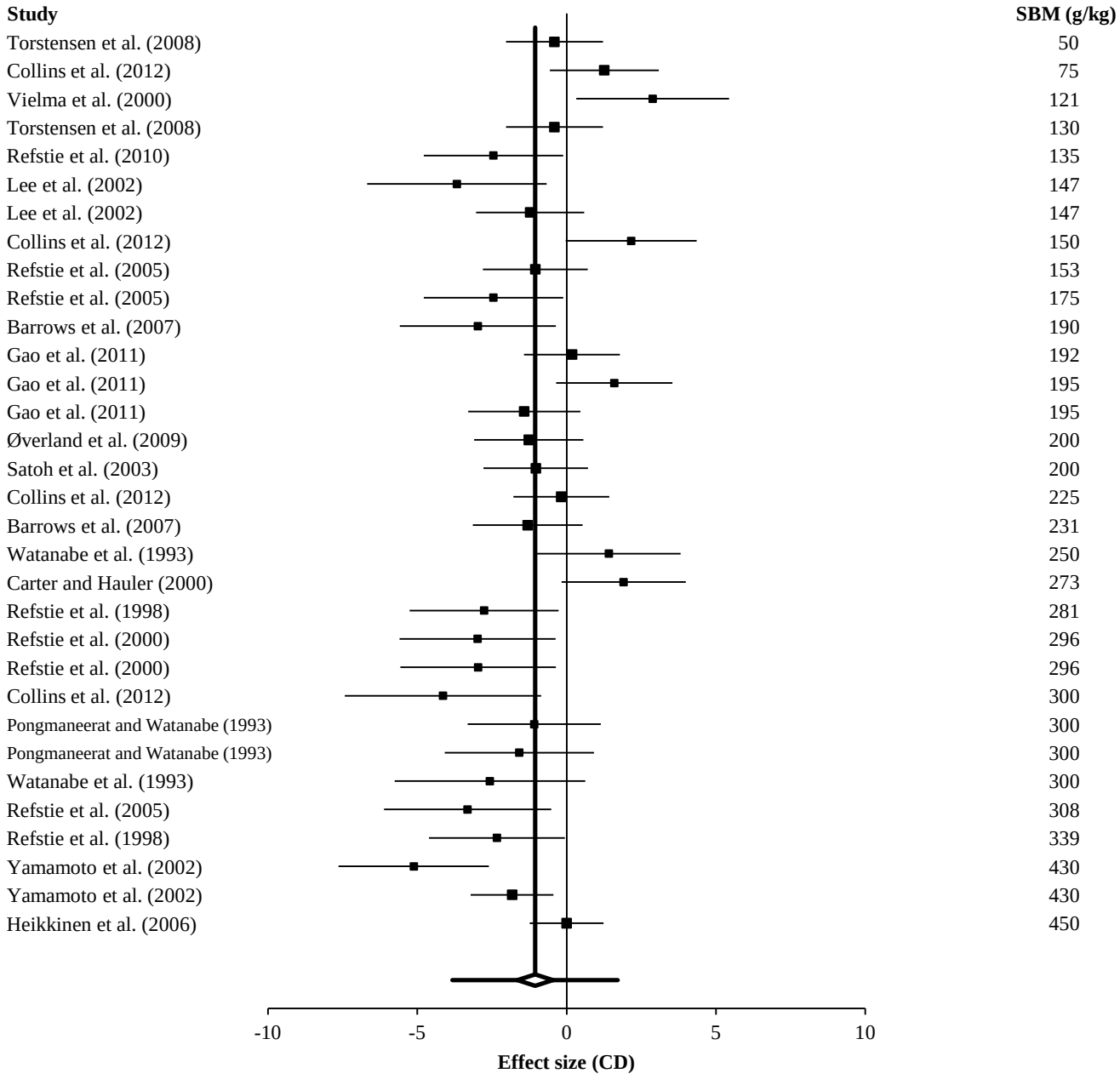

Figure 3.4.4. Forest plot of treatment effect sizes (CD) by soybean meal (SBM) dietary concentration.

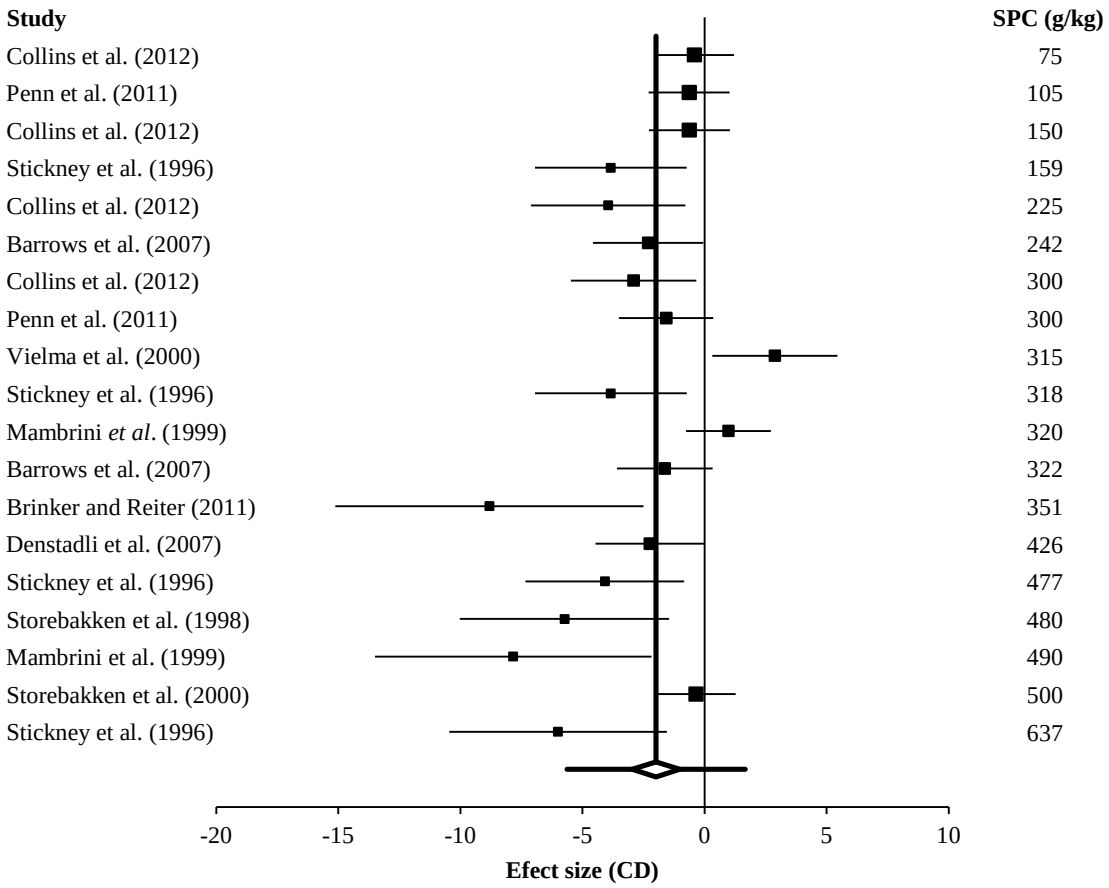

Figure 3.4.5. Forest plot of treatment effect sizes (CD) by soy protein concentrate (SPC) dietary concentration.

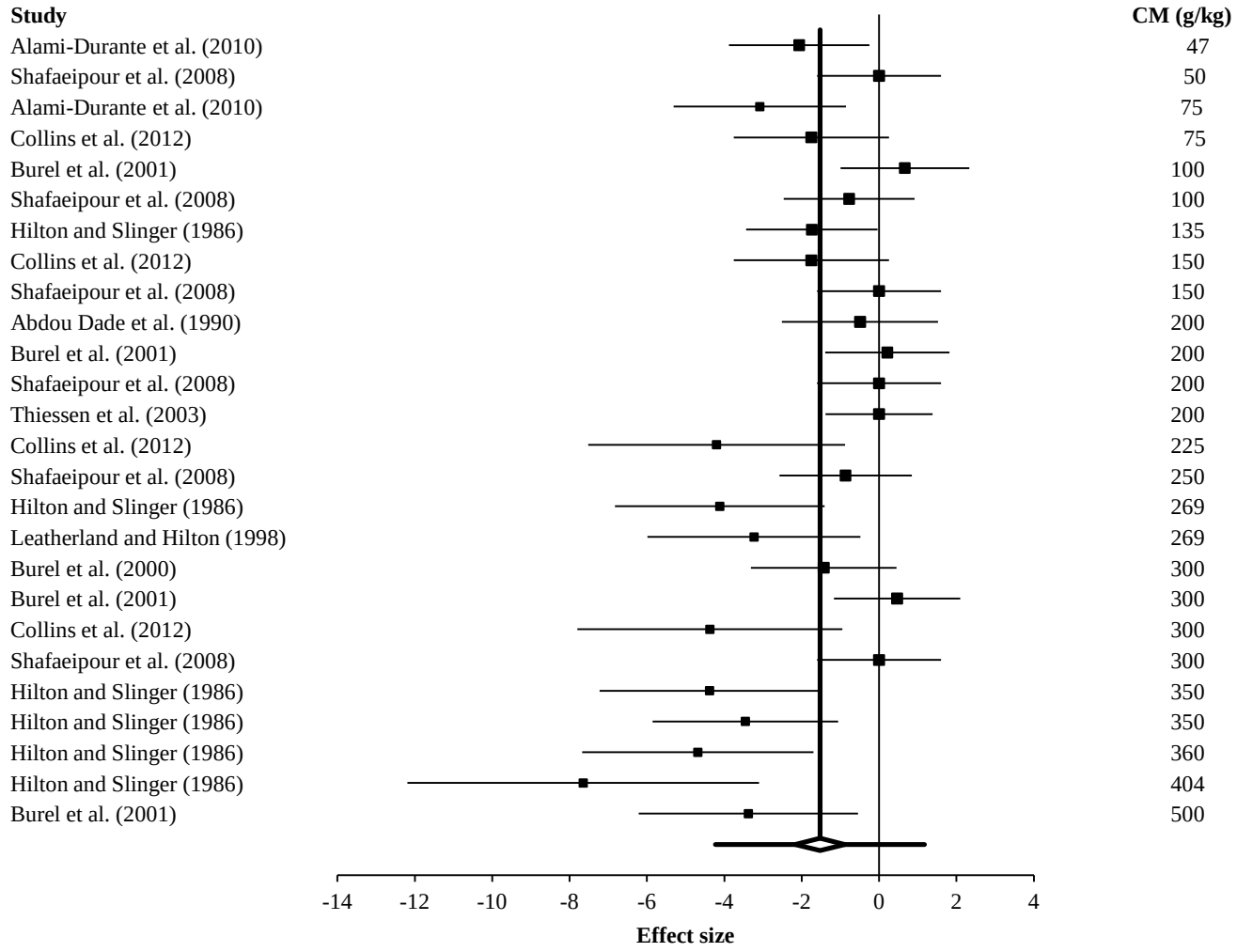

Figure 3.4.6. Forest plot of treatment effect sizes by canola / rapeseed meal (CM) dietary concentration.

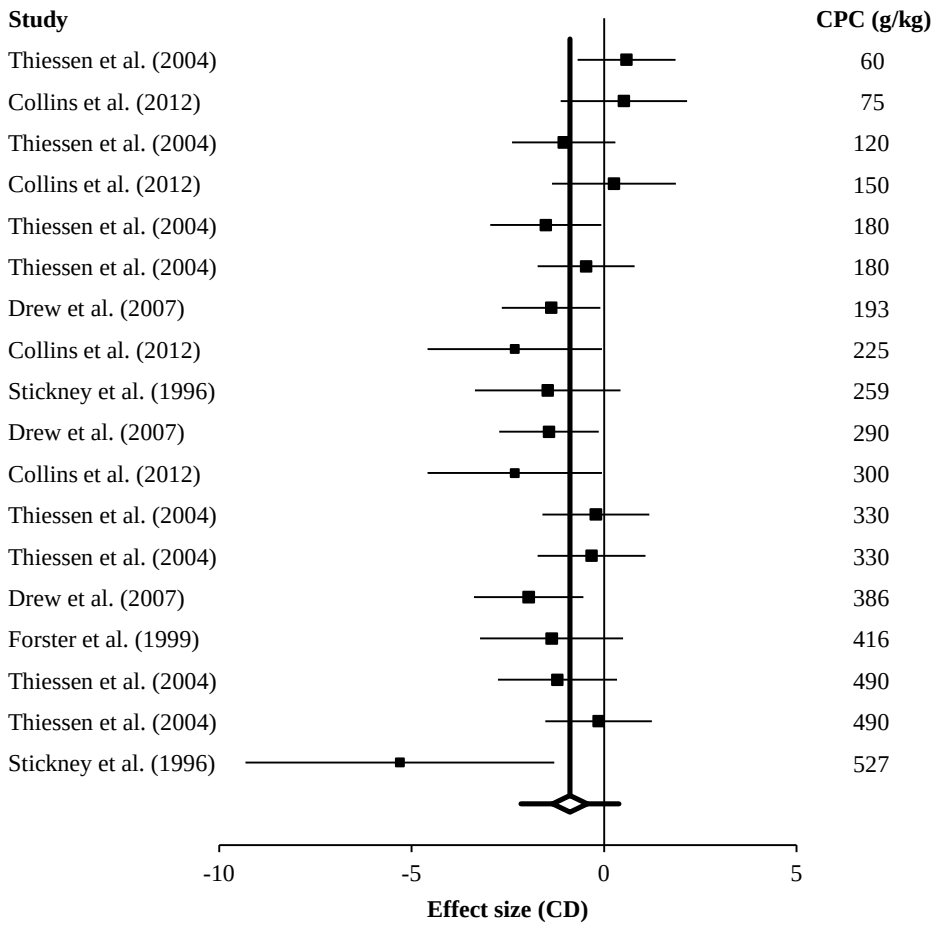

Figure 3.4.7. Forest plot of treatment effect sizes (CD) by dietary canola / rapeseed protein concentrate (CPC) concentration.

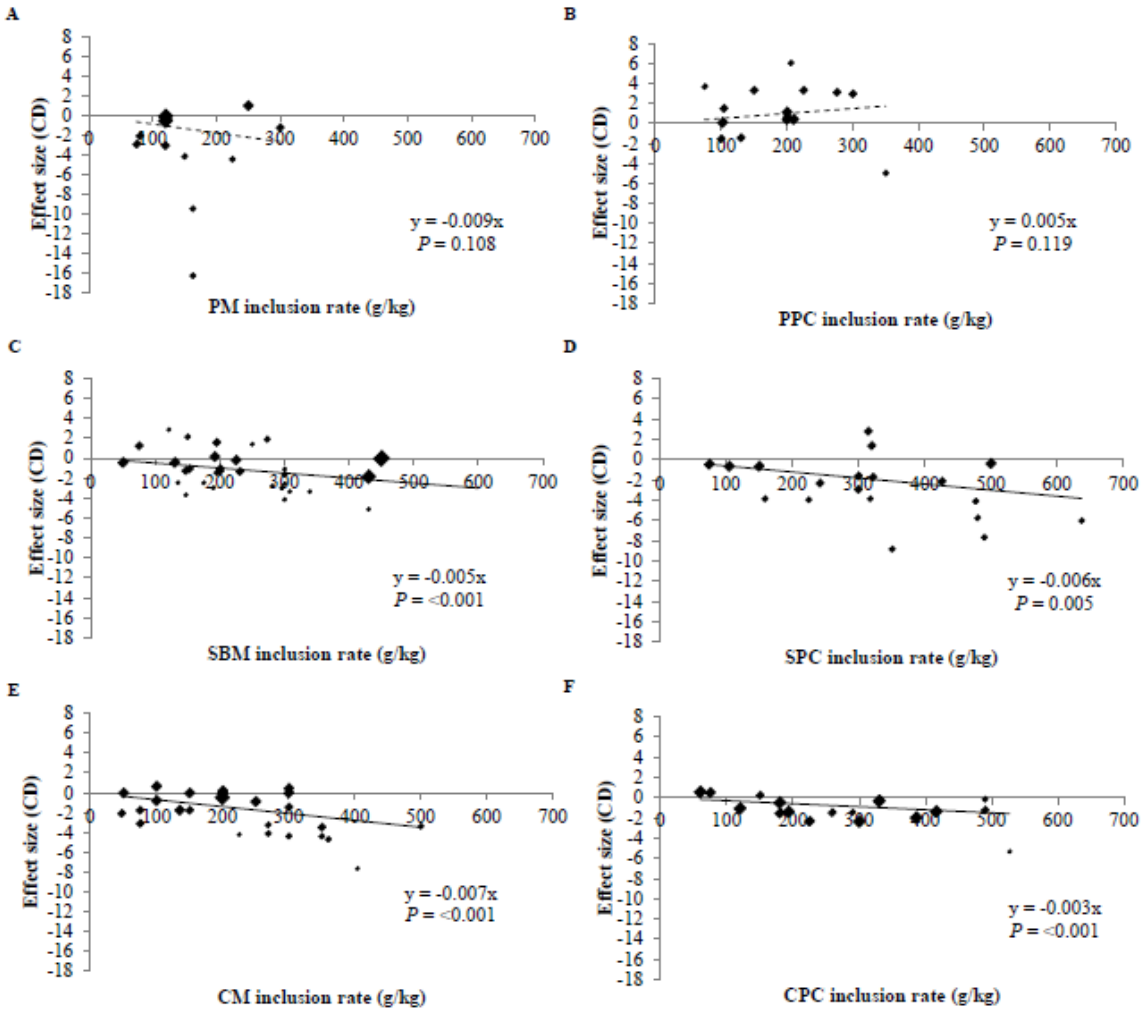

Figure 3.4.8. Weighted regression of effect size (CD) on dietary inclusion of plant ingredients on specific growth rate (SGR): (A) pea meal; PM (B) pea protein concentrate; (PPC) (C) soybean meal; SBM (D) soy protein concentrate; SPC (E) canola / rapeseed meal; CM (F) canola / rapeseed protein concentrate; CPC.

3.4.2. Pea meal

Eleven data points from five studies were included in the analysis (Table 2.6.2). Only rainbow trout were used in these studies. Effect size (Table 2.6.8; Figure 2.6.2) ranged from -16.31 (163 g/kg) to 0.99 (250 g/kg). All but one data point showed negative CD values for SGR. The overall mean of the PM meta-analysis was -1.98 (95% CI: -3.24 to 0.71; P = 0.002). The weighted linear and quadratic regressions were not significant (Figure 2.6.8A).

Table 3.4.7. Meta-analysis synthesis details for the effect size (CD) for specific growth rate (SGR) when including pea meal (PM) in salmonid diets.

Author	PM (g/kg)	Effect Size (CD)	*P*-value	Weight (%)
Collins et al. (2012)	75	-2.92	0.027	9.30
Alami-Durante et al. (2010)	80	-2.07	0.026	11.57
Alami-Durante et al. (2010)	120	-3.08	0.007	10.32
Drew et al. (2005)	120	-0.54	0.361	13.47
Drew et al. (2005)	120	-0.09	0.877	13.53
Collins et al. (2012)	150	-4.18	0.013	7.45
Alami-Durante et al. (2010)	163	-16.31	0.001	1.64
de Francesco et al. (2004)	163	-9.49	0.050	1.59
Collins et al. (2012)	225	-4.46	0.012	7.07
Thiessen et al. (2003)	250	0.99	0.194	12.52
Collins et al. (2012)	300	-1.25	0.178	11.55
Overall	-	-1.98	0.002	100.00

3.4.3. Pea protein concentrate

Fifteen data points from seven studies were used in the analysis of PPC (Table 2.6.3). Three studies used Atlantic salmon and four used rainbow trout. Ingredient effect size ranged from -4.95 (350 g/kg) to 6.07 (276 g/kg) (Table 2.6.9; Figure 2.6.3). The overall mean of effect size for the meta-analysis was 1.05 (95% CI: 0.09 to 2.01; P = 0.004). The

linear weighted regression of inclusion rate on CD (Figure 2.6.8B) was not significant (P = 0.119).

Table 3.4.8. Meta-analysis synthesis details for the effect size (CD) for specific growth rate (SGR) when including pea protein concentrate (PPC) in salmonid diets.

Study	PPC (g/kg)	Effect size (CD)	*P*-value	Weight (%)
Collins et al. (2012)	75	3.71	0.016	5.20
Gao et al. (2011)	100	-1.50	0.124	7.56
Gao et al. (2011)	102	0.11	0.896	8.29
Gao et al. (2011)	104	1.53	0.118	7.53
Penn et al. (2011)	130	-1.41	0.140	7.63
Collins et al. (2012)	150	3.34	0.020	5.59
Øverland et al. (2009)	200	0.42	0.611	8.23
Øverland et al. (2009)	200	0.70	0.410	8.12
Thiessen et al. (2003)	200	1.16	0.140	8.46
Carter and Hauler (2000)	206	6.07	0.008	3.20
Moreno-Rojas et al. (2008)	210	0.43	0.554	8.76
Collins et al. (2012)	225	3.34	0.020	5.59
Carter and Hauler (2000)	276	3.14	0.023	5.82
Collins et al. (2012)	300	2.97	0.026	6.00
Penn et al. (2011)	350	-4.95	0.010	4.02
Overall	-	1.05	0.003	100.00

3.4.4. Soybean meal

Fifty-eight data points from 24 studies were used in the analysis of SBM (Table 2.6.4). Rainbow trout constituted 43 data points from 17 studies, while Atlantic salmon represented 15 data points from eight studies. Ingredient effect size (Table 2.6.10; Figure 2.6.4) ranged from -5.12 (430 g/kg) to 2.88 (121 g/kg). The overall effect size was -1.05 (95% CI: -1.65 to -0.46; P = 0.001). The slope of the weighted linear regression of inclusion rate on CD (Figure 2.6.8C) was significantly negative (P = <0.001).

Table 3.4.9. Meta-analysis synthesis details for the effect size (CD) for specific growth rate (SGR) when including soybean meal (SBM) in salmonid diets.

Study	SBM (g/kg)	Effect size (CD)	*P*-value	Weight (%)
Torstensen et al. (2008)	50	-0.41	0.623	3.78
Collins et al. (2012)	75	1.26	0.175	3.52
Vielma et al. (2000)	121	2.88	0.027	2.66
Torstensen et al. (2008)	130	-0.41	0.623	3.78
Refstie et al. (2010)	135	-2.45	0.040	2.91
Lee et al. (2002)	147	-3.67	0.017	2.24
Lee et al. (2002)	147	-1.22	0.185	3.54
Collins et al. (2012)	150	2.16	0.053	3.07
Refstie et al. (2005)	153	-1.05	0.241	3.60
Refstie et al. (2005)	175	-2.45	0.040	2.91
Barrows et al. (2007)	190	-2.97	0.026	2.61
Gao et al. (2011)	192	0.18	0.828	3.80
Gao et al. (2011)	195	1.60	0.108	3.37
Gao et al. (2011)	195	-1.42	0.139	3.45
Øverland et al. (2009)	200	-1.27	0.174	3.52
Satoh et al. (2003)	200	-1.03	0.248	3.61
Collins et al. (2012)	225	-0.18	0.826	3.80
Barrows et al. (2007)	231	-1.31	0.164	3.50
Watanabe et al. (1993)	250	1.41	0.248	2.83
Carter and Hauler (2000)	273	1.91	0.072	3.20
Refstie et al. (1998)	281	-2.76	0.030	2.73
Refstie et al. (2000)	296	-2.98	0.025	2.61
Refstie et al. (2000)	296	-2.96	0.026	2.62
Collins et al. (2012)	300	-4.14	0.014	2.02
Pongmaneerat and Watanabe (1993)	300	-1.08	0.340	3.02
Pongmaneerat and Watanabe (1993)	300	-1.58	0.215	2.73
Watanabe et al. (1993)	300	-2.57	0.114	2.09
Refstie et al. (2005)	308	-3.32	0.020	2.43
Refstie et al. (1998)	339	-3.34	0.044	2.97
Yamamoto et al. (2002)	430	-5.12	0.000	2.71
Yamamoto et al. (2002)	430	-1.83	0.001	4.09
Heikkinen et al. (2006)	450	0.00	1.000	4.28
Overall	-	-1.05	0.001	100.00

3.4.5. Soy protein concentrate

Twenty-one data points from ten studies were used in the analysis of the effect of SPC on growth performance (Table 2.6.5). Six studies with 16 data points using rainbow trout and four studies with five data points using Atlantic salmon were represented in the data set. Ingredient effect size (Table 2.6.11; Figure 2.6.5) ranged from -8.81 (351 g/kg) to

2.83 (315 g/kg). The overall effect size was -1.98 (95% CI: -2.95 to 1.02; P = <0.001). The slope of the weighted linear regression (Figure 2.6.8D) was significantly negative (P = 0.001).

Table 3.4.10. Meta-analysis synthesis details for the effect size (CD) for specific growth rate (SGR) when including soy protein concentrate (SPC) in salmonid diets.

Study	SPC (g/kg)	Effect size (CD)	*P*-value	Weight (%)
Collins et al. (2012)	75	-0.42	0.617	7.11
Penn et al. (2011)	105	-0.63	0.458	7.05
Collins et al. (2012)	150	-0.62	0.461	7.05
Stickney et al. (1996)	159	-3.84	0.015	4.64
Collins et al. (2012)	225	-3.94	0.015	4.56
Barrows et al. (2007)	242	-2.29	0.046	5.99
Collins et al. (2012)	300	-2.91	0.027	5.46
Penn et al. (2011)	300	-1.58	0.112	6.56
Vielma et al. (2000)	315	2.83	0.027	5.49
Stickney et al. (1996)	318	-3.84	0.015	4.64
Mambrini et al. (1999)	320	1.42	0.269	6.91
Barrows et al. (2007)	322	-1.70	0.104	6.52
Brinker and Reiter (2011)	351	-8.81	0.006	1.86
Denstadli et al. (2007)	426	-2.11	0.049	6.05
Stickney et al. (1996)	477	-4.08	0.014	4.44
Storebakken et al. (1998)	480	-5.73	0.009	3.25
Mambrini et al. (1999)	490	-7.65	0.007	2.20
Storebakken et al. (2000)	500	-0.35	0.675	7.12
Stickney et al. (1996)	637	-6.00	0.008	3.09
Overall	-	-1.98	<0.001	100.00

3.4.6. Canola meal

The data set for CM contained 30 data points from 12 studies (Table 2.6.6). All studies were performed using rainbow trout except for one study with two data points that used Chinook salmon, which were both found to be outliers and not included in the final meta-analysis. Ingredient effect size ranged from -7.65 (404 g/kg) to 0.67 (100 g/kg) (Table 2.6.1.4.12; Figure 2.6.6). The overall effect size was -1.53 (95% CI: -2.17 to -0.89; P = <0.001). The weighted quadratic regression of

inclusion rate on CD was significant (P = <0.001) and showed feeding CM at dietary inclusion levels of 300 g/kg and higher decreases effect size (Figure 2.6.8E).

Table 3.4.11. Meta-analysis synthesis details for the effect size (CD) for specific growth rate (SGR) when including canola / rapeseed meal (CM) in salmonid diets.

Study	CM (g/kg)	Effect size (CD)	*P*-value	Weight (%)
Alami-Durante et al. (2010)	47	-2.07	0.026	4.35
Shafaeipour et al. (2008)	50	0.00	1.000	4.71
Alami-Durante et al. (2010)	75	-3.08	0.007	3.69
Collins et al. (2012)	75	-1.75	0.088	4.03
Burel et al. (2001)	100	0.67	0.433	4.59
Shafaeipour et al. (2008)	100	-0.78	0.368	4.56
Hilton and Slinger (1986)	135	-1.74	0.045	4.54
Collins et al. (2012)	150	-1.75	0.088	4.03
Shafaeipour et al. (2008)	150	0.00	1.000	4.71
Abdou Dade et al. (1990)	200	-0.49	0.634	4.01
Burel et al. (2001)	200	0.21	0.794	4.70
Shafaeipour et al. (2008)	200	0.00	1.000	4.71
Thiessen et al. (2003)	200	0.00	1.000	5.08
Collins et al. (2012)	225	-4.20	0.013	2.39
Shafaeipour et al. (2008)	250	-0.87	0.321	4.52
Hilton and Slinger (1986)	269	-4.11	0.003	3.04
Leatherland and Hilton (1998)	269	-3.23	0.021	2.99
Burel et al. (2001)	300	-1.43	0.136	4.23
Burel et al. (2001)	300	0.47	0.575	4.65
Collins et al. (2012)	300	-4.37	0.012	2.29
Shafaeipour et al. (2008)	300	0.00	1.000	4.71
Hilton and Slinger (1986)	350	-4.38	0.003	2.89
Hilton and Slinger (1986)	350	-3.46	0.005	3.45
Hilton and Slinger (1986)	360	-4.68	0.002	2.72
Hilton and Slinger (1986)	404	-7.65	0.001	1.53
Burel et al. (2001)	500	-3.38	0.020	2.89
Overall	-	-1.53	<0.001	100.00

3.4.7. Canola protein concentrate

The CPC data set had 18 data points from five studies (Table 2.6.7), all with rainbow trout. Ingredient effect size (Table 2.6.13; Figure 2.6.7) ranged from -5.31 (527 g/kg) to 0.58 (60 g/kg). The overall effect size was -0.84 (95% CI: -1.20 to -0.48; P = <0.001). The weighted linear regression (Figure 2.6.8F) was significantly negative (P = <0.001).

Table 3.4.12. Meta-analysis synthesis details for the effect size (CD) for specific growth rate (SGR) when including canola / rapeseed protein concentrate (CPC) in salmonid diets.

Study	CPC (g/kg)	Effect size (CD)	*P*-value	Weight (%)
Thiessen et al. (2004)	60	0.58	0.370	6.05
Collins et al. (2012)	75	0.52	0.538	4.49
Thiessen et al. (2004)	120	-1.05	0.126	5.71
Collins et al. (2012)	150	0.26	0.754	4.59
Thiessen et al. (2004)	180	-1.51	0.040	5.25
Thiessen et al. (2004)	180	-0.47	0.469	6.11
Drew et al. (2007)	193	-1.37	0.036	6.00
Collins et al. (2012)	225	-2.32	0.045	2.81
Stickney et al. (1996)	259	-1.46	0.130	3.68
Drew et al. (2007)	290	-1.43	0.030	5.95
Collins et al. (2012)	300	-2.32	0.045	2.81
Thiessen et al. (2004)	330	-0.21	0.765	5.48
Thiessen et al. (2004)	330	-0.33	0.648	5.45
Drew et al. (2007)	386	-1.96	0.007	5.35
Forster et al. (1999)	416	-1.36	0.151	3.79
Thiessen et al. (2004)	490	-1.21	0.125	4.83
Thiessen et al. (2004)	490	-0.14	0.842	5.50
Stickney et al. (1996)	527	-5.31	0.010	1.06
Overall	-	-0.84	<0.001	100.00

3.4.8. Additional factors influencing specific growth rate

The effects of additional factors on SGR are shown in Table 2.6.14. Initial weight of the fish had a significant impact on PPC and CM-fed fish, as did dietary processing conditions on CM-fed fish. Because one or more groups had fewer than two cases, post-

hoc analysis was not possible, although CD tended to decrease as initial weight increased for the PPC-fed fish. For the CM-fed fish, the CD was lower in fish with an initial weight of 2.0 g and it tended to increase with fish size.

Among available data points of CM-fed fish, CDs were significantly lower when diets were steam-pelleted (-4.18) than in cases where the diets were cold-pressed (-1.57). The experiment with extruded diets had the highest CD, but this was for only one data point in the CM data set.

Whether or not the diet was balanced for nutrients had a significant impact on the CD of fish fed CM and PPC. In the CM trials, fish fed diets that were not balanced for nutrient content had a significantly lower average CD (-4.34) than fish fed diets balanced for nutrient content (-0.66). In the PPC trials, fish fed unbalanced diets had an average CD of -0.70, which was significantly lower than the CD of fish fed balanced diets (2.96). Feeding regime also had a significant impact on the CD of fish fed SBM and CM. In the SBM data set, the CD was highest in fish fed 1x / day (2.88), although this was only for one data point. After this, fish with the highest CD (-0.75) were fed 2x / day, followed by fish who were fed 3x / day (-0.94) and those who were fed continuously (-2.29). In the CM data set, fish fed continuously had a significantly lower average CD (-4.18) than fish fed 2 or 3x / day (-1.63 and -0.28, respectively).

In the CM data set, when fish were fed fish meal at an inclusion level equal to or lower than the fish meal level in the control diet, the CD was significantly lower (-3.20 vs -1.37). For all other test ingredients and factors, results were either not significant or there was insufficient data to determine whether or not they had an impact on SGR.

Table 3.4.13. Additional factors influencing specific growth rate (SGR).

	PM	PPC	SBM	SPC	CM	CPC
Initial weight	*P* = 0.370	*P* = 0.006	*P* = 0.452	*P* = 0.173	*P* = <0.001	*P* = 0.163
Species	All rainbow trout	*P* = 0.493	*P* = 0.380	*P* = 0.570	All rainbow trout	All rainbow trout
Water environment	All fresh	*P* = 0.342	*P* = 0.224	*P* = 0.570 (mirrors species)	All fresh	All fresh
Dietary processing conditions	All pelleted, except for one data point	*P* = 0.069	*P* = 0.834	All extruded (information missing for one data point)	*P* = 0.009	*P* = 0.881
Diet balanced for nutrients	All yes	*P* = 0.004	*P* = 0.289	*P* = 0.671	*P* = 0.001	All yes
Palatability enhancers	No palatability enhancers	All no, except for one data point	*P* = 0.541	All no, except for one data point	*P* = 0.122	*P* = 0.354
Feeding regime	All fed 2x / day	*P* = 0.605	*P* = 0.025	*P* = 0.206	*P* = 0.001	All fed 2x / day, except for one data point
Blended diets	*P* = 0.729	One blended diet	One blended diet	One blended diet	*P* = 0.177	All no
Mismatched diets	*P* = 0.387	*P* = 0.799	*P* = 0.452	*P* = 0.962	*P* = 0.914	*P* = 0.456
More fish meal in control diet relative to test diet	All yes, except for one data point	All yes	All yes (information missing for one data point)	All yes	*P* = 0.031	*P* = 0.953

3.5. Discussion

3.5.1. Plant ingredients

The urgent need to include fish meal replacements in salmonid diets has prompted extensive research into alternative plant protein sources. While plant ingredients are generally thought to have a negative effect on salmonid growth performance, the different methodologies used in published studies make it difficult to come to a definitive conclusion. In the present study, the data on SBM and CM comprised a large number of studies, whereas our findings with PM, PPC, SPC and CPC were based on fewer studies and should be considered to be less conclusive.

The papers used in this study represent over 25 years of research. In this time span, several factors affecting fish growth responses to plant ingredients may have occurred, such as changes in the chemical and physical properties of the ingredients due to advances in plant breeding. For example, the glucosinolate content of canola has decreased since the 1970s, but still has a high level of variation. In 2011, canola glucosinolate levels in western Canada varied from 4.3 to 17.0 μmoles/g (Barthet, 2011). Differences among studies would be expected to be due to feed-related factors, such as feed ingredient characteristics and the nutritional and physical properties of the diets.

Reduced growth performance may be partly explained by reduced protein, amino acid, lipid and energy digestibility, if not compensated by increased feed intake. The negative effects of plant ingredients on salmonid growth are generally ascribed to antinutritional factors. In these meta-analyses, data is not specified on antinutrient content, due to a lack of available information. Noteworthy, PM and PPC had no significant effect on SGR in the present study, which may be related to lower levels of

critical antinutrients in diets with pea products than in diets with products from soybeans and canola/rapeseed.

3.5.2. Diet formulation

The protein quality of the control diet may have had an impact on the results of some studies. The amino acid composition of the diet, either through supplementation or dietary formulation can influence SGR. Unfortunately, information on ingredient and dietary amino acid composition is lacking in many papers. Mundheim et al. (2004) found that replacing high-quality fish meal with a plant protein blend reduced the growth of Atlantic salmon to a greater extent than when a medium-quality fish meal was replaced. Carter and Hauler (2000) replaced fish meal with plant protein and had positive effect sizes in both the SBM and PPC data sets. In addition, these authors conducted a digestibility experiment and found that SBM, PPC and lupin protein concentrate had a significantly higher protein digestibility than their control diet, which is indicative of a poor quality fish meal. Oliva-Teles et al. (1994) obtained higher digestibility and improved growth in rainbow trout by replacing brown fish meal with SBM. This suggests that the quality of the fish meal being replaced will affect the SGR of the test ingredient. In the majority of the studies used for meta-analysis, there was more fish meal in the control diet than the test diet. Unfortunately, information regarding the quality of the fish meal used in these experiments was limited and insufficient for subgroup meta-analysis.

Fish meal may contain nutritional components that are often overlooked and enhance growth beyond what would be expected on the basis of digestible nutrient content alone, such as taurine and low molecular weight compounds (Li et al., 2009;

Aksnes et al., 2006; Gaylord et al., 2006). These dietary components may be lacking or suboptimal in diets with high plant ingredient content, which may explain some of the results when assessing the effects of plant proteins.

In a number of studies, plant protein blends are used as a fish meal substitute. According to Kaushik and Seiliez (2010), mixtures of ingredients are required to completely replace fish meal in salmonid diets, as no single plant ingredient can equally support fish growth. The results of our meta-analyses may concur with this view, although growth impairment due to the inclusion of plant protein was greatly dependent on individual plant protein source, as well as the level applied in the diets and there was no statistically significant effect of feeding blended or mismatched diets (Table 14).

For future research projects determining the impact of a dietary ingredient on growth, we recommend ensuring dietary nutrients are balanced and that this balance is maintained for all treatments involved in the study. With unbalanced diets, it is possible that data obtained would be invalid, as it could be an illustration of the effects of feeding a nutrient-deficient diet, rather than the effects of feeding the test ingredient (Hua and Bureau, 2012).

3.5.3. Effect of processing on specific growth rate

The description of the dietary processing conditions in many studies is lacking and limited to pelleting, steam pelleting and extrusion. Therefore, dietary processing conditions were considered as one of three separate methods as stated in each respective study. However, the processing conditions applied within each category can drastically change feed characteristics and quality. Using the heat treatment involved in the extrusion

process as an example, the nutrient quality of a feed could be impaired if heat-labile vitamins are damaged (Barrows et al., 2008) or if amino acids, such as lysine or cysteine, are made unavailable via Maillard reaction (Singh et al., 2007). Conversely, this heat could positively affect the nutrient quality of a feed by inactivating heat-labile antinutrients (Francis et al., 2001).

Changes in processing methods for creating protein concentrates, as well as conditions during processing of the complete feed may have significantly affected the nutrient and antinutrient content of these products. In this regard, equal processing conditions for the control diet and the diets containing the plant protein source do not necessarily imply the same effect of processing on nutritional and physical quality. While it is generally assumed that the use of protein concentrates provides better growth performance than using conventional meals, the meta-analysis results showed that SPC and CPC both had significant negative effects on the growth of salmonid fish.

3.5.4. Specific growth rate as the growth parameter

Growth in fish is a complex trait expressed by growth rate estimates. The most common expression of fish growth is SGR (Bureau et al., 2002). The exponential growth assumed in the logarithmic SGR equation is suitable for young, rapidly growing fish over short time periods, but not as suitable for large fish with slower relative growth rate and lower SGR, especially over long periods (Hopkins, 1992). Furthermore, SGR is influenced by water temperature. The TGC proposed by Iwama and Tautz (1981) is regarded as more precise than SGR (Bureau et al., 2002; Cho, 1992) and less affected by body size and

temperature, although certain limitations of the model have been reported (Jobling, 2003).

In the present study, SGR was used to express growth due to more available data than for TGC and because our meta-analyses applied within-study data, where initial weight and experimental conditions were similar. Differences in SGR among salmonid species should be considered, although SGR may be similar in Atlantic salmon and rainbow trout (Austreng et al., 1987). Refstie et al. (2000) showed a higher tolerance to SBM in rainbow trout than in Atlantic salmon. A previous meta-analysis of fish meal replacement with SBM showed no significant difference in effect size between carnivorous and omnivorous fish (Sales, 2009). Our own investigation showed no significant effect of species on SGR, although this analysis was only possible when sufficient data were available. For PM, CM and CPC, we cannot rule out species variation, as the fish in these studies were all rainbow trout.

We observed substantial differences in effect size among different studies for each individual plant protein source. The unexplained variation can be attributed to various factors not included in our analysis, such as differences in design, methodology, fish age, genetics (Overturf et al., 2012) and environment, in addition to nutritional composition and quality of ingredients. For instance, the effect of partial replacement of fish meal with plant proteins may depend on the length of the experimental period. Refstie et al. (1997) reported rainbow trout with an initial weight of 33 g were able to adapt to high dietary inclusion of SBM following 29-56 days of feeding, which was illustrated by their higher SGR relative to fish fed a fish meal control diet. This was not observed after only 28 days of feeding.

3.5.5. Feeding methods

For the SBM and CM data sets, feeding regime had a significant effect on CD. When fish were fed continuously, the CD was lower than when they were fed between one and three times per day. Increased feeding rate increases gastric emptying rate (Jobling, 1981; Grove et al., 1978), which may have influenced growth. Less frequent, and more controlled feeding periods may have been beneficial in the case of these plant-based diets, which are commonly less digestible than fish meal diets, by slowing the rate of gastrointestinal passage. A longer retention time in the digestive tract could increase access to digestive enzymes and fermentative microorganisms, thus freeing more nutrients than would be released with a more rapid gastrointestinal transit. This is a topic that would benefit from future research with focus on feed intake, feed efficiency and nutrient digestibility of plant ingredient-based diets.

3.5.6. Additional considerations

Weighted statistics were used to reduce the possibility of statistical bias, as they take into account the relative contribution of each data point to the entire data set. The influence of publication bias on the availability of data has also been considered. With publication bias, studies with statistically significant results are more likely to be published than those lacking significant results (Easterbrook et al., 1991). In the case of growth studies, such as those included in these meta-analyses, this is not likely, due to the nature of their design. When the focus of an experiment is to replace traditional fish feed ingredients using plant-based feed ingredients and examine growth, non-significant results would be

considered equally as important as significant results and would not be left unpublished due to lack of interest on the part of the researcher.

3.6. Conclusion

Meta-analysis made it possible to compare growth data from available literature on salmonids fed six plant proteins of varying dietary inclusion levels. By assessing existing information sources, SGR for salmonids fed varying dietary inclusion levels of these six plant proteins was calculated and with the use of CD, growth data were compared among studies. These meta-analyses and regression models showed that there are differences in the effect of plant ingredients on the growth performance of salmonid fish. Increasing the inclusion level of SBM, SPC, CM and CPC decreases SGR. However, there may be potential for further studies involving PM and PPC, as no significant adverse effect of these two feed ingredients was noted, although this may be due to the fact that they have not been studied at inclusion levels as high as those used for the other four ingredients. This is does not necessarily mean that these feed ingredients will reduce the growth of salmonid fish. Rather, they may be more effective at low inclusion levels when using a mixture of several plant ingredients.

4. NUTRIENTS, ANTINUTRIENTS AND NUTRIENT DIGESTIBILITY IN RAINBOW TROUT: ASSESSMENT OF SOYBEAN, PEA AND CANOLA MEALS AND PROTEIN CONCENTRATES

This chapter reports the nutrient composition and digestibilities of nutrients in the ingredients used to prepare the diets fed in the experiments reported in Chapters 5 and 6, as well as the antinutrient composition of these feed ingredients, which were used for the statistical equation modeling performed in Chapter 6.

4.1. Abstract

Chemical analysis was conducted to determine the nutrient and antinutrient composition of soybean meal (SBM), soy protein concentrate (SPC) pea meal (PM), pea protein concentrate (PPC), canola meal (CM) an aqueous-extracted canola protein concentrate (CPC) and a high phytate CPC (PCPC). Total tract digestibility of the proximate and amino acid values of each feed ingredient was measured in rainbow trout (*Oncorhynchus mykiss*) in two separate trials (Trial 1: legume products, Trial 2: canola products). Digestibility was calculated indirectly, using high-purity flux-calcined diatomaceous earth (Celite 545, Celite Co., World Minerals Co., Lompoc, CA, USA) at an inclusion ration of 10 g/kg as an indigestible marker. To make test diets, the reference mash was combined with each test ingredient at a ratio of 7:3. Diets were fed to triplicate tanks of fish, which were acclimated to the diets for six days and then over three weeks, feces were collected via settling column, centrifuged, freeze-dried, ground and analyzed for digestible energy, amino acids, phosphorus and antinutrient levels. In the legume products trial, dry matter (DM), crude protein (CP), gross energy (GE), lipid and amino acid digestibilities of the soy products were significantly higher than in the pea products. These parameters were also significantly improved in protein concentrates as compared with the meals. In the canola products trial, DM (0.81) and GE (0.84) apparent digestibility coefficients (ADC) for fish fed CPC were significantly higher than those in fish fed CM (0.68 and 0.77, respectively). Fish fed CPC also had a significantly higher phosphorus ADC (0.78) than fish fed CM (0.28), which is likely due to the CPC being completely devoid of phytate, whereas CM had a phytate content of 38.8 g/kg. Nutrient, digestible nutrient and ANF composition of these plant proteins will be useful to the trials

following these studies with respect to the larger scope of the long-term goals for this experiment.

4.2. Introduction

The increasing demand for a static supply of marine protein sources has resulted in rising costs and decreasing availability of these products for use in aquafeeds. Thus, the replacement of marine proteins in aquafeeds is one of the central problems facing aquaculture. A large number of studies have been performed to examine the effect of replacing marine products with plant protein ingredients. The best studied of these ingredients are soybeans, field pea and canola/rapeseed. A general conclusion of these studies is that antinutritional factors (ANF) present in these ingredients limits their use in salmonid diets due to decreased growth, intestinal inflammation, low nutrient concentration and low digestibility (Hart et al., 2010; Drew et al., 2007; Denstadli et al., 2006a; Sajjadi and Carter, 2004; Francis et al., 2001) compared to marine ingredients. Fractionation of these ingredients to produce protein concentrates has been investigated as a method to decrease ANF, increase nutrient concentration and digestibility. This has generally resulted in improved digestibility and growth.

Identified ANF in these three ingredients are known to bind nutrients (tannins, phytate), increase gut viscosity thus reducing nutrient absorption, decrease the activity of intestinal enzymes (trypsin inhibitor, chymotrypsin inhibitor) alter metabolism (glucosinolates) or cause intestinal damage reducing nutrient absorption, which can occur in the presence of ANF such as saponins and lectins (Drew et al., 2007; Kraugerud et al., 2007; Singh et al., 2003; Abd El-Hady and Habiba, 2003; Vielma et al., 2002; Francis et

al., 2001; Carter and Hauler, 2000). However, the ANF responsible for decreasing ingredient digestibility have not been conclusively identified. Studies where single ANF are fed do not generally replicate the effects of feeding native ingredients (Krogdahl et al., 2010).

These studies examine the effect of ingredient, level of processing and levels of ANF on nutrient digestibility in rainbow trout.

4.3. Materials and methods

4.3.1. Ingredients and diets

The test ingredients used in this experiment were: soybean meal (SBM; Federated Cooperatives Limited, Saskatoon, SK, Canada), soy protein concentrate (SPC; Soycomil K; ADM Specialty Ingredients (Europe) BV, Koog aan de Zaan, The Netherlands), pea meal (PM; yellow field pea, CDC Mozart, Crop Development Centre, Saskatoon, SK, Canada), pea protein concentrate (PPC; Yellow field pea, prestige protein, Parrheim Foods, Saskatoon, SK, Canada), canola meal (CM; Canola Meal-35; Federated Co-Operative Ltd., Saskatoon, SK, Canada) an aqueous-extracted canola protein concentrate (CPC; Can Pro IP; CanPro Ingredients Ltd., Arborfield, SK, Canada) and a high phytate CPC (PCPC; CanPro Ingredients Ltd., Arborfield, SK, Canada).

The reference diet and experimental diets are described in Table 3.1.1. The reference diet was that of Bureau and Cho (1994). Celite 545 high-purity flux-calcined diatomaceous earth (Celite Co., World Minerals Co., Lompoc, CA, USA) was used as a non-absorbable indicator for indirect digestibility analysis. The reference mash was combined with the test ingredients at a mass ratio of 7:3 to make experimental diets.

Diets mixed with a Hobart mixer (Hobart Corporation; Model L-800; Troy, OH, USA) and were cold extruded on a Hobart mixer (Hobart Corporation; Model 4822; Troy, OH, USA) through a 5 mm die. Pellets were dried in a forced air oven for 12 h at 55 ºC, then chopped and screened.

Table 4.3.1. Ingredient composition of experimental diets (g/kg).

Ingredient	Control	SBM	SPC	PM	PPC	CM	CPC	PCPC
Fish meal[a]	300.000	210.000	210.000	210.000	210.000	210.000	210.000	210.000
Soybean meal[b]	170.000	419.000	119.000	119.000	119.000	119.000	119.000	119.000
Soy protein concentrate[c]	0.000	0.000	300.000	0.000	0.000	0.000	0.000	0.000
Pea meal[d]	0.000	0.000	0.000	300.000	0.000	300.000	0.000	0.000
Pea protein concentrate[e]	0.000	0.000	0.000	0.000	300.000	0.000	0.000	0.000
Canola meal[f]	0.000	0.000	0.000	0.000	0.000	300.000	0.000	0.000
CPC[g]	0.000	0.000	0.000	0.000	0.000	0.000	300.000	0.000
PCPC[h]	0.000	0.000	0.000	0.000	0.000	0.000	0.000	300.000
Wheat flour[i]	280.000	196.000	196.000	196.000	196.000	196.000	196.000	196.000
Corn gluten meal	130.000	91.000	91.000	91.000	91.000	91.000	91.000	91.000
Fish oil[j]	100.000	70.000	70.000	70.000	70.000	70.000	70.000	70.000
Celite[k]	10.000	7.000	7.000	7.000	7.000	7.000	7.000	7.000
Vitamin premix[l]	4.750	3.325	3.325	3.325	3.325	3.325	3.325	3.325
Mineral premix[m]	4.750	3.325	3.325	3.325	3.325	3.325	3.325	3.325
Vitamin C[n]	0.500	0.350	0.350	0.350	0.350	0.350	0.350	0.350

[a] Nova Scotia herring meal; Shur-Gain Aquaculture, Truro, NS, Canada.
[b] Soybean meal; Federated Cooperatives Limited, Saskatoon, SK, Canada.
[c] Soycomil K; ADM Specialty Ingredients (Europe) BV, Koog aan de Zaan, The Netherlands.
[d] Yellow field pea, CDC Mozart; Crop Development Centre, Saskatoon, SK, Canada.
[e] Pea protein concentrate, yellow field pea, prestige protein; Parrheim Foods, Saskatoon, SK, Canada.
[f] Canola Meal-35; Federated Co-Operative Ltd., Saskatoon, SK, Canada.
[g] Can Pro IP; CanPro Ingredients Ltd., Arborfield, SK, Canada.
[h] High phytate canola protein concentrate; CanPro Ingredients Ltd., Arborfield, SK, Canada.
[i] Robin Hood All-Purpose Flour; Robin Hood Multifoods Corporation, Markham, ON, Canada.
[j] Danish Fish Oil; FF of Denmark, Skagen, Denmark.
[k] Celite 545, <125µm; Celite Corporation, World Minerals Co., Lompoc, CA, USA.
[l] Vitamin premix, commercial (EWOS FISH-STR VIT PX, Surrey, BC; closed formulation), formulated to meet the requirements of juvenile rainbow trout; BASF Canada, Surrey, BC, Canada.
[m] Mineral premix, commercial (EWOS FISH MINERAL PX#2, Surrey, BC; closed formulation), formulated to meet the requirements of juvenile rainbow trout; BASF Canada, Abbotsford, BC, Canada.
[n] Ascorbic acid, pharmaceutical grade; NOW Foods, Bloomingdale, IL, USA.

4.3.2. Fish husbandry and digestibility trial design

Triploid female rainbow trout (*Oncorhynchus mykiss*) were purchased from Wild West Steelhead (Lucky Lake, SK, Canada) and subjected to one of two digestibility trials at the Prairie Aquaculture Research Centre (University of Saskatchewan, Saskatoon, SK), an indoor, biologically filtered, recirculating aquaculture system. Water temperature was kept at 15 ± 2 °C and photoperiod was a 14 h light:10 h dark cycle. Fish maintenance followed the guidelines set by the Canadian Council on Animal Care (CCAC, 2005), which included careful monitoring of environmental and water quality indicators.

Fish were housed in 120 L tanks with three replicates per treatment and were hand-fed twice daily to apparent satiation. Trial 1 was for the legume products (SBM, SPC, PM, PPC) and had 14 fish per tank (103.6 g; average weight). Trial 2 was for the canola products (CM, CPC, PCPC) and had 14 fish per tank (235.8 g; average weight). Diets were randomly assigned to the tanks and fed for three weeks.

4.3.3. Sample preparation

Fish were fed the experimental diets for an acclimation period of six days, which was followed by a three-week period of fecal collection using a settling column (Hajen *et al*., 1993). Fecal matter was centrifuged at 5000 x g for 15 min, frozen and freeze-dried. Test ingredients, experimental diets and feces were ground with an ultra centrifugal mill (Model ZM 100; Retsch Inc., Newtown, PA, USA) to pass through a 1.0 mm screen and were subjected to proximate, amino acid and antinutrient testing. The exceptions were for samples analyzed for phytate, total starch and non-starch polysaccharides, which were ground to 0.5 mm.

4.3.4. Digestibility analysis

Experimental feeds, ingredients and feces were analyzed for moisture (AOAC, 1990, method no. 934.01), DM (100-moisture), GE (oxygen bomb calorimetry; Parr Adiabatic Calorimeter, Model 1281, Parr Instrument Co., Moline, IL, USA), CP, acid ether extract (lipid) (AOAC, 1995; method no. 954.02) and acid insoluble ash (Newkirk *et al*., 2003). Nitrogen content was determined using the combustion method on a Leco protein / N analyzer (Model FP-528, Leco Corp., St. Joseph, MI, USA) (AOAC, 1995; method no. 990.03) and multiplied by 6.25 to determine CP. Amino acid content of all samples was determined at Evonik Industries (Essen, Germany). Apparent digestibility coefficients (ADC) for the experimental diets and test ingredients were calculated using the equations of Cho et al. (1982) and Sugiura et al. (1998), as recommended by Bureau and Cho (1999).

4.3.5. Antinutritional factor analysis

Glucosinolates in CM and CPC (AOCS, 1998, method Ak 1-92) and isoflavones in SBM, SPC, PM and PPC were determined (AACC International, 2001; method 20-20.01) by POS Pilot Plant Corporation (Saskatoon, SK, Canada). Test ingredients were analyzed for tannins at Nutrilab B.V. (Giessen, Netherlands) using the Folis Denis method. Phytates in the test ingredients and diets were extracted using the method of Newkirk and Classen (1998). Samples were analyzed via high performance liquid chromatography at MCN BioProducts, Inc. (Saskatoon, SK, Canada).

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) in the test ingredients and diets were determined using an Ankom fibre analyzer (model ANKOM^{200}, Ankom Technology, Fairport, NY, USA). Sodium sulfite and alpha-amylase were used in the NDF procedure (Van Soest *et al.*, 1991). NDF and ADF were not adjusted for ash. Total starch was analyzed using the Megazyme total starch analysis kit (AOAC, 1995; method no. 996.11).

A modified version of the procedure developed by Englyst and Hudson (1987) was used to analyze the total, soluble and insoluble non-starch polysaccharide (NSP) content of the test ingredients, as described by Reveco et al. (2011). Saponin content of the test ingredients was based on values found in the literature, with the exception of the values for CPC and PCPC, which were obtained from MCN BioProducts (personal communication).

4.3.6. Statistical analysis

For statistical analysis, tanks were the experimental units. Analysis of the digestibility results was performed using the General Linear Model procedure of SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Ingredient data from the two digestibility trials were analyzed separately. Results from the legume product trial were additionally analyzed using a 2x2 factorial design with product (soy vs pea) and processing (meal vs protein concentrate) as the two independent factors. Differences between means were determined using the Ryan-Einot-Gabriel-Welsh F test. Significance of results were determined when $P < 0.05$.

4.4. Results

Starch was highest in PM and PPC and present at much lower values in the other four ingredients. Phytates were highest in PPC and absent from CPC. Glucosinolates were only present in the canola products and CM had levels more than five times higher than CPC. Tannin levels were relatively consistent in the six ingredients, ranging from 4.9 g/kg in PM to 10.6 g/kg in CM. Isoflavones were present at levels less than 1 g/kg in all ingredients except SBM, which had a concentration of 53.7 g/kg. The highest saponin levels were reported for SBM.

The non-starch polysaccharide composition of the ingredients is shown in Table 3.3.3. PPC had the highest level of soluble NSP (10.95 g/kg), while the other five ingredients had relatively similar soluble NSP concentrations ranging from 5.38-6.81 g/kg. Insoluble NSP concentrations were higher than soluble NSP values for all six ingredients. CM had the highest level of insoluble NSP. CM also had the highest levels of total NSP of the ingredients (34.42 g/kg), while PM had the lowest total NSP (18.18 g/kg).

Based on the factorial analysis of the soy and pea product digestibility trial (Table 7), DM, CP, GE, lipid and amino acid digestibilities of the soy products were higher ($P < 0.05$) than those of the pea products. These parameters were also significantly improved in protein concentrates as compared with the meals.

Apparent digestibility coefficients (ADC) of DM and GE for fish fed CPC were higher ($P < 0.05$) in fish fed CPC than in those fed CM. Additionally, fish fed CPC had a phosphorus ADC that was significantly higher than fish fed CM or PCPC. CM had

superior cysteine, glutamine, glycine, histidine and methionine digestibilities to PCPC and CPC. PCPC had a higher cysteine digestibility than PCPC.

Table 4.4.1. Nutrients (g/kg, unless otherwise stated) in SBM, SPC, PM, PPC, CM, PCPC and CPC.

	SBM	SPC	PM	PPC	CM	PCPC	CPC
DM	922.0	949.7	901.2	938.5	918.2	960.5	954.3
CP	527.5	720.6	247.7	496.0	399.2	482.5	666.5
GE (kcal / kg)	4583.0	4736.4	4312.4	4698.1	4486.4	5230.0	5310.5
Ash	72.5	61.6	26.9	47.7	115.4	67.2	53.9
Lipid	35.5	10.5	24.9	48.6	58.9	172.6	74.2
Phosphorus	6.9	9.3	4.6	6.9	10.0	10.5	8.2
Total amino acids	486.1	678.8	220.3	441.2	350.7	530.5	585.9
Alanine	22.5	31.3	10.2	20.5	17.4	27.1	30.7
Arginine	38.0	53.4	21.7	43.0	23.9	35.8	40.4
Asparagine	59.6	83.7	28.2	55.7	28.0	43.7	51.6
Cystine	7.6	9.8	3.4	6.4	8.3	12.5	13.7
Glutamine	95.3	132.2	40.3	79.5	70.6	105.4	110.7
Glycine	22.0	30.6	10.4	20.5	19.8	30.4	34.9
Histidine	14.0	18.8	5.9	12.0	11.2	17.2	19.3
Isoleucine	23.7	33.6	9.9	29.7	15.5	25.4	28.7
Leucine	40.1	56.4	17.3	35.1	27.8	45.4	51.2
Lysine	32.6	46.2	17.8	36.2	22.3	30.2	32.5
Methionine	7.2	10.1	2.3	4.5	9.7	11.1	10.4
Phenylalanine	26.4	36.6	11.6	23.9	15.7	25.6	29.4
Proline	25.4	35.6	9.5	19.5	26.4	37.8	38.9
Serine	26.4	36.6	11.6	24.2	17.1	25.5	28.7
Threonine	20.4	28.5	9.0	18.3	17.3	25.3	29.0
Valine	24.8	35.3	11.2	22.2	19.8	31.8	36.7

Table 4.4.2. Antinutrients (g/kg, unless otherwise stated) in SBM, SPC, PM, PPC, CM, PCPC and CPC.

	SBM	SPC	PM	PPC	CM	PCPC	CPC
ADF[a]	60.4	131.3	77.4	76.2	178.9	n.a.[b]	34.8
NDF[c]	91.8	294.5	27.3	137.1	247.2	n.a.	61.8
Starch	51.2	25.4	486.8	307.9	26.9	3.4	9.4
Phytic acid	22.3	29.0	16.0	23.9	38.8	13.7	0.0
Glucosinolates (mmoles/kg)	n.a.	n.a.	n.a.	n.a.	71.4	n.a.	12.5
3-butenyl	n.a.	n.a.	n.a.	n.a.	20.4	n.a.	6.3
4-pentenyl	n.a.	n.a.	n.a.	n.a.	1.5	n.a.	0.8
2-OH-3-butenyl	n.a.	n.a.	n.a.	n.a.	49.2	n.a.	5.4
CH3-thiobutenyl	n.a.	n.a.	n.a.	n.a.	1.6	n.a.	0.0
Phenylethyl	n.a.	n.a.	n.a.	n.a.	1.0	n.a.	0.6
3-CH3-indolyl	n.a.	n.a.	n.a.	n.a.	2.6	n.a.	0.0
4-OH-3-CH3-indolyl	n.a.	n.a.	n.a.	n.a.	10.5	n.a.	1.5
Tannins	8.4	5.4	4.9	7.6	10.6	n.a.	6.2
Isoflavones	53.7	0.3	0.2	0.7	n.a.	n.a.	n.a.
Daidzin	21.0	0.1	0.0	0.0	n.a.	n.a.	n.a.
Glycitin	3.5	0.0	0.0	0.2	n.a.	n.a.	n.a.
Genistin	27.9	0.2	0.0	0.1	n.a.	n.a.	n.a.
Daidzein	0.2	0.0	0.2	0.0	n.a.	n.a.	n.a.
Glycitein	1.1	0.0	0.0	0.4	n.a.	n.a.	n.a.
Genistein	0.0	0.0	0.0	0.0	n.a.	n.a.	n.a.
7-Hydroxy-4-methoxyisoflavone	0.0	0.0	0.0	0.0	n.a.	n.a.	n.a.
5,7-Dihydroxy-4-methoxyisoflavone	0.0	0.0	0.0	0.0	n.a.	n.a.	n.a.
Total NSP[d]	241.3	256.3	181.8	224.2	344.2	n.a.	251.8
Saponins	3.5[e]	0.0[f]	1.8[g]	5.4[e]	3.6[h]	0.0[i]	0.0[i]

[a] Acid detergent fibre
[b] Not analyzed
[c] Neutral detergent fibre
[d] Non-starch polysaccharide
[e] Curl et al., 1985
[f] Ireland et al., 1986
[g] Heng et al., 2006
[h] Barrón-Yánez et al., 2009
[i] MCN BioProducts Inc. Saskatoon SK Canada

Table 4.4.3. Constituent sugars and non-starch polysaccharide (NSP) content of SBM, SPC, PM, PPC, CM and CPC.

	Neutral sugars (g/kg, DM basis)								Uronic acids	Total NSP
	Rha[a]	Fuc[b]	Ara[c]	Xyl[d]	Man[e]	Gal[f]	Glc[g]			
							NC[h]	Cel[i]		
Soluble NSP										
Wheat	0.00	0.00	0.55	0.92	0.08	0.23	0.54	0.00	0.23	2.55
SBM	0.11	0.03	0.47	0.23	0.36	0.65	0.21	0.00	4.51	6.58
SPC	0.00	0.03	0.49	0.12	0.26	0.41	0.00	0.00	4.62	5.93
PM	0.00	0.00	0.77	0.16	0.00	0.25	1.25	0.00	4.38	6.81
PPC	0.00	0.00	1.43	0.15	0.00	0.59	0.00	0.00	8.78	10.95
CM	0.00	0.00	0.61	0.24	0.25	0.20	0.00	0.00	5.55	6.86
CPC	0.11	0.11	0.68	0.35	0.00	0.18	0.05	0.00	3.90	5.38
Insoluble NSP										
Wheat	0.00	0.00	1.75	3.17	0.18	0.18	0.46	2.06	0.26	8.05
SBM	0.00	0.25	1.65	0.87	0.00	3.15	0.00	2.66	9.20	17.77
SPC	0.00	0.27	2.03	0.93	0.00	3.24	1.01	0.88	11.56	19.92
PM	0.00	0.00	1.94	0.85	0.00	0.27	0.16	1.54	6.62	11.37
PPC	0.00	0.00	4.57	0.48	0.00	0.52	1.94	0.00	5.90	13.41
CM	0.00	0.00	2.95	1.59	0.00	1.01	2.24	2.02	18.53	28.33
CPC	0.00	0.00	2.98	1.50	0.00	0.80	1.31	1.28	11.94	19.81
Total NSP										
Wheat	0.00	0.00	2.30	4.09	0.25	0.41	1.00	2.06	0.49	10.60
SBM	0.11	0.27	2.12	1.10	0.36	3.80	0.21	2.66	13.71	24.34
SPC	0.00	0.30	2.52	1.05	0.26	3.65	1.01	0.88	16.19	25.86
PM	0.00	0.00	2.71	1.01	0.00	0.52	1.41	1.54	11.00	18.18
PPC	0.00	0.00	6.00	0.63	0.00	1.11	1.94	0.00	14.67	24.36
CM	0.00	0.00	3.56	1.83	0.25	1.21	2.24	2.02	24.08	35.19
CPC	0.11	0.11	3.66	1.85	0.00	0.98	1.36	1.28	15.84	25.18

[a]Rhamnose
[b]Fucose
[c]Arabinose
[d]Xylose
[e]Mannose
[f]Galactose
[g]Glucose
[h]Non-cellulosic
[i]Cellulosic

Table 4.4.4. Apparent digestibility coefficients of proximate and amino acid values for the two trials. Statistics for legume product trial and canola product trial have been run separately.

	Legume product trial					Canola product trial			
	SBM	SPC	PM	PPC	SEM	CM	PCPC	CPC	SEM
DM	0.78^{c}	0.74^{bc}	0.27^{a}	0.64^{b}	0.032	0.68^{a}	0.79^{b}	0.81^{b}	0.032
CP	0.95^{c}	0.93^{bc}	0.80^{a}	0.90^{b}	0.000	0.88	0.86	0.86	0.000
GE	0.74^{b}	0.78^{b}	0.27^{a}	0.67^{b}	0.026	0.77^{a}	0.85^{b}	0.84^{b}	0.018
Lipid	0.21^{a}	1.86^{b}	0.16^{a}	0.74^{a}	0.158	0.95	1.08	0.95	0.047
Ash	0.62	0.42	0.87	0.59	0.114	0.25	0.48	0.49	0.066
Phosphorus	0.47	0.04	0.40	0.29	0.151	0.28^{a}	0.50^{a}	0.78^{b}	0.066
Total amino acids	0.96^{c}	0.94^{bc}	0.80^{a}	0.90^{b}	0.000	0.92	0.90	0.89	0.000
Alanine	0.93^{c}	0.90^{bc}	0.74^{a}	0.85^{b}	0.018	0.92	0.88	0.86	0.018
Arginine	0.97^{b}	0.97^{b}	0.88^{a}	0.95^{b}	0.000	0.93	0.91	0.92	0.000
Asparagine	0.97^{c}	0.95^{bc}	0.83^{a}	0.93^{b}	0.000	0.91	0.92	0.91	0.000
Cysteine	0.96^{b}	0.92^{b}	0.73^{a}	0.76^{a}	0.018	0.94^{c}	0.90^{b}	0.87^{a}	0.000
Glutamine	0.96^{c}	0.96^{c}	0.83^{a}	0.92^{b}	0.000	0.95^{b}	0.92^{a}	0.91^{a}	0.000
Glycine	0.95^{b}	0.91^{b}	0.82^{a}	0.90^{b}	0.018	0.91^{b}	0.88^{a}	0.86^{a}	0.000
Histidine	0.97^{c}	0.95^{bc}	0.84^{a}	0.91^{b}	0.000	0.93^{b}	0.89^{a}	0.89^{a}	0.000
Isoleucine	0.95^{c}	0.94^{c}	0.76^{a}	0.88^{b}	0.018	0.90	0.89	0.88	0.000
Leucine	0.93^{c}	0.91^{bc}	0.70^{a}	0.85^{b}	0.018	0.91	0.90	0.88	0.000
Lysine	0.97^{c}	0.96^{bc}	0.86^{a}	0.94^{b}	0.000	0.92	0.88	0.88	0.000
Methionine	0.95^{c}	0.90^{c}	0.64^{a}	0.76^{b}	0.018	0.96^{b}	0.91^{a}	0.91^{a}	0.000
Phenylalanine	0.95^{c}	0.93^{bc}	0.75^{a}	0.88^{b}	0.000	0.88	0.86	0.86	0.000
Proline	0.94^{c}	0.93^{c}	0.74^{a}	0.84^{b}	0.018	0.90	0.88	0.86	0.000
Serine	0.95^{c}	0.93^{bc}	0.78^{a}	0.89^{b}	0.000	0.90	0.89	0.88	0.000
Threonine	0.95^{c}	0.92^{bc}	0.77^{a}	0.89^{b}	0.000	0.89	0.90	0.89	0.000
Valine	0.95^{c}	0.93^{c}	0.78^{a}	0.87^{b}	0.018	0.90	0.90	0.88	0.000

^{ab} Means in the same row with different superscripts are significantly different ($P < 0.05$)
SEM=Standard error of the mean

Table 4.4.5. Apparent digestibility coefficients of proximate and amino acid values for soybean meal, soy protein concentrate, pea meal and pea protein concentrate in rainbow trout.

	Product			Processing			SEM	Product*processing
	Soybean	Pea	*P*-value	Meal	PC	*P*-value		*P*-value
Dry matter	0.76[b]	0.45[a]	0.00	0.53[a]	0.69[b]	0.00	0.032	0.00
Crude protein	0.94[b]	0.85[a]	0.00	0.88[a]	0.92[b]	0.01	0.000	0.00
Gross energy	0.76[b]	0.47[a]	0.00	0.50[a]	0.73[b]	0.00	0.026	0.00
Lipid	1.06 [b]	0.45 [a]	0.00	0.21[a]	1.30[b]	0.00	0.151	0.01
Ash	0.52	0.73	0.10	0.75	0.50	0.07	0.114	0.74
Phosphorus	0.25	0.41	0.34	0.15	0.11	0.27	0.107	0.19
Total amino acids	0.95[b]	0.85[a]	0.00	0.88[a]	0.92[b]	0.01	0.000	0.00
Alanine	0.91[b]	0.79[a]	0.00	0.83[a]	0.88[b]	0.04	0.018	0.00
Arginine	0.97[b]	0.92[a]	0.00	0.93[a]	0.96[b]	0.00	0.000	0.00
Asparagine	0.96[b]	0.88[a]	0.00	0.90[a]	0.94[b]	0.00	0.000	0.00
Cysteine	0.94[b]	0.75[a]	0.00	0.85	0.84	0.71	0.018	0.18
Glutamine	0.96[b]	0.87[a]	0.00	0.90[a]	0.94[b]	0.00	0.000	0.00
Glycine	0.93[b]	0.86[a]	0.00	0.88	0.90	0.21	0.018	0.00
Histidine	0.96[b]	0.88[a]	0.00	0.90[a]	0.93[b]	0.04	0.000	0.00
Isoleucine	0.95[b]	0.82[a]	0.00	0.86[a]	0.91[b]	0.01	0.018	0.00
Leucine	0.92[b]	0.78[a]	0.00	0.81[a]	0.88[b]	0.00	0.018	0.00
Lysine	0.97[b]	0.90[a]	0.00	0.91[a]	0.95[b]	0.00	0.000	0.00
Methionine	0.92[b]	0.70[a]	0.00	0.80	0.83	0.23	0.018	0.00
Phenylalanine	0.94[b]	0.81[a]	0.00	0.85[a]	0.90[b]	0.00	0.000	0.00
Proline	0.93[b]	0.79[a]	0.00	0.84[a]	0.88[b]	0.01	0.018	0.00
Serine	0.94[b]	0.84[a]	0.00	0.87[a]	0.91[b]	0.01	0.000	0.00
Threonine	0.93[b]	0.83[a]	0.00	0.86[a]	0.90[b]	0.01	0.000	0.00
Valine	0.94[b]	0.82[a]	0.00	0.86[a]	0.90[b]	0.02	0.018	0.00

[ab] Means in the same row with different superscripts are significantly different ($P < 0.05$)

PC=Protein concentrate

SEM=Standard error of the mean

4.5. Discussion

The nutrient digestibilities of these ingredients have been previously studied in rainbow trout. These ingredients have not all been compared with each other in the same experiment, and not all digestibility studies have been conducted in the same manner. Some differences in the way digestibility studies are conducted in aquaculture include the use of digestibility markers, such as chromic oxide, cholestane, yttrium oxide and acid insoluble ash (Allameh et al., 2007; Thiessen et al., 2004; Carter et al., 2003; Austreng et al., 2000). There are also different methods employed for fecal collection. These include fecal stripping, total collection through the bottom of the tank, the use of a settling column and the use of a rotating device that collects fish feces on a screen (Allameh et al., 2007; Glencross et al., 2005; Austreng, 1978; Bureau and Cho, 1999; Gomes et al., 1995). Slight variations also exist in the calculation of ADC for feed ingredients (Bureau and Cho, 1999; Forster, 1999; Sugiura et al., 1998; Cho et al., 1982).

Taking these differences in study approaches into consideration, the nutrient digestibility of these feed ingredients was similar to other digestibility values found in the literature (Table 3.1.7). The SBM used by Mansfield et al. (2010) was from the same lot as the SBM used in this study, with a different (fish meal-free) control diet formulation. The DM and CP digestibility values remained the same between the two trials. The energy digestibility was slightly lower in this study but within the range reported by other studies. The ingredients with the least amount of digestibility information for rainbow trout are PPC, CM and CPC. The digestibility of all macronutrients in this study, excluding ash and phosphorus, which was higher in PPC than PM, were similar to those of Thiessen et al. (2003), who found that CP, acid ether extract, starch, energy and DM

digestibility was higher in autoclaved PPC than in raw, whole peas. The DM, energy and lipid digestibilities of PM were lower than the values reported in the literature as were the DM and lipid digestibility in PPC, although this was in comparison with an autoclaved product. Similar to what was found in this study, Mwachireya et al. (1999) found the digestibility values of fish fed a canola protein isolate was higher than when they were fed CM.

The production of protein concentrates from pea, soy and canola has been shown to improve nutrient digestibility, growth performance and intestinal factors, such as morphology and microflora (Collins et al., 2012; Mansfield et al., 2010; Drew et al., 2007; Escaffre et al., 2007). In addition to increasing the protein content of a feed ingredient, protein concentration decreases many ANF, which is why protein concentrates have superior nutrient digestibilities to their respective plant meals (Drew et al., 2007).

In the soy and pea products, protein concentration also led to phytic acid concentration. This is a general event that results from protein concentration and is also seen in other feed ingredients, including CPC. The most common and effective method of eliminating phytic acid from a feed is with the use of a microbial phytase enzyme (Wang et al., 2009; Denstadli et al., 2006b; Forster, 1999). Sajjadi and Carter (2004) reported a significant improvement in phosphorus digestibility when microbial phytase was added to CM-based diets fed to Atlantic salmon. As the CPC used in this study was treated with phytase during the production process, this problem was avoided and improved the phosphorus ADC of fish fed CPC as opposed to those fed CM or PCPC.

The effect protein concentration had on tannins varied between ingredients. SPC and CPC had lower tannin values than SBM and CM, respectively, whereas the tannin level of PPC were higher than that of PM. The condensation of tannins in PPC, as opposed to SPC and CPC is expected, as free tannins are solvent-extractable (Chavan et al., 2001). SPC and CPC are both formed using aqueous solvent extraction, whereas PPC is produced via air-classification. The tannins in SPC and CPC were not completely eliminated, as some tannins also bind to protein and fibre and require a more extensive extraction procedure (Terrill et al., 1992). The proclivity tannins have for proteins also explains their condensation in PPC, as compared with PM.

Protein concentration also increased the soluble NSP in PPC, which could have been a result of protein concentration via air classification, rather than solvent extraction, as soluble NSP were not increased in SPC or CPC. The DM digestibility of CM was significantly lower than PCPC or CPC. CM contained high levels of insoluble NSP, ADF and NDF, which likely an impact on nutrient digestibility. NSP increase the viscosity of intestinal contents, cause changes in the gut microbial population, and physiologically and morphometrically altering the gastrointestinal tract, all of which impair nutrient digestibility (Sinha et al., 2011; Refstie et al., 1999). The glucosinolate levels of CM were also five times higher than those of CPC, although their mode of action is more likely to affect growth, feed intake and metabolism, not digestibility (Tripathi and Mishra, 2007; Pereira et al., 2002; Burel et al., 2001).

The CP and total amino acid digestibilities of PM were significantly lower than those of PPC, as well as the two soy ingredients, and PM tannin levels were the lowest of these four plant proteins. PM also exhibited significantly low DM, GE and lipid

digestibilities. Starch is commonly cited as an ANF that decreases the feed intake and growth rate of salmonid fish (Storebakken et al., 1998; Krogdahl et al., 2004). However, starch digestibility can be improved with moist heat, such as what is used in the extrusion process to gelatinize starch (Krogdahl et al., 2004; Pfeffer et al., 1991; Bergot and Breque, 1983). Starch was present at high levels in PM and PPC, and as the diets they were included in were cold pelleted, the starch gelatinization that would accompany extrusion did not occur. Peas are commonly high in starch (585 g/kg; Thiessen et al., 2003), but PPC starch values can be much more variable, depending on the product. Gunawardena et al. (2010) reported 681 g/kg starch in PPC, whereas the PPC used by Thiessen et al. (2003) contained 223 g/kg starch. Protein concentration reduced starch in all of the protein concentrates, although the starch content of the PPC used in this experiment was still six times that of SBM.

In spite of pre-existing digestibility values existing in the literature, there are still variations between sources of these feed ingredients. Because of this experiment, future studies involving the same lots of these ingredients were made possible (Collins et al., 2012). The results of this study were used for diet formulations in Section 3.3 of this manuscript, enabling diet formulation on a digestible nutrient basis. Antinutrient values from this experiment were also used for the subsequent nutritional modeling reported in Section 3.4, comparing dietary antinutrient composition with feed intake and growth in rainbow trout.

Relationships between antinutrients and nutrient digestibility have been established for some time. Direct effects between these factors remain to be quantified. Future studies utilizing the information obtained in this data series will involve

examining direct relationships between antinutrients and nutrient digestibility, health, and other nutritional responses seen in rainbow trout fed plant proteins, such as intestinal morphology and gut microbial populations.

Table 4.5.1. Compilation of literature values of nutrient digestibility results (DM, CP, GE) of plant-based feed ingredients fed to rainbow trout.

Ingredient	Fish weight (g)	Internal marker / collection method	Reference for digestibility calculation	DM	CP	Energy	Lipid	Reference
SBM	120	Chromic oxide / total collection through tank drain	Jobling (1994), NRC (1993), Willoughby (1993)	-	-	0.6915	-	Allameh et al. (2007)
SBM	220	Chromic oxide / total collection through tank drain	Jobling (1994), NRC (1993), Willoughby (1993)	-	-	0.6650	-	Allameh et al. (2007)
SBM	266	Chromic oxide / fecal stripping	Sugiura et al. (1998)	0.610[a]	0.921[b]	0.721	-	Glencross et al. (2005)
SBM	266	Chromic oxide / fecal stripping	Sugiura et al. (1998)	0.773[a]	0.990[b]	0.833	-	Glencross et al. (2004)
SBM	99	Yttrium oxide / fecal stripping	Austreng et al. (1978)	-	0.902[b]	0.829	0.829	Refstie et al. (2000)
SBM	42.5-170.3	Yttrium oxide / settling column	Sugiura et al. (1998)	0.712	0.901	-	-	Sugiura et al. (1998)
SBM	50	Chromic oxide / settling column	Cho and Slinger (1979)	0.692	0.873	-	0.785[c]	Dadgar et al. (2010)
SBM	40	Chromic oxide / settling column	Cho et al. (1985), Morales et al. (1994)	-	0.901	-	-	Sanz et al. (1994)
SBM	691.2	Celite / settling column	Bureau and Cho (1999)	0.79	0.95	0.83	-	Mansfield et al. (2010)
SBM	100-400	Chromic oxide / fecal stripping	Aksnes et al. (1996)	-	0.911	0.560	-	Aksnes and Opstvedt (1998)
SPC	266	Chromic oxide / fecal stripping	Sugiura et al. (1998)	0.672[a]	0.979[b]	0.873	-	Glencross et al. (2005)
SPC	266	Chromic oxide / fecal stripping	Sugiura et al. (1998)	0.820[a]	1.069[b]	0.873	-	Glencross et al. (2004)
SPC	100-400	Chromic oxide / fecal stripping	Aksnes et al. (1996)	-	0.912	0.646	-	Aksnes and Opstvedt (1998)
PM	-	Chromic oxide / rotating collection apparatus	Gomes et al. (1995)	0.661	0.804	0.592	-	Gomes et al. (1995)
Pea[d]	100	Chromic oxide / settling column	Maynard and Loosli (1969)	0.663	0.879	0.689	-	Burel et al. (2000)
PM	300	Celite / settling column	Thiessen et al. (2003)	0.421	0.909	0.546	0.718[e]	Thiessen et al. (2003)
PPC[e]	300	Celite / settling column	Thiessen et al. (2003)	0.840	0.946	0.870	0.860[e]	Thiessen et al. (2003)
CM	74.1	Chromic oxide / settling column	Cho et al. (1982)	0.498	0.881	0.556	-	Mwachireya et al. (1999)
RSM[f]	100	Chromic oxide / settling column	Maynard and Loosli (1969)	0.708	0.962	0.770	-	Burel et al. (2000)
CPI[g]	74.1	Chromic oxide / settling column	Cho et al. (1982)	0.771	0.976	0.847	-	Mwachireya et al. (1999)
CPC	106	Celite / settling column	Kleiber (1961)	0.817	0.899	0.861	-	Thiessen et al. (2004)

[a]Organic matter
[b]Energy
[c]Crude fat
[d]Extruded
[e]Acid ether extract
[e]Autoclaved
[f]Rapeseed meal (solvent-extracted)
[g]Canola protein isolate

4.6. Conclusion

In aquaculture research, particularly regarding carnivorous fish species, the general purpose of investigating plant proteins is as a fish meal replacement. These studies often include directly replacing dietary fish meal with plant proteins, or formulating these ingredients into the diet on the basis of total nutrient content of the diet. The variability in the nutrient digestibility of the seven plant-based feed ingredients investigated in this study indicates the importance of formulating aquafeeds on a digestible nutrient basis, taking into account the actual nutritional contribution each ingredient will make to the diet.

5. THE EFFECT OF INCREASING INCLUSION RATES OF SOYBEAN, PEA AND CANOLA MEALS AND THEIR PROTEIN CONCENTRATES ON THE GROWTH OF RAINBOW TROUT: CONCEPTS IN DIET FORMULATION AND EXPERIMENTAL DESIGN FOR INGREDIENT EVALUATION

This chapter was originally published in Aquaculture and is reprinted in this thesis with the permission of Elsevier. The citation for this journal article is: Collins, S.A., Desai, A.R., Mansfield, G.S., Hill, J.E., Van Kessel, A.G, Drew, M.D. 2012. The effect of increasing inclusion rates of soybean, pea and canola meals and their respective protein concentrates on the growth performance of rainbow trout: Concepts in diet formulation and experimental design for ingredient evaluation. Aquaculture. 344-349: 90-99. The purpose of this study was to determine the effects of PM, PPC, SBM, SPC, CM and CPC at inclusion rates of 0, 75, 150, 225 and 300 g/kg on rainbow trout growth performance and feed intake. These studies provide a model that allows fish nutritionists to predict rainbow trout growth when formulating aquafeeds, based on dietary inclusion levels of these plant-based feed ingredients. The information obtained in this chapter was analyzed in conjunction with the antinutrient information reported in chapter 4 to create a nutritional model showing the effects of dietary antinutrients on rainbow trout growth and feed intake, which is presented in chapter 6.

5.1. Abstract

A series of six growth experiments were conducted to assess the effects of feeding pea meal (PM), pea protein concentrate (PPC), soybean meal (SBM), soy protein concentrate (SPC), canola meal (CM) and canola protein concentrate (CPC) on the growth of rainbow trout. The nutrient digestibility of the experimental ingredients was determined prior to commencement of this experiment. Based on these digestibility values, diets containing 0, 75, 150, 225 or 300 g/kg of each test ingredient were formulated. All diets contained 17.6 MJ/kg digestible energy, 386.2 g/kg digestible crude protein and were balanced for digestible essential amino acids to meet or exceed the requirements of rainbow trout. The inclusion of fish meal in the diets was kept as constant as possible within the constraints of balancing digestible nutrients. Experiments for each ingredient were conducted consecutively over a 361-day period. During each growth experiment, three tanks of rainbow trout per treatment were fed twice daily to apparent satiety for 56 days. Fish were weighed on days 0 and 56 and total feed intake was measured. Linear and quadratic regression equations of the growth parameters on ingredient inclusion rate were calculated. The calculated regression equations for inclusion rate on average daily feed intake (ADFI), specific growth rate (SGR), feed conversion ratio (FCR) or protein efficiency ratio (PER), were not significant for PM, SPC or CPC ($P > 0.05$). PPC inclusion had a positive linear relationship with ADFI ($P < 0.05$). SBM inclusion had a significantly negative quadratic relationship with SGR and FCR, while for PER, both the linear and quadratic regressions were negative ($P < 0.05$). CM inclusion rate had a significantly negative linear and quadratic relationship with SGR and FCR. The *P*-value for the linear regression was lower than for the quadratic regression. CM also had a

negative linear relationship with PER ($P < 0.05$). The results suggest there are no significant negative ingredient effects of PM, PPC, SPC and CPC on rainbow trout growth. Thus, growth can be predicted on the basis of digestible nutrients for these ingredients, provided the nutritional standards set for this experiment are followed. SBM and CM have significantly negative ingredient effects, which must be taken into account when using these ingredients in rainbow trout diets.

5.2. Introduction

The replacement of fish meal with plant proteins is one of the major challenges facing the aquaculture industry. This is an even greater problem in carnivorous fish, such as rainbow trout. While a large number of plant protein sources are available, a high proportion of research has concentrated on soybean meal (SBM). SBM contains 462-562 g/kg crude protein (Oliva-Teles et al., 1994; Øverland et al., 2009; Refstie et al., 2005, 1998, 1997) and is high in lysine (58.8-75 g/kg) (Øverland et al., 2009; Refstie et al., 2005; Watanabe et al., 1993). More than 65 studies have examined the effect of SBM on the growth of salmonid fish. Most of these studies report increasing SBM inclusion in rainbow trout diets decreases growth, feed intake and feed efficiency (Gao et al., 2011; Refstie et al., 2001, 1998; Torstensen et al., 2008). It should be noted, however, that this trend is not universally observed (Selden et al., 2001; Vielma et al., 2000). The negative effect of SBM on growth is attributed to the anti-nutritional factors (ANF) in SBM such as protease inhibitors, tannins, lectins and non-starch polysaccharides (Francis et al., 2001). The specific ANF responsible for these effects and their mode of action are poorly characterized (Barrows et al., 2007; Penn et al., 2011). In addition to reducing growth, high inclusion levels of SBM in rainbow trout diets also affect gut health negatively

(Krogdahl et al., 2003) and there is an extensive body of research investigating which ANF are the causative agents (Barrows et al., 2007; Knudsen et al., 2008; Olli and Krogdahl, 2008; Penn et al., 2011; Sørensen et al., 2011). The primary mode of action appears to be initiating a cascade of intestinal inflammatory responses (Mansfield et al., 2010), including the infiltration of the lamina propria by inflammatory cells (Burrells et al., 1999; Refstie et al., 2000), shortening of villi (Heikkinen et al., 2006; Merrifield et al., 2009) and increased enterocyte turnover (Baeverfjord and Krogdahl, 1996; Bakke-McKellep et al., 2000; Krogdahl et al., 2003).

The nutritive value of SBM can be improved using fractionation to produce soy protein concentrate (SPC), which contains approximately 700 g/kg crude protein (Vielma et al., 2002). Furthermore, the concentrations of ANF in SPC are lower than for SBM (Bureau et al., 1998; Deng et al., 2006; Refstie et al., 2001). Thus, the growth of rainbow trout is less affected by the inclusion of SPC into diet formulations (Barrows et al., 2007; Storebakken et al., 2000, 1998).

While SBM is the most extensively studied of plant protein sources, other ingredients have also been widely used in diets fed to rainbow trout. Field peas are grown throughout the world and pea meal (PM) has an amino acid balance similar to SBM; however, PM contains only 156-325 g/kg crude protein and is high in starch (557-584 g/kg) (Castell et al., 1996; Thiessen et al., 2003), a putative ANF for rainbow trout. Both SBM and PM are legumes and contain similar ANF, such as non-starch polysaccharides, protease inhibitors, lectins, phytic acid and tannins (Castell et al., 1996; Francis et al., 2001). The levels of these ANF in PM are generally lower than in SBM (Castell et al., 1996).

Studies investigating the effects of feeding PM to rainbow trout have reported both positive (Thiessen et al., 2003) and negative (Alami-Durante et al., 2010; De Francesco et al., 2004; Drew et al., 2005) effects on growth, although these negative growth effects are not as marked as those seen when SBM is fed. The low protein content of PM can be increased by air classification to produce pea protein concentrate (PPC), which contains 359-502 g/kg crude protein and 79-248 g/kg starch (Øverland et al., 2009; Thiessen et al., 2003). Studies on the use of PPC in salmonid diets have shown a mostly neutral (Gao et al., 2011; Moreno-Rojas et al., 2008) or positive (Carter and Hauler, 2000; Øverland et al., 2009) effects on growth, although at high inclusion levels, growth is negatively impacted (Carter & Hauler, 2000) and sub-acute intestinal enteritis is a consequence (Penn et al., 2011).

Canola (low glucosinolate rapeseed) meal (CM) has also been widely investigated as a protein source for rainbow trout. CM contains 355-453 g/kg crude protein (Burel et al., 2000; Hilton and Slinger, 1986; Leatherland and Hilton, 1988; Satoh et al., 1998; Shafaeipour et al., 2008) and has one of the best amino acid balances of commercially available plant proteins (protein efficiency ratio (PER) = 3.29; Friedman, 1996). Canola and rapeseed are of the *Brassica* genus and as such, have some different ANF, such as glucosinolates and euricic acid, and phenolic compounds, such as free phenolic acid and sinapic acid (Enami, 2011; Naczk et al., 1998; Webster et al., 1997). It also contains approximately 130 g/kg crude fibre, which reduces its nutritional value (Brown et al., 2003; Burel et al., 2000; McCurdy and March, 1992; Shafaeipour et al., 2008). CM has been extensively researched and its effect on the growth of rainbow trout has been found to be almost exclusively negative (Alami-Durante et al., 2010; Burel et al., 2001, 2000;

De Francesco et al., 2004; Drew et al., 2005; Hilton and Slinger, 1986; Leatherland and Hilton, 1988; Satoh et al., 1998). A few studies are exceptions to this trend (Burel et al., 2001; Shafaeipour et al., 2008).

Protein fractionation of CM yields canola protein concentrate (CPC), a product with 464-724 g/kg crude protein and reduced levels of ANF, particularly fibre (Forster et al., 1999; McCurdy and March, 1992; Thiessen et al., 2004). CPC positively (Thiessen et al., 2004) and negatively (Drew et al., 2007; Forster et al., 1999; Stickney et al., 1996; Thiessen et al., 2004) affects the growth of rainbow trout. The composition of CPC used in various experiments varies widely depending on the method used to concentrate the protein, as can be seen when comparing the products studied by Higgs et al. (1982), Forster et al. (1999), McCurdy and March (1992) and Thiessen et al. (2004).

While soy, pea and canola are not an exhaustive list of plant protein ingredients used in rainbow trout diets, these three ingredients may serve well as model ingredients for examining the effect of plant proteins on the growth of rainbow trout and other species of fish. PM and SBM have a similar amino acid balance and a number of ANF in common, but they differ in protein and ANF concentrations. CM is different in almost all respects from SBM and PM. Their respective protein concentrates also provide interesting contrasts in nutritive and anti-nutritive content.

Many studies have investigated feeding these ingredients to rainbow trout, but have used many different methodologies, making direct comparisons of the effects of these ingredients difficult. Differences in test ingredient inclusion rate, control diet composition (including level of fish meal inclusion) and the ingredient(s) replaced by the

addition of the plant protein make it difficult to determine the overall nutritional value of these ingredients.

We hypothesized measuring the effect of increasing inclusion rates of SBM, PM and CM and their respective protein concentrates, while maintaining the level of fish meal in the diets would allow a better comparison of the effects of these ingredients on the growth of rainbow trout. Therefore, an experiment was performed to determine the effect of inclusion rates of 0, 75, 150, 225 and 300 g/kg of PM, PPC, SBM, SPC, CM and CPC on the growth of rainbow trout.

5.3. Materials and methods

5.3.1. Fish husbandry

The experiment was conducted at the Prairie Aquaculture Research Centre (University of Saskatchewan, Saskatoon, SK, Canada), a biologically filtered, semi-closed recirculating aquaculture system operating under the University of Saskatchewan Committee on Animal Care and Supply Protocol #19980142. The fish were triploid female rainbow trout (*Oncorhynchus mykiss*), acquired from Wild West Steelhead (Lucky Lake, SK, Canada). Water temperature was maintained at 15 ± 1 °C and the photoperiod was 14 h light:10 h dark. Environmental and water quality indicators were closely monitored over the course of the experiment. A commercial fish meal diet was fed for two weeks prior to the experiment to acclimate the fish to their environment and was also fed to the fish for a minimum of one week between trials. The guidelines set by the CCAC (1993, 2005) were followed in the maintenance of all fish for the duration of this experiment. No major health issues were encountered over the course of this experiment. There were no disease outbreaks, no problems with feed acceptance and mortality was below 8%.

5.3.2. Experimental design

The experimental ingredients were: SBM (Federated Cooperatives Limited, Saskatoon, SK, Canada), SPC (Soycomil K; ADM Specialty Ingredients (Europe) BV, Koog aan de Zaan, The Netherlands), PM (yellow field pea, CDC Mozart, Crop Development Centre, Saskatoon, SK, Canada), PPC (yellow field pea, prestige protein, Parrheim Foods, Saskatoon, SK, Canada), CM (canola meal-35; Federated Co-Operative Ltd., Saskatoon, SK, Canada) and an aqueous-extracted CPC (Can Pro IP; CanPro Ingredients Ltd., Arborfield, SK, Canada).

Six growth trials were conducted, with one for each of the six ingredients. The order of plant protein sources was randomized and the meals and their respective protein concentrates were fed in succession (Table 5.3.1). In each trial, the fish were fed five diets with identical nutritional compositions but increasing levels of the test ingredient (0, 75, 150, 225 or 300 g/kg) (Tables 5.3.2 and 5.3.3). The 0 and 300 g/kg diets were formulated independently, then combined in relative proportions to yield the 75, 150 and 225 g/kg diets. All diets were formulated on a digestible nutrient basis, using the digestibility data reported in section 4.0. Diets contained 17.6 MJ/kg digestible energy and 386.2 g/kg digestible crude protein (Table 5.3.4). Diets were balanced for essential amino acids according to Mambrini and Guillaume (1999) and met or exceeded rainbow trout nutrient requirements (NRC, 1993). The level of fish meal in the diets was kept as constant as possible, within the constraints of balancing the diets on digestible nutrient levels.

Diets were mixed on a Hobart mixer (Hobart Corporation; Model L-800; Troy, OH, USA) and cold extruded through a 5-mm die on a Hobart mixer (Hobart Corporation; Model 4822; Troy, OH, USA), dried in a forced air oven for 12 h at 55ºC, then chopped and screened to form pellets of uniform size.

For each 56-day trial, fifteen 360 L tanks were used, with three replicates per treatment. The diets were randomly assigned to the tanks and hand-fed twice daily to apparent satiation. The amount of feed consumed per tank was recorded for the entirety of each trial. The same fish were used throughout the experiment and were re-randomized by tank and diet before each experiment. For each trial, each tank of fish was weighed on days 0 and 56 and total feed intake was measured. Following the feeding period, three fish per tank were euthanized by a sharp blow to the head. Each fish and their liver were weighed to calculate hepatosomatic index ([wet liver weight/wet body weight] × 100).

Table 5.3.1. Timeline, distribution of fish per tank and average weights (g) of fish during the six growth trials.

Trial	n[a]	Start (d)	End (d)	Initial weight ± SD[b]
PM[c]	22	0	56	235.2 ± 17.1
PPC[d]	22	1	57	237.7 ± 21.7
SBM[e]	17	130	186	553.0 ± 44.6
SPC[f]	17	166	222	610.8 ± 22.7
CM[g]	16	192	248	639.8 ± 77.5
CPC[h]	17	305	361	824.6 ± 82.8

[a] n=fish/tank.
[b] SD=Standard deviation.
[c] Pea meal.
[d] Pea protein concentrate.
[e] Soybean meal.
[f] Soy protein concentrate.
[g] Canola meal.
[h] Canola protein concentrate.

Table 5.3.2. Ingredient composition of experimental diets for SBM, PM and CM growth trials.

Ingredient (g/kg)	0 g/kg	75 g/kg	150 g/kg	225 g/kg	300 g/kg
SBM					
Soybean meal[a]	0.00	75.00	150.00	225.00	300.00
Fish meal[b]	390.00	367.41	344.82	322.23	299.64
Meat and bone meal[c]	224.80	180.06	135.32	90.57	45.83
Fish oil[d]	141.34	142.64	143.94	145.24	146.54
Alpha-cellulose[e]	117.94	111.00	104.07	97.13	90.19
Wheat flour[f]	100.00	100.00	100.00	100.00	100.00
Corn gluten meal	11.16	9.03	6.90	4.76	2.63
Vitamin premix[g]	4.75	4.75	4.75	4.75	4.75
Mineral premix[h]	4.75	4.75	4.75	4.75	4.75
Choline chloride[i]	4.00	4.00	4.00	4.00	4.00
DL-Methionine[j]	0.75	0.86	0.96	1.07	1.17
Vitamin C[k]	0.50	0.50	0.50	0.50	0.50
PM					
Pea meal[l]	0.00	75.00	150.00	225.00	300.00
Fish meal[b]	390.00	389.28	388.55	387.83	387.10
Meat and bone meal[c]	224.80	168.60	112.40	56.20	0.00
Fish oil[d]	141.34	145.08	148.83	152.57	156.31
Alpha-cellulose[e]	117.94	88.46	58.97	29.49	0.00
Wheat flour[f]	100.00	86.89	73.78	60.67	47.56
Corn gluten meal	11.16	32.13	53.10	74.07	95.04
Vitamin premix[g]	4.75	4.75	4.75	4.75	4.75
Mineral premix[h]	4.75	4.75	4.75	4.75	4.75
Choline chloride[i]	4.00	4.00	4.00	4.00	4.00
DL-Methionine[j]	0.75	0.56	0.38	0.19	0.00
Vitamin C[k]	0.50	0.50	0.50	0.50	0.50
CM					
Canola meal[m]	0.00	75.00	150.00	225.00	300.00
Fish meal[b]	400.00	413.20	426.40	439.60	452.80
Meat and bone meal[c]	304.00	228.00	152.00	76.00	0.00
Fish oil[d]	120.50	111.28	102.05	92.83	83.60
Wheat flour[e]	100.00	100.00	100.00	100.00	100.00
Alpha-cellulose[f]	57.80	54.40	51.00	47.60	44.20
Vitamin premix[g]	4.75	4.75	4.75	4.75	4.75
Mineral premix[h]	4.75	4.75	4.75	4.75	4.75
Choline chloride[i]	4.00	4.00	4.00	4.00	4.00
L-Lysine[n]	1.90	2.73	3.55	4.38	5.20
DL-Methionine[k]	1.80	1.40	1.00	0.60	0.20
Vitamin C[l]	0.50	0.50	0.50	0.50	0.50

[a] Soybean meal; Federated Cooperatives Limited, Saskatoon, SK, Canada.
[b] SBM and PM diets: Nova Scotia herring meal; Shur-Gain Aquaculture, Truro, NS, Canada. CM diets: West Coast Fish Meal; Federated Co-Operative Ltd., Saskatoon, SK, Canada.
[c] Saskatoon Processing Co.; Saskatoon Processing Co., Saskatoon, SK. Canada.
[d] SBM and PM diets: Danish Fish Oil; FF of Denmark, Skagen, Denmark. CM diets: Mixed Species Fish Oil; Bioriginal Food and Science Corp., Saskatoon, SK, Canada.
[e] Solka-floc, 200 FCC; International fiber corporation, North Tonawanda, NY, USA.
[f] Robin Hood All-Purpose Flour; Robin Hood Multifoods Corporation, Markham, ON, Canada.
[g] DL-methionine, feed grade. Degussa Corporation, Theodore, AL, USA.
[h] Vitamin premix, commercial (EWOS FISH-STR VIT PX, Surrey, BC; closed formulation), formulated to meet the requirements of juvenile rainbow trout; BASF Canada, Surrey, BC, Canada.
[i] 60% Choline Chloride; Chinook Group Limited Partnership, Sombra, ON, Canada.
[j] DL-methionine, feed grade. Degussa Corporation, Theodore, AL, USA.
[k] Ascorbic acid, pharmaceutical grade; NOW Foods, Bloomingdale, IL, USA.
[l] Yellow field pea, CDC Mozart; Crop Development Centre, Saskatoon, SK, Canada.
[m] Canola Meal-35; Federated Co-Operative Ltd., Saskatoon, SK, Canada.
[n] L-lysine HCl 788 g/kg; Archer Daniels Midland Company, Decatur, IL, USA.

Table 5.3.3. Ingredient composition of experimental diets for SPC, PPC and CPC growth trials.

Ingredient (g/kg)	0 g/kg	75 g/kg	150 g/kg	225 g/kg	300 g/kg
SPC					
Soy protein concentrate[l]	0.00	75.00	150.00	225.00	300.00
Fish meal[b]	400.00	354.29	308.59	262.88	217.17
Meat and bone meal[c]	304.00	250.41	196.82	143.22	89.63
Fish oil[d]	120.50	132.84	145.18	157.52	169.86
Wheat flour[e]	100.00	100.00	100.00	100.00	100.00
Alpha-cellulose[f]	57.80	69.18	80.57	91.95	103.33
Vitamin premix[g]	4.75	4.75	4.75	4.75	4.75
Mineral premix[h]	4.75	4.75	4.75	4.75	4.75
Choline chloride[i]	4.00	4.00	4.00	4.00	4.00
L-Lysine[j]	1.90	2.17	2.45	2.72	2.99
DL-Methionine[k]	1.80	2.11	2.41	2.72	3.02
Vitamin C[l]	0.50	0.50	0.50	0.50	0.50
PPC					
Pea protein concentrate[m]	0.00	75.00	150.00	225.00	300.00
Fish meal[b]	390.00	361.35	332.71	304.06	275.41
Meat and bone meal[c]	224.80	182.66	140.52	98.38	56.24
Fish oil[d]	141.34	136.51	131.69	126.86	122.03
Alpha-cellulose[e]	117.94	105.36	92.79	80.21	67.63
Wheat flour[f]	100.00	100.00	100.00	100.00	100.00
Corn gluten meal	11.16	24.05	36.93	49.82	62.70
Vitamin premix[g]	4.75	4.75	4.75	4.75	4.75
Mineral premix[h]	4.75	4.75	4.75	4.75	4.75
Choline chloride[i]	4.00	4.00	4.00	4.00	4.00
DL-Methionine[j]	0.75	1.06	1.38	1.69	2.00
Vitamin C[k]	0.50	0.50	0.50	0.50	0.50
CPC					
Canola protein concentrate[n]	0.00	75.00	150.00	225.00	300.00
Fish meal[b]	400.00	401.96	403.92	405.87	407.83
Meat and bone meal[c]	304.00	228.00	152.00	76.00	0.00
Fish oil[d]	120.50	108.95	97.39	85.84	74.28
Wheat flour[e]	100.00	100.00	100.00	100.00	100.00
Alpha-cellulose[f]	57.80	67.31	76.82	86.33	95.84
Vitamin premix[g]	4.75	4.75	4.75	4.75	4.75
Mineral premix[h]	4.75	4.75	4.75	4.75	4.75
Choline chloride[i]	4.00	4.00	4.00	4.00	4.00
L-Lysine[j]	1.90	3.08	4.27	5.45	6.63
DL-Methionine[k]	1.80	1.71	1.61	1.52	1.42
Vitamin C[l]	0.50	0.50	0.50	0.50	0.50

[a] Soycomil K; ADM Specialty Ingredients (Europe) BV, Koog aan de Zaan, The Netherlands.
[b] PPC diets: Nova Scotia herring meal; Shur-Gain Aquaculture, Truro, NS, Canada. SPC and CPC diets: West Coast Fish Meal; Federated Co-Operative Ltd., Saskatoon, SK, Canada.
[c] Saskatoon Processing Co.; Saskatoon Processing Co., Saskatoon, SK. Canada.
[d] SPC diets: Danish Fish Oil; FF of Denmark, Skagen, Denmark. PPC and CPC diets: Mixed Species Fish Oil; Bioriginal Food and Science Corp., Saskatoon, SK, Canada.
[e] Solka-floc, 200 FCC; International fiber corporation, North Tonawanda, NY, USA.
[f] Robin Hood All-Purpose Flour; Robin Hood Multifoods Corporation, Markham, ON, Canada.
[g] DL-methionine, feed grade. Degussa Corporation, Theodore, AL, USA.
[h] Vitamin premix, commercial (EWOS FISH-STR VIT PX, Surrey, BC; closed formulation), formulated to meet the requirements of juvenile rainbow trout; BASF Canada, Surrey, BC, Canada.
[i] 60% Choline Chloride; Chinook Group Limited Partnership, Sombra, ON, Canada.
[j] L-lysine HCl 788 g/kg; Archer Daniels Midland Company, Decatur, IL, USA.
[k] DL-methionine, feed grade. Degussa Corporation, Theodore, AL, USA.
[l] Ascorbic acid, pharmaceutical grade; NOW Foods, Bloomingdale, IL, USA.
[m] Pea protein concentrate, yellow field pea, prestige protein; Parrheim Foods, Saskatoon, SK, Canada.
[n] Can Pro IP; CanPro Ingredients Ltd., Saskatoon, SK, Canada.

Table 5.3.4. Digestible nutrient composition unless otherwise stated of reference (0 g/kg test ingredient inclusion) and 300 g/kg test ingredient inclusion diets for each growth trial[a].

	Reference[b]	PM	PPC	SBM	Reference[c]	SPC	CM	CPC
Total dry matter (g/kg)	963.7	974.2	970.1	957.1	979.8	968.3	960.6	949.3
Crude protein (g/kg)	386.2	386.2	386.2	386.2	386.2	386.2	386.2	386.2
Gross energy (MJ / kg)	17.6	17.6	17.6	17.6	17.6	17.6	17.6	17.6
Total ash (g/kg)	138.8	65.2	69.8	77.1	100.7	82.0	118.3	100.8
Total lipid (g/kg)	228.5	195.7	187.0	205.1	211.6	220.9	161.9	151.7
Phosphorus (g/kg)	20.7	12.0	12.0	10.0	24.6	10.0	11.7	10.5
Amino acids (g/kg)								
Arginine	25.9	26.1	28.7	27.3	25.7	27.4	24.0	23.7
Cystine	3.6	4.2	4.3	4.6	3.4	4.5	4.5	4.2
Isoleucine	16.6	17.4	17.0	17.0	15.7	17.2	15.8	15.4
Lysine	29.2	29.2	29.2	29.2	29.2	29.2	29.2	29.2
Methionine	10.8	10.9	8.9	9.4	9.9	7.7	6.7	6.1
Threonine	16.9	17.3	16.6	17.0	16.1	15.7	16.2	16.4
Valine	21.5	21.4	20.8	21.1	20.7	20.0	19.2	19.3
Total starch (g/kg)	146.2	253.4	207.4	134.6	127.0	139.8	146.4	130.2

[a] All intermediate diets were produced by combining the reference and 300 g/kg diets in relative proportions.
[b] Reference diet fed as control with PM, PPC and SBM.
[c] Reference diet fed as control with SPC, CM and CPC.

5.3.3. Statistical analysis

Tank was considered the experimental unit. Linear and quadratic regression equations of the growth parameters on ingredient inclusion rate were calculated using SPSS and the regressions were considered significant when $P < 0.05$. Data were further analyzed as a completely randomized design using the General Linear Model procedure of IBM SPSS Statistics (Version 19.0.0, SPSS Inc., Chicago, IL, USA). The Ryan-Einot-Gabriel-Welsh F test was used to determine differences between means, with significance being attributed to $P < 0.05$.

5.4. Results

Linear and quadratic regression equations of ADFI, SGR, FCR and PER on inclusion rate were not significant for PM, SPC or CPC (Figures 3.2.1, 3.2.2, 3.2.3 and 3.2.4). PPC inclusion had a significantly positive linear relationship with ADFI ($P < 0.05$). SBM inclusion had a significantly negative quadratic relationship with SGR and FCR while for

PER, both the linear and quadratic regressions were negative and significant ($P < 0.05$). The inclusion rate of SBM resulting in 95% of the control SGR was 22 g/kg; for PER 33 g/kg and the inclusion rate of SBM resulting in 105% of the control FCR was 27 g/kg. CM inclusion had a significantly negative linear and quadratic relationship with SGR and FCR and the *P*-value for the linear regression was lower than for the quadratic regression. CM also had a significant, negative linear relationship with PER ($P < 0.05$). The inclusion rate of CM resulting in 95% of the control SGR was 3 g/kg; for PER, 4 g/kg and the inclusion rate of CM resulting in 105% of the control FCR was 2 g/kg. There was no significant relationship between inclusion rates of any of the ingredients and HSI.

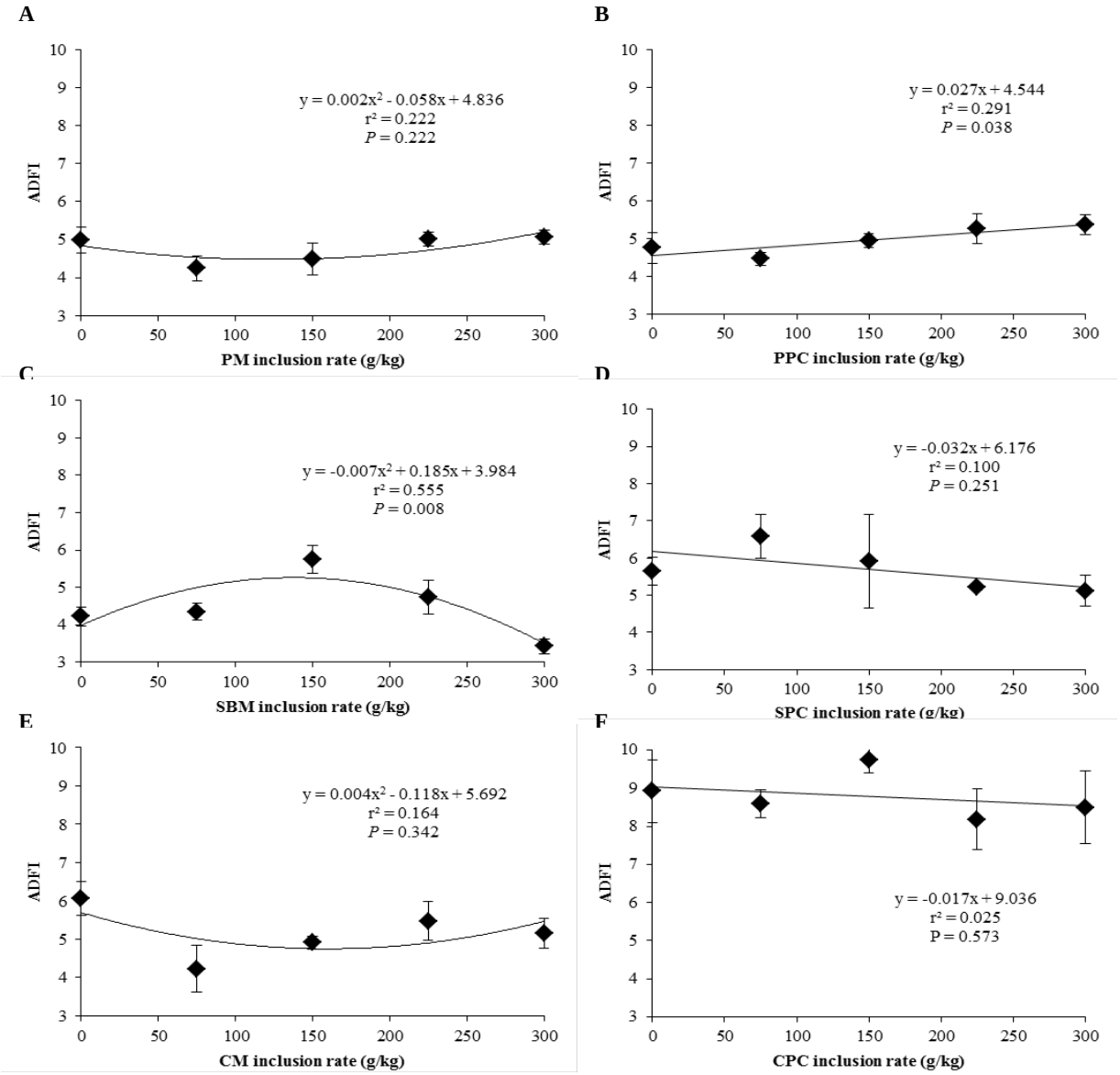

Figure 5.4.1. Regression analysis of ingredient inclusion level on ADFI±SEM in rainbow trout. (A) PM (B) PPC (C) SBM (D) SPC (E) CM (F) CPC.

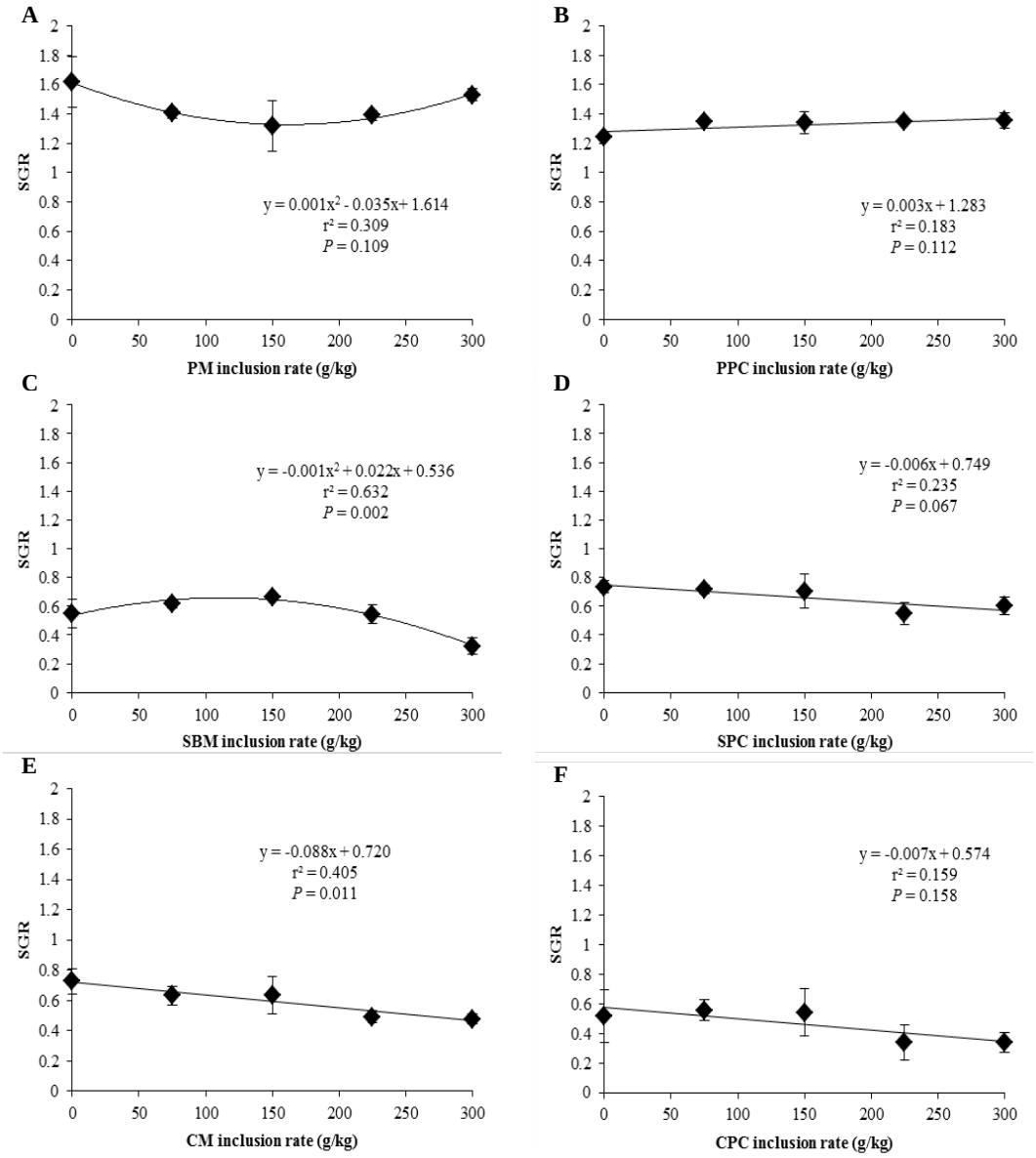

Figure 5.4.2. Regression analysis of ingredient inclusion level on SGR±SEM in rainbow trout. (A) PM (B) PPC (C) SBM (D) SPC (E) CM (F) CPC.

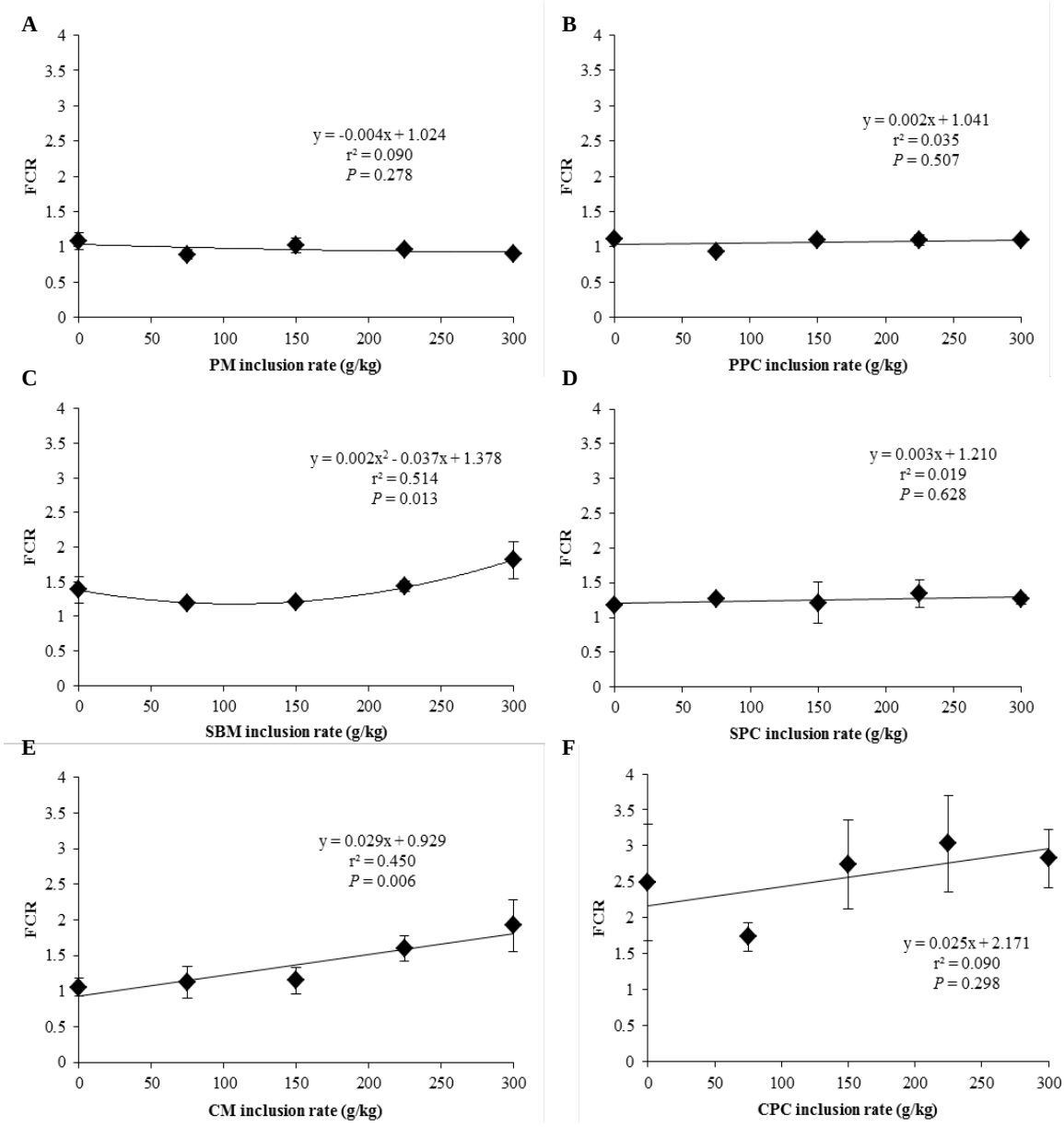

Figure 5.4.3. Regression analysis of ingredient inclusion level on FCR±SEM in rainbow trout. (A) PM (B) PPC (C) SBM (D) SPC (E) CM (F) CPC.

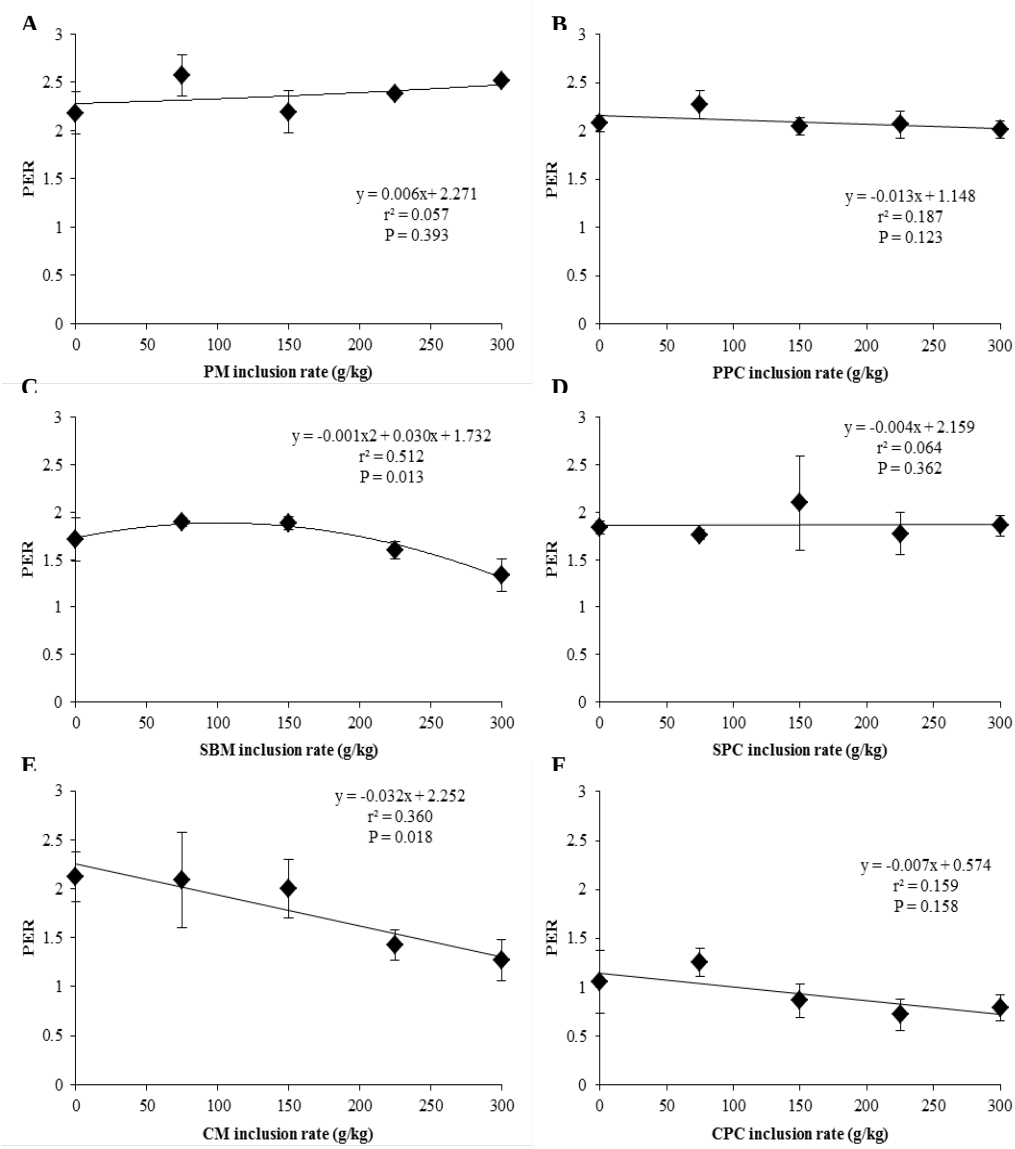

Figure 5.4.4. Regression analysis of ingredient inclusion level on PER±SEM in rainbow trout. (A) PM (B) PPC (C) SBM (D) SPC (E) CM (F) CPC.

5.5. Discussion

The same fish were used throughout the experiment and start weights ranged from 235.2 g for the PM experiment to 824.6 g for the CPC experiment. Generally, SGR decreased as body weight increased. This agrees with previous studies (Jobling, 1983a, 1983b). Because the effect of fish age and body weight on growth was constant within each trial, while the means of growth parameters cannot be compared, the slopes of the regression equations should not have been affected by initial body weight and are most probably comparable.

The effect of diet on the growth of rainbow trout can be attributed to nutrient and ingredient effects. Two diets that are equivalent in digestible nutrients would be expected to support the same level of growth. Ingredients also have significant effects on the growth of rainbow trout. Fish meal is a highly palatable and digestible source of proteins and lipids and is also reported to contain pro-nutritional factors, which promote the health and growth of fish and are absent in plant-based ingredients. Known factors include taurine (Gaylord et al., 2006; Lunger et al., 2007), cholesterol (Gómez-Requeni et al., 2004; Kaushik et al., 1995), nucleotides (Burrells et al., 2001a, 2000b; Li and Gatlin, 2006) and essential fatty acids (Steffens, 1997). There are also uncharacterized factors associated with low molecular weight fractions in fish meal (Aksnes et al., 2006a, 2006b, 2006c; Kousoulaki et al., 2009) that appear to have beneficial effects on fish growth. In contrast, plant ingredients contain ANF (Francis et al., 2001), which reduce fish growth to a level below that which would be predicted based on dietary digestible nutrient composition.

The diets used in the current studies were formulated to keep fish meal inclusion as constant as possible, within the constraint of balancing diets on digestible nutrients. Corn gluten meal and meat and bone meal were replaced with increasing inclusion levels of the test ingredients. Both of these ingredients were assumed to have neutral effects on fish growth, due to their reported lack of pro- and antinutritional factors (Alexis et al., 1985; Bureau et al., 2000; Hardy, 2000; Yu, 2004).

Most previously conducted studies on these six ingredients replaced fish meal with the respective ingredient (Alami-Durante et al., 2010; Carter and Hauler, 2000; Forster et al., 1999; Gao et al., 2011; Gomes et al., 1993; Hilton and Slinger, 1986; Satoh et al., 1998; Shafaeipour et al., 2008; Stickney et al., 1996). This has the potential to confound the effect of the plant and marine ingredients on growth. Furthermore, many studies balance diets for levels of total nutrients (Denstadli et al., 2007; Mambrini et al., 1999; Refstie et al., 2000; Rumsey et al., 1994; Stickney et al., 1996; Yamamoto et al., 2002), rather than digestible nutrients. This complicates the interpretation of data, as extensive research (Burel et al., 2000; Drew et al., 2007, 2005; Øverland et al., 2009) shows the variability in nutrient digestibility for different ingredients.

In the present study, only SBM and CM had negative effects on growth. This agrees with most previous studies evaluating these ingredients. SBM has been reported to decrease the growth of rainbow trout significantly at inclusion levels from 14-780 g/kg (Barrows et al., 2007; Brinker and Reiter, 2011; Davies and Morris, 1997; Lee et al., 2002; Refstie et al., 2005; Rumsey et al., 1994; Torstensen et al., 2008). Likewise, CM has generally been reported to decrease the growth of rainbow trout at dietary inclusion levels from 47-500 g/kg, (Alami-Durante et al., 2010; Burel et al., 2000; De Francesco et

al., 2004; Drew et al., 2005; Hilton and Slinger, 1986; Satoh et al., 1998). However, several studies have reported that CM inclusion from 100-300 g/kg had no effect on growth (Abdou Dade et al., 1990; Burel et al., 2001; Thiessen et al., 2003).

Studies on the use of PM in rainbow trout also report conflicting results. The effect of PM on SGR was reported to be negative at levels ranging from 80-163 g/kg (Alami-Durante et al., 2010; De Francesco et al., 2004). Other studies have reported no effect of PM on the SGR of rainbow trout at inclusion rates of 120-250 g/kg (Drew et al., 2005; Thiessen et al., 2003). In the present study, PM had no significant effect on SGR or other growth parameters at levels up to 300 g/kg in the diet. This effect may have been due to the relatively constant level of fish meal in the test diets. In comparison, previous studies have replaced fish meal with PM and thus, the reported decreases in growth might have been due to decreased levels of fish meal in the diets. Thus, the neutral effect of PM on the growth of rainbow trout in this study may be more representative of the true feeding value of PM.

The three protein concentrates had no effects on rainbow trout growth at levels of up to 300 g/kg. Studies on the effect of PPC on the growth of rainbow trout generally report no significant effects at levels up to 210 g/kg (Gao et al., 2011; Moreno-Rojas et al., 2008; Øverland et al., 2009; Penn et al., 2011; Thiessen et al., 2003). At inclusion levels from 276-350 g/kg, PPC does have a negative effect on growth (Carter and Hauler, 2000; Penn et al., 2011). The present study reported no significant effect of PPC on SGR at levels up to 300 g/kg while feed intake did increase significantly with increasing inclusion of PPC. This did not result in a significant increase in FCR. In combination

with previous results, the present results suggest 300 g/kg may be the maximum inclusion rate for PPC in rainbow trout diets.

In the present study, SPC had no effect on the growth of rainbow trout. Previous studies on SPC report variable results. Vielma et al. (2000) fed 315 g/kg SPC to rainbow trout and reported a significant increase in growth compared to controls. Mambrini et al. (1999) fed 320 g/kg of SPC and reported no effect on fish growth. A large number of studies reported at inclusion levels of 159-637 g/kg, SPC reduced the growth of rainbow trout compared to controls (Barrows et al., 2007; Brinker and Reiter, 2011; Stickney et al., 1996). Several different methods are used to produce SPC, including aqueous and ethanol fractionation. This results in SPCs with varying nutrient and ANF content, which may explain conflicting results between studies.

Variability in the production and chemical composition of CPC is even greater than for SPC. Many different methods of fractionation have been used to produce CPC for rainbow trout growth experiments. This variation in CPC is reflected in marked differences in the effect of CPC on the growth of rainbow trout. Thiessen et al. (2004) reported that inclusion rates of 60-490 g/kg CPC had no significant effect on SGR. Other studies have reported that CPC inclusion levels from 193-527 g/kg reduce rainbow trout growth, as compared with a control (Forster et al., 1999; Stickney et al., 1996). The major difference between these studies is the use of dephytinized CPC by Thiessen et al. (2004). This suggests that phytate, when present in CPC, may be an important ANF, inducing negative effects on growth. In support of this notion, dephytinized CPC was used in the current study and no negative effect of CPC on was seen at inclusions up to 300 g/kg.

The large body of literature on the use of plant proteins to replace marine proteins in salmonid diets is difficult to assess due to differences in experimental methodologies. The current study attempted to compare all of the six ingredients using diets with identical concentrations of digestible nutrients and similar levels of fish meal. This allowed a comparison of these ingredients in the absence of confounding dietary factors. However, the goal of practical diet formulation is the replacement of fish meal with plant ingredients. Thus, the inclusion level of fish meal will decrease with increasing levels of plant proteins. This will result in an interaction between the pronutritional effects of fish meal and the antinutritional effects of plant. Moreover, the effect of feeding two or more plant protein sources may result in significant interactions between plant ingredients and further complicate things. To successfully address these issues, the development of consistent experimental methods to determine the true effects of and interactions between ingredients need to be developed. This will allow a better comparison of results generated by different laboratories and increase the rate of progress in this important are of research.

5.6. Conclusions

The equations formulated in this experiment can be used to predict the ADFI, SGR, FCR and PER of rainbow trout, provided the diets are formulated on a digestible nutrient basis and the nutritional standards set in this experiment are followed. Problematic performance curves (ADFI, SGR, FCR and PER) were seen in the SBM and CM trials, which indicate dietary formulation on a digestible nutrient basis is not adequate to maintain performance similar to that of fish fed a control diet. In the cases of the PM, PPC, SPC and CPC trials, no significant regressions were noted, excluding PPC, which

had a positive effect on ADFI. As SPC and CPC did not exhibit the same drop in performance as that seen in fish fed SBM and CM, it is apparent that nutrient digestibility is not the only germane factor requiring consideration for these two plant-based feed ingredients. Plant meals and plant protein concentrates differ in their ANF content and it is this difference that made influenced whether or not the fish who consumed them were capable of performing as well as fish fed a control. Although the six trials were not conducted during the same moment in time, nor with fish of identical start weights, many of the consistencies in experimental practices, such as dietary formulation and experimental design produced results that are comparable in ways not possible between data from trials with grossly differing experimental methodologies. The impact of these feed ingredients as individual entities on the parameters measured in these fish illustrate how they influence performance. When viewed in conjunction, the full effect and implications of feeding these six plant-based feed ingredients and variations in their influence can be evaluated.

6. STRUCTURAL EQUATION MODELING OF ANTINUTRIENTS IN RAINBOW TROUT DIETS AND THEIR IMPACT ON FEED INTAKE AND GROWTH

This chapter has been accepted for publication in Aquaculture as the following article: Collins, S.A., Mansfield, G.S., Desai, A.R., Hill, J.E., Van Kessel, A.G, Drew, M.D. 2013. Structural equation modeling of antinutrients in rainbow trout diets and their impact on feed intake and growth. Aquaculture. Article in Press. doi: 10.1016/j.aquaculture.2013.09.020. It is included as a chapter in this thesis with the permission of the publisher, Elsevier. The purpose of this study was to compare dietary ANF composition based on the analysis conducted in chapter 4 with the growth and feed intake data for rainbow trout fed diets containing PM, PPC, SBM, SPC, CM and CPC, as reported in chapter 6 to determine the effect of ANF on growth performance and feed intake. This chapter provides a model displaying the relationship between ANF and fish performance, which may be taken into consideration by fish nutritionists when formulating future rainbow trout diets.

6.1. Abstract

The inclusion of plant proteins in rainbow trout diets significantly impacts growth and feed intake. This effect has been ascribed to the presence of antinutritional factors (ANF) present in plant ingredients, although the magnitude of the effects of ANF has not been determined. A series of six 56-day growth trials were performed to determine the effect of feeding 0, 75, 150, 225 or 300 g/kg of pea meal (PM), pea protein concentrate (PPC), soybean meal (SBM), soy protein concentrate (SPC), canola meal (CM) and canola protein concentrate (CPC) on the growth of rainbow trout. Diets were formulated to be equal in digestible nutrient content, with 17.6 MJ/kg digestible energy and 386.2 g/kg digestible crude protein. For each ingredient trial, specific growth rates (SGR) and average daily feed intakes (ADFI) of fish fed experimental diets were transformed to a percentage of the response of fish fed the 0 g/kg diet for each ingredient trial, (resulting in tSGR and tADFI, respectively). SGR and ADFI values were transformed to enable comparisons between experiments (tSGR and tADFI, respectively).

Structural equation modeling was used to determine the highest likelihood model for the effect of ANF (starch, phytic acid, glucosinolates, tannins, isoflavones, total NSP, soluble NSP, insoluble NSP and saponins) on tADFI and tSGR, as well as the effect of tADFI on tSGR. All possible models were examined. The Akaike Information Criteria$_0$ was used to determine the model with the highest likelihood. This model contained 29 parameters and six degrees of freedom. Insoluble NSP, glucosinolates, saponins, tannins and phytic acid were statistically significant within the model. There were significant correlations between all ANFs in the structural equation model, except between phytic acid and saponins. Glucosinolates, saponins and phytic acid in the diet had a significantly negative

impact on tADFI, whereas the tannins had a significantly positive impact on tADFI. SGR was negatively influenced by saponins and positively influenced by tADFI. This model provides a basis for the design of experiments to determine the effect of dietary ANF on the growth performance of rainbow trout. It can be expanded in the future using additional ingredients and ANF and can be tested by feeding diets containing varying levels of these ANF to determine if the resulting growth and feed intakes are the same as would be predicted by the model.

6.2. Introduction

Numerous studies have investigated the inclusion of plant plant proteins in aquafeeds. Challenges are associated with feeding plant-based ingredients such as soy, pea and canola to rainbow trout and other salmonid fish. Production-related impacts are observed in nutrient digestibility, growth performance, feed intake and feed conversion (Gao et al., 2011; Torstensen et al., 2008; Drew et al., 2005; Forster et al., 1999; Refstie et al., 1998). Additional influences of plant protein sources include detrimental impacts on gut histology and morphology, altered liver morphology, increased expression of inflammatory marker genes and shifts in intestinal microbial populations (Desai et al., 2012; Sørensen et al., 2011; Mansfield et al., 2010; Merrifield et al., 2009; Krogdahl et al., 2003; Bakke-McKellep et al., 2000; Burrells et al., 1999). These effects have been largely attributed to the presence of antinutritional factors (ANF) found in plant-based ingredients.

Antinutritional factors are primarily metabolic or protective mechanisms of plants (Bennett and Wallsgrove, 1994) and as such, can have detrimental (sometimes toxic)

effects when eaten (Francis et al., 2001; Novak and Haslberger, 2000). When single ANF such as phytic acid, saponins and protease inhibitors are fed to salmonids, the results mimic the effects seen when ingredients containing these ANF are fed (Sørensen et al., 2011; Denstadli et al., 2006a; Bureau et al., 1998; Krogdahl et al., 1994), supporting the suggestion that ANF are responsible for the negative effects of feeding plant ingredients.

The functionality of plant-based feed ingredients is markedly improved for use in aquafeeds by processing plant meals into protein concentrates. Protein is concentrated from plant meals using aqueous extraction or air classification. Depending on the processing method used, ANF can be eliminated (e.g. fibre and phytic acid) or activated (e.g. glucosinolates) (Grub and Abel, 2006; Thiessen et al., 2004, 2003; Bennett and Wallsgrove, 1994). Despite many nutritional improvements, there are still differences seen in salmonids when protein concentrates are fed, as opposed to feeding fish meal (Penn et al., 2011; Barrows et al., 2007; Drew et al., 2007). This indicates that not all detrimental ANF have been removed.

While ANF with negative effects on the growth performance of salmonids have been identified, the relative magnitudes of these effects have not been quantified. This is due to several factors. Firstly, ANF are present in mixtures in feed ingredients making it impossible to separate the effects of individual ANF using only a single ingredient. It is also difficult to discern whether they are due to the independent action(s) of one or more ANF or the result of an interaction between two or more ANF. The addition of individual ANF to diets does not account for the interactions between ANF present in plant ingredients and thus, cannot be used to provide a reliable estimate of the effect size.

Lastly, the inclusion of fish meal is usually reduced when a plant ingredient is added. This confounds the effects of the plant ingredient with those of fish meal.

Structural equation models are used to simplify complex relationships between interconnected variables by describing them in the form of mathematical equations. With structural equation modeling, both direct and indirect causal relationships can be examined (Lamb et al., 2011). Observed data is fitted with unobserved, latent variables in such a way that assumptions about the interrelationships between factors can be simultaneously explored (Schumacker and Lomax, 2010), suggesting cause and effect. Structural equation modeling is commonly used for psychological and social studies (Yeh et al., 2010; Golob, 2003), whereas its usefulness to other branches of science has only begun to be realized. Structural equation modeling is now employed in the plant sciences (Lamb et al., 2011; Lamb and Cahill, 2008) and recently in the field of aquaculture (Abou et al., 2012). As there is still much mystery surrounding ANF in aquaculture research, we concluded that the simultaneous effect of multiple ANF on SGR and ADFI might be best explored using structural equation modeling.

Using this approach, we began the process of identifying the direct impact of dietary ANF and their interactions. We applied this technique to growth and feed intake data from a set of six experiments, where PM, PPC, SBM, SPC, CM or CPC were fed at inclusion rates of 0, 75, 150, 225 and 300 g/kg to rainbow trout. Fish meal concentrations were kept relatively constant among diets (Collins et al., 2012). The ANF content of all diets used in Collins et al. (2012) was calculated and a structural equation model was created to describe the relationship between the ANF content of diets and the growth performance of rainbow trout. With this method, we hypothesize that it will be possible

to identify a testable, causal relationship between the ANF present in intact feed ingredients and the ADFI and SGR of rainbow trout.

6.3. Materials and methods

6.3.1. Fish growth studies

The growth and feed intake data used to perform this experiment originate from the results reported by Collins et al. (2012). Five diets containing 0, 75, 150, 225 or 300 g/kg of PM, PPC, SBM, SPC, CM and CPC were fed in a series of six experiments (Table 6.3.1). Diets were formulated to contain the same concentrations of digestible nutrients (17.6 MJ/kg digestible energy and 386.2 g/kg of digestible crude protein). The diets met or exceeded the nutritional requirements of rainbow trout (National Research Council, 1993). Dietary fish meal levels among diets were kept as constant as possible, given the constraints of formulating on a digestible nutrient basis, to reduce potential nutritional effects of this ingredient (Gómez-Requeni et al., 2004; Kaushik et al., 1995; Burrells et al., 2001a, b; Li and Gatlin, 2006). Meat and bone meal and corn gluten meal were substituted for the test ingredients. All diets met the nutritional requirements of rainbow trout as mandated by the National Research Council (1993).

Triploid, female rainbow trout (*Oncorhynchus mykiss*) were purchased from Wild West Steelhead (Lucky Lake, SK, Canada) and housed at the Prairie Aquaculture Research Centre (University of Saskatchewan, Saskatoon, SK, Canada) in 360 L tanks. This facility is a semi-closed recirculation system, where water temperature was kept at 15 ± 1 °C and the photoperiod was a 14 h light:10 h dark cycle. Water was filtered biologically and environmental and water quality indicators were closely monitored. This

trial was operated under the University of Saskatchewan Committee on Animal Care and Supply Protocol #19980142. Fish husbandry followed the guidelines of the Canadian Council on Animal Care (1993, 2005).

The six test ingredients were fed in separate trials over a period of 361 days. In each experiment, the diets were randomly assigned to 15 tanks (3 tanks/treatment) and fed for 56 days. Feedings occurred twice daily by hand and fish were fed to apparent satiation. The distribution of fish per tank and average weight of fish in each trial are as follows: PM (n=22; mean weight, 235.2 g), PPC (n=22; mean weight, 237.7 g), SBM (n=17; mean weight, 553.1 g), SPC (n=17; mean weight 610.8 g), CM (n=16; mean weight, 639.8 g) or CPC (n=17; mean weight, 822.6 g). The same fish were used in all trials. Between trials, the fish were re-randomized within tanks and fed a commercial diet.

For each trial, the fish were weighed on day 0 and day 56. SGR was calculated for each experimental unit using the following calculation: SGR = (ln final weight – ln initial weight) / time (days) x 100. Feed intake was recorded daily and ADFI was calculated using the following equation: ADFI = feed consumed/fish/day. SGR and ADFI data were transformed so the mean SGR and ADFI of the 0 g/kg control diet for each ingredient trial was equal to 100.0 (transformed SGR and ADFI = tSGR and tADFI). All other ADFI and SGR values for fish fed experimental diets were transformed to be a percentage of their respective controls.

Table 6.3.1. Ingredient composition of control (0 g/kg test ingredient inclusion) and 300 g/kg test ingredient inclusion diets for each growth trial[a].

Ingredient (g/kg)	Ctrl1[b]	300 g/kg PM	300 g/kg PPC	300 g/kg SBM	Ctrl 2[c]	300 g/kg SPC	300 g/kg CM	300 g/kg CPC
Pea meal[d]	0.00	300.00	0.00	0.00	0.00	0.00	0.00	0.00
Pea protein concentrate[e]	0.00	0.00	300.00	0.00	0.00	0.00	0.00	0.00
Soybean meal[f]	0.00	0.00	0.00	300.00	0.00	0.00	0.00	0.00
Soy protein concentrate[g]	0.00	0.00	0.00	0.00	0.00	300.00	0.00	0.00
Canola meal[h]	0.00	0.00	0.00	0.00	0.00	0.00	300.00	0.00
Canola protein concentrate[i]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	300.00
Fish meal[j]	390.00	387.10	275.41	299.64	400.00	217.17	452.80	407.83
Meat and bone meal[k]	224.80	0.00	56.24	45.83	304.00	89.63	0.00	0.00
Fish oil[l]	141.34	156.31	122.03	146.54	120.50	169.86	83.60	74.28
Alpha-cellulose[m]	117.94	0.00	67.63	90.19	57.80	103.33	44.20	95.84
Wheat flour[n]	100.00	47.56	100.00	100.00	100.00	100.00	100.00	100.00
Corn gluten meal	11.16	95.04	62.70	2.63	0.00	0.00	0.00	0.00
Vitamin premix[o]	4.75	4.75	4.75	4.75	4.75	4.75	4.75	4.75
Mineral premix[p]	4.75	4.75	4.75	4.75	4.75	4.75	4.75	4.75
Choline chloride[q]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
L-Lysine[r]	0.00	0.00	0.00	0.00	1.90	2.99	5.20	6.63
DL-Methionine[s]	0.75	0.00	2.00	1.17	1.80	3.02	0.20	1.42
Vitamin C[t]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[a] All intermediate diets were produced by combining the reference and 300 g/kg diets in relative proportions.
[b] Control 1 fed as control for PM, PPC and SBM trials.
[c] Control 2 fed as control for SPC, CM and CPC trials.
[d] Yellow field pea, CDC Mozart; Crop Development Centre, Saskatoon, SK, Canada.
[e] Pea protein concentrate, yellow field pea, prestige protein; Parrheim Foods, Saskatoon, SK, Canada.
[f] Soybean meal; Federated Cooperatives Limited, Saskatoon, SK, Canada.
[g] Soycomil K; ADM Specialty Ingredients (Europe) BV, Koog aan de Zaan, The Netherlands.
[h] Canola Meal-35; Federated Co-Operative Ltd., Saskatoon, SK, Canada.
[i] Can Pro IP; CanPro Ingredients Ltd., Saskatoon, SK, Canada.
[j] Control 1, PM, PPC and SBM diets: Nova Scotia herring meal; Shur-Gain Aquaculture, Truro, NS, Canada.. Control 2, SPC, CM and CPC diets: West Coast Fish Meal; Federated Co-Operative Ltd., Saskatoon, SK, Canada.
[k] Saskatoon Processing Co.; Saskatoon Processing Co., Saskatoon, SK. Canada.
[l] Control 1, PM, PPC and SBM diets: Danish Fish Oil; FF of Denmark, Skagen, Denmark. Control 2, SPC, CM and CPC diets: Mixed Species Fish Oil; Bioriginal Food and Science Corp., Saskatoon, SK. Canada.
[m] Solka-floc, 200 FCC; International fiber corporation, North Tonawanda, NY, USA.
[n] Robin Hood All-Purpose Flour; Robin Hood Multifoods Corporation, Markham, ON, Canada.
[o] Vitamin premix, commercial (EWOS FISH-STR VIT PX, Surrey, BC; closed formulation), formulated to meet the requirements of juvenile rainbow trout; BASF Canada, Surrey, BC, Canada.
[p] Mineral premix, commercial (EWOS FISH MINERAL PX#2, Surrey, BC; closed formulation), formulated to meet the requirements of juvenile rainbow trout; BASF Canada, Surrey, BC, Canada.
[q] 60% Choline Chloride; Chinook Group Limited Partnership, Sombra, ON, Canada.
[r] L-lysine HCL 788 g/kg; Archer Daniels Midland Company, Decatur, IL, USA.
[s] DL-methionine, feed grade. Degussa Corporation, Theodore, AL, USA.
[t] Ascorbic acid, pharmaceutical grade; NOW Foods, Bloomingdale, IL, USA.

6.3.2. Chemical analysis

Total starch was analyzed using the Megazyme total starch analysis kit (AOAC, 1995; method no. 996.11). Glucosinolates were analyzed in CM and CPC (AOCS, 1998, method Ak 1-92) and isoflavones in SBM, SPC, PM and PPC were determined (AACC International, 2001; method 20-20.01) by POS Pilot Plant Corporation (Saskatoon, SK, Canada). Test ingredients were analyzed for tannins at Nutrilab B.V. (Giessen, Netherlands) using the Folis Denis method. Phytic acid (inositol hexakisphosphate and inositol pentakisphosphate) in the test ingredients was extracted using the method of Newkirk and Classen (1998). Samples were analyzed via high performance liquid chromatography at MCN BioProducts, Inc. (Saskatoon, SK, Canada). A modified version of the procedures developed by Englyst and Hudson (1987) and Englyst et al. (1994), as described by Reveco et al. (2011) was used to analyze total, soluble and insoluble non-starch polysaccharides (NSP) in the test ingredients. Values for saponins were based on values found in the literature for all ingredients, except for CPC, which was obtained from MCN BioProducts (Table 6.3.2).

Table 6.3.2. ANF[a] content (g/kg unless otherwise stated) of test ingredients (dry matter basis).

	SBM[c]	PM[e]	CM[g]	SPC[d]	PPC[f]	CPC[h]
Starch	51.2	486.8	26.9	25.4	307.9	9.4
Phytic acid	22.3	16.0	38.8	29.0	76.2	0.0
Glucosinolates (mmol/kg)	0.00	0.00	7.14	0.00	0.00	1.25
Tannins	8.4	4.9	10.6	5.4	7.6	6.2
Isoflavones	53.7	0.2	0.0	0.3	0.7	0.0
Total NSP[l]	24.2	18.2	34.4	25.6	24.4	25.2
Soluble NSP	6.6	6.8	6.1	5.7	11.0	5.4
Insoluble NSP	17.6	11.4	28.3	19.9	13.4	19.8
Saponins	3.5[i]	1.8[j]	3.6[k]	0.0[l]	5.4[i]	0.0[m]

[a] Antinutritional factor.
[b] Soybean meal.
[c] Pea meal.
[d] Canola meal.
[e] Soy protein concentrate.
[f] Pea protein concentrate.
[g] Canola protein concentrate.
[h] Non-starch polysaccharide.
[i] Curl et al., 1985.
[j] Heng et al., 2006.
[k] Barrón-Yánez et al., 2009.
[l] Ireland et al., 1986.
[m] MCN BioProducts Inc. Saskatoon SK Canada.

6.3.3. Antinutrients in the diets

Dietary ANF were calculated based on the ANF in the test ingredients in relation to their inclusion level in each diet. The contribution of ANF to each diet was considered solely on the basis of the test ingredient. Thus, the diets containing 0 g/kg of the test ingredients were considered to contain no ANF and diets containing 300 g/kg test ingredient had the highest proportion of ANF, with those of 75, 150 and 225 g/kg being intermediary. Dietary ANF values for experimental diets with 300 g/kg ingredient inclusion are shown in Table 6.3.3.

Table 6.3.3. Calculated ANF[a] content (g/kg unless otherwise stated) of test diets at 300 g/kg ingredient inclusion level[b] (dry matter basis).

	SBM[c]	PM[e]	CM[g]	SPC[d]	PPC[f]	CPC[h]
Starch	15.4	146.0	8.1	7.6	92.4	2.8
Phytic acid	6.7	4.8	11.6	8.7	7.2	0.0
Glucosinolates (mmol/kg)	0.00	0.00	2.14	0.00	0.00	0.38
Tannins	2.5	1.5	3.2	1.6	2.3	1.9
Isoflavones	16.1	0.1	0.0	0.1	0.2	0.0
Total NSP[l]	7.2	5.5	10.3	7.7	6.7	7.6
Soluble NSP	2.0	2.0	1.8	1.7	3.3	1.6
Insoluble NSP	5.3	3.4	8.5	6.0	4.0	6.0
Saponins	1.1	0.5	1.1	0.0	1.6	0.0

[a] Antinutritional factor
[b] Diets with ingredient inclusion of 0 g/kg are considered to contain no ANF due to experimental ingredients and diets with 75, 150 and 225 g/kg have an intermediary antinutrient composition.
[c] Soybean meal.
[d] Pea meal.
[e] Canola meal.
[f] Soy protein concentrate.
[g] Pea protein concentrate.
[h] Canola protein concentrate.
[l] Non-starch polysaccharide.

6.3.4. Statistical analysis

Tank was considered the experimental unit. IBM SPSS Amos (version 16.0, SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. Structural equations with observed variables (path modeling) was used on this model to determine direct causal relationships between ANF, tSGR and tADFI. A saturated model consisting of all possible linear regression weights and covariances investigated the effects of the concentration of phytic acid, starch, glucosinolates, tannins, isoflavones, total NSP, soluble NSP, insoluble NSP and saponins on tADFI and tSGR, as well as the effect of tADFI on tSGR. A specification search was used to determine the best-fit models containing 1 to 34 parameters among all possible models. The Akaike Information Criteria (AIC) of 0 (AIC_0) was used to determine the best-fit parameter model.

6.4. Results

The levels of ANF in each ingredient and the ANF in the experimental diets containing the test ingredients at 300 g/kg are shown in Tables 2 and 3, respectively. Phytic acid was highest in PPC, while CPC was devoid of phytic acid. The diets containing 300 g/kg of the test ingredients fed in the six growth trials had concentrations of 6.7, 4.8, 11.6, 8.7, 22.9 and 0 g/kg phytic acid for SBM, PM, CM, SPC, PPC and CPC, respectively. Glucosinolates were only present in the canola products and CM had levels more than five times higher than CPC (2.13 and 0.38 mmol/kg, respectively). Tannin levels were relatively consistent in the six ingredients, ranging from 4.9 g/kg in PM to 10.6 g/kg in CM. Isoflavones were present at levels less than 1 g/kg in all ingredients except SBM, which had a concentration of 53.7 g/kg. The highest saponin levels were reported for SBM and PPC.

PPC had the highest level of soluble NSP (11.0 g/kg), while the other five ingredients had relatively similar soluble NSP concentrations ranging from 5.4-6.8 g/kg. Insoluble NSP concentrations were higher than soluble NSP values for all six ingredients. CM had the highest level of insoluble NSP. CM also had the highest levels of total NSP of the ingredients (34.4 g/kg), while PM had the lowest total NSP (18.2 g/kg). As additional information, the wheat flour used to make these diets contained 10.3 g/kg insoluble NSP, 1.4 g/kg soluble NSP (11.7 g/kg total NSP.

The untransformed ADFI and SGR of the controls in the six growth trials are shown in Table 6.4.1. The tSGR for the fish fed 300 g/kg of the test ingredients ranged from 58.2% for the SBM-fed fish to 106.4% for the PPC-fed fish. Likewise, the tADFI

for the fish fed 300 g/kg of the test ingredients ranged from 81% for SBM-fed fish to 114.9% for the PPC-fed fish.

The possible statistical equation models (with 25 to 34 parameters) describing the interrelationships between ANF, tSGT and tADFI are shown in Table 6.4.2. Positive values indicate positive relationships between variables, where if one variable occurs or is present, the other synchronously occurs or is present. Negative values indicate inverse relationships between values. The AIC_0 was a minimum for the 29 parameter model, making it the best-fit model. $Error_1$ represented the unexplained variation for tADFI with a factor of 0.15 and $Error_2$ represented the unexplained variation for tSGR with a factor of 0.19 (Figure 6.4.1).

In this model, glucosinolates, saponins and phytic acid had a significant, negative, impact on tADFI and tannins had a significant, positive effect on tADFI. Saponins had a negative impact on tSGR. It was the only ANF that had a direct effect on tSGR and of all the ANF, had the greatest effect on tADFI. The only factor that had a direct, positive effect on tSGR was tADFI. Insoluble NSP are included in the best-fit model, but they have no direct effect on either tADFI or tSGR. There were significant covariances between all five ANF in the structural equation model, except between phytic acid and saponins (Table 6.4.2).

Table 6.4.1. Transformed ADFI[a] and SGR[b] and of rainbow trout fed SBM, PM, CM, SPC, PPC and CPC at inclusion levels of 0, 75, 150, 225 and 300 g/kg. Untransformed SGR and ADFI of fish fed control diet (0 g/kg).

Ingredient		Ingredient inclusion rate (g/kg)					SEM[c]
		0	75	150	225	300	
	Untransformed SGR of control	Transformed SGR (% of SGR of 0 g/kg control group)					
SBM	0.6	100.0	112.7	121.8	98.2	58.2	0.04
PM	1.6	100.0	87.0	81.5	85.8	94.4	0.05
CM	0.7	100.0	86.3	86.3	67.1	65.8	0.04
SPC	0.7	100.0	97.3	95.9	74.3	81.1	0.03
PPC	1.3	100.0	108.0	107.2	107.2	106.4	0.02
CPC	0.5	100.0	107.7	103.8	65.4	65.4	0.06
	Untransformed ADFI of control	Transformed ADFI (% of ADFI of 0 g/kg control group)					
SBM	4.2	100.0	104.8	135.7	111.9	81.0	0.31
PM	5.0	100.0	84.0	90.0	100.0	102.0	0.31
CM	6.1	100.0	68.9	80.3	90.2	85.2	0.45
SPC	5.6	100.0	117.9	105.4	92.9	91.1	0.67
PPC	4.7	100.0	95.7	106.4	112.8	114.9	0.30
CPC	8.9	100.0	96.6	109.0	92.1	95.5	0.30

[a] Average daily feed intake (g feed / fish / d).
[b] Specific growth rate ([ln final weight – ln initial weight] / time (days) x 100).
[c] Standard error of the mean of untransformed data.

Table 6.4.2. Best-fit models for 25-34 parameters (10-1 degrees of freedom) based on the minimum Akaike Information Criteria of 0 (AIC_0). The first 14 parameters consist of variable means and error terms.

	Parameters									
	25	26	27	28	**29**	30	31	32	33	34
Covariances										
Insol NSP[a] <> Saponins	0.68	0.68	0.68	0.73	**0.68**	0.73	0.73	0.73	0.73	0.73
Insol NSP<>Tannins	1.53	1.53	1.53	1.73	**1.53**	1.73	1.73	1.73	1.73	1.73
Insol NSP<>Glucosinolates	0.61	0.61	0.61	0.70	**0.61**	0.70	0.70	0.70	0.70	0.70
Insol NSP<>Phytic acid	6.78	6.78	6.78	8.31	**6.78**	8.31	8.31	8.31	8.31	8.31
Saponins<>Tannins	0.20	0.20	0.20	0.23	**0.20**	0.23	0.23	0.23	0.23	0.23
Saponins<>Glucosinolates	0.07	0.07	0.07	0.09	**0.07**	0.09	0.09	0.09	0.09	0.09
Saponins<>Phytic acid				0.32		0.32	0.32	0.32	0.32	0.32
Tannins<>Glucosinolates	0.21	0.21	0.21	0.24	**0.21**	0.24	0.24	0.24	0.24	0.24
Tannins<>Phytic acid	3.72	3.72	3.72	4.19	**3.72**	4.19	4.19	4.19	4.19	4.19
Glucosinolates<>Phytic acid	1.85	1.85	1.85	2.02	**1.85**	2.02	2.02	2.02	2.02	2.02
Regression weight (tADFI[b])										
Insol NSP								0.06	-0.03	-0.06
Saponins					**-0.53**	-0.53	-0.85	-0.85	-0.85	-0.85
Tannins			0.04	0.04	**0.35**	0.35	0.37	0.37	0.37	0.37
Glucosinolates	-0.26	-0.26	-0.26	-0.30	**-0.18**	-0.18	-0.17	-0.17	-0.17	-0.17
Phytic acid					**-0.03**	-0.03	-0.04	-0.04	-0.04	-0.04
Regression weight (tSGR[c])										
Insol NSP							0.06	0.06	0.06	0.06
Saponins		-0.27	-0.27	-0.27	**-0.27**	-0.27	-0.27			-0.20
Tannins									-0.08	-0.12
Glucosinolates										
Phytic acid								0.01	0.01	0.02
tADFI	0.43	0.29	0.29	0.29	**0.28**	0.28	0.28	0.30	0.34	0.37
AIC_0	21.69	2.64	1.86	2.41	**0.00**	0.56	1.29	2.55	4.05	5.82
Relative likelihood	<0.01	0.268	0.395	0.299	**1.000**	0.758	0.525	0.279	0.132	0.055

[a] Water-insoluble non-starch polysaccharide.
[b] Transformed average daily feed intake.
[c] Transformed specific growth rate.

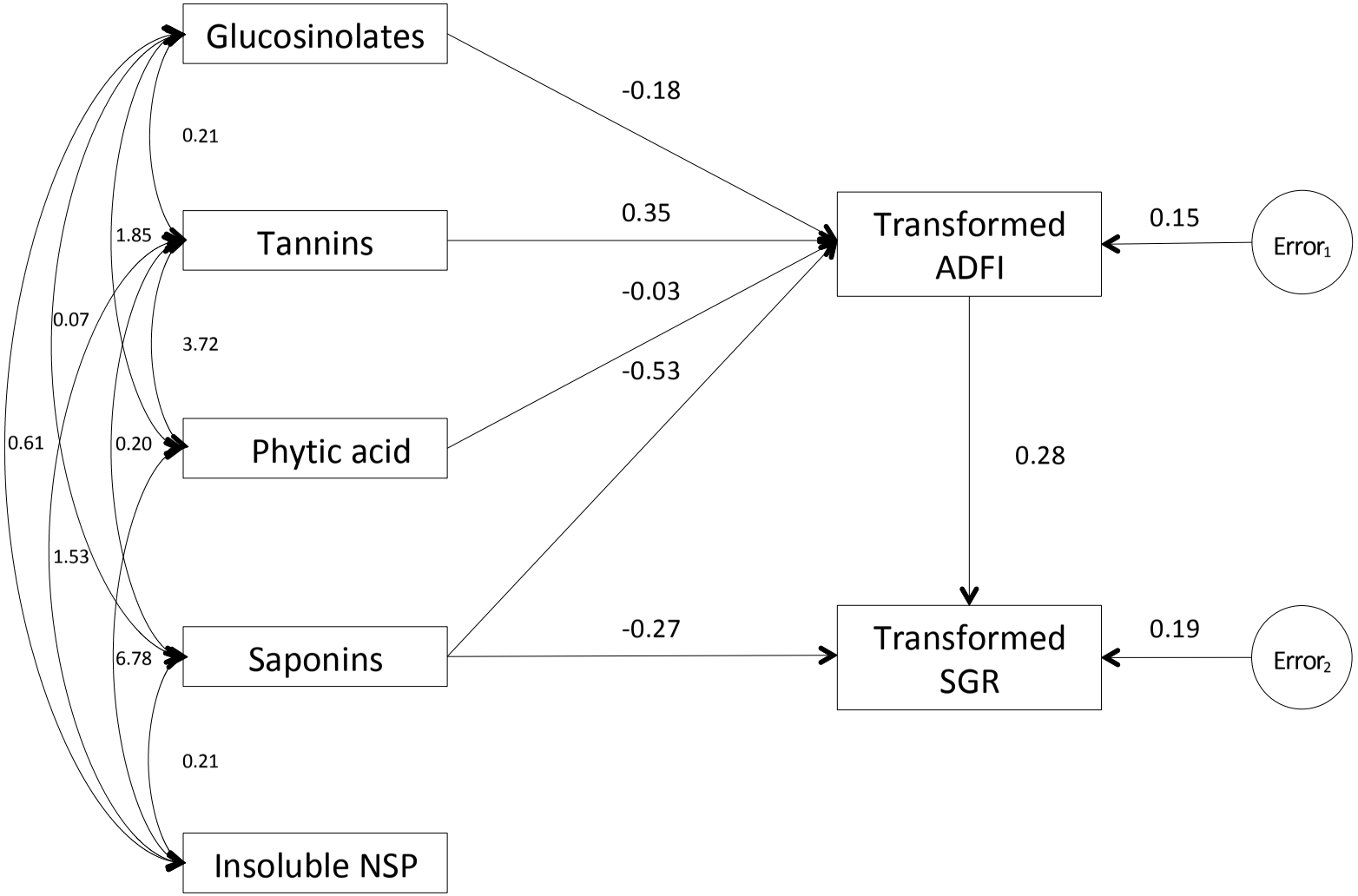

Figure 6.4.1. Best-fit structural equation model (29 parameters; 6 degrees of freedom, AIC_0) for direct causal relationships between plant antinutritional factors (ANF) on transformed specific growth rate (SGR) and transformed average daily feed intake (ADFI). $Error_1$ and $Error_2$ represent unexplained variation in the model.

6.5. Discussion

Antinutritional factors are generally acknowledged to be responsible for decreases in the growth performance of rainbow trout when plant ingredients are added to diets. However, a model of the magnitude of the effects of ANF on growth performance and the relationships between ANF has not been established. This is due to the complexity of such a model and the difficulty of conducting an experiment that provides unbiased data on the relationships between ANF and growth performance.

The objective of this study was to identify the relationships between the ANF in these six ingredients and their impact on the ADFI and SGR of rainbow trout. Structural equation modeling was selected as the demonstrative approach, due to its capability of illustrating the potentially expansive web of interrelationships between these factors.

The isoflavones in soybeans have antioxidant (Rüfer and Kulling, 2006) and anti-inflammatory capabilities (Droke et al., 2007) and modulate inflammatory signaling pathways (Dijsselbloem et al., 2004; Kim et al., 1998). However, none of the best-fit models with less than 29 parameters included isoflavones as a significant factor. While isoflavones may not affect growth, they have been shown to affect estrogen metabolism and gametogenesis in salmonids and more markedly in sturgeon (Gontier-Latonnelle et al., 2007; Ng et al., 2006; Bennetau-Pelissero et al., 2001). Thus, they might still have significant effects on the overall productivity of aquaculture systems, and may be more suitable for structural equation models involving the relationship between ANF and fish reproduction.

Starch has been cited as an ANF that decreases the feed intake and growth rate of salmonid fish. Starch is more readily digestible when gelatinized using hydrothermal

processing such as extrusion (Krogdahl et al., 2004; Pfeffer et al., 1991; Bergot and Breque, 1983). The diets used in these trials were cold pelleted and starch would not have been significantly gelatinized. The diets in the present experiment were balanced based on digestible energy. The results suggest that starch is a readily utilized energy substrate for rainbow trout at the concentrations used in these trials and does not have significant antinutritional effects in rainbow trout diets.

Although protein concentration has the benefit of reducing the ANF levels of the concentrate as compared with those found in a meal, this is not true for phytic acid. Carnovale et al. (1988) reported 8.5 and 9.4 g/kg phytic acid in two pea cultivars. Phytic acid in the protein concentrates of these same cultivars was 19.0 and 13.2 g/kg, respectively. CPC can contain phytic acid levels from 53 to 75 g/kg, a concentrated value when compared with CM, which necessitates the use of phytase (Forster et al., 1999).

Phytic acid impairs growth and feed intake in Atlantic salmon when present in the diet at a level of 4.6 g/kg (Denstadli et al., 2006a). Except for CPC, all ingredients provided concentrations of phytic acid in the 300 g/kg diets at higher levels, with the lowest in the PM treatment at 4.8 g/kg and the highest in the PPC diet at 22.7 g/kg. The CPC used in the current study was dephytinized, serving as a reference ingredient for the effect of phytic acid on feed intake and growth. While tADFI was directly impaired by phytic acid, there was no significant regression weight for phytic acid on tSGR. This suggests that phytic acid decreases feed intake, possibly by altering olfaction or gustation of diets. Soluble amino acids are potent olfactory and gustatory stimulators in rainbow trout (Atema, 1980; Carpio, 1984; Jones, 1990). Given that phytic acid binds to free

amino acids, they may decrease their concentration and reduce the overall palatability of the diet.

Like phytic acid, tannins bind to proteins, reducing amino acid digestibility (Mariscal-Landín et al., 2004; Francis et al., 2001). Moreover, the astringent flavour of the tannins might affect the gustation of feed negatively (Bravo, 1998; Kumar and Vaithiyanathan, 1990), reducing tADFI. However, in this study, the effect of tannins on tADFI was significantly positive. There are no published studies on the effect of tannins on feed intake in salmonids. However, Grosjean et al., (1991) reported that pigs ate significantly more of a two diets containing pea varieties with 3.6 and 3.9 g/kg of tannins, than two other diets that contained pea varieties with 0 g/kg tannins. As the diets fed in these rainbow trout trials were balanced on digestible amino acids, any reduction in amino acid digestibility by tannins would be compensated for during diet formulation. These results suggest that in diets where the lowered digestibility of amino acids due to tannins is adjusted for, the tannins in these six plant proteins are not an ANF and may actually have a small positive effect on feed intake.

Glucosinolates and their derivative products impair thyroidal iodine uptake and growth performance (Tripathi and Mishra, 2007; Pereira et al., 2002). For growth performance, the dietary tolerance level of rainbow trout for glucosinolates is 1.4-19.3 mmol/kg, with values lower than 3.7 mmol/kg most highly recommended (Tripathi and Mishra, 2007; Burel et al., 2001). Glucosinolates are present almost exclusively in plant products of Brassica origin (Tripathi and Mishra, 2007), in the case of this experiment, CM and CPC. Glucosinolate concentrations in CM and CPC diets were 2.14 and 0.38 mmol/kg, respectively. These concentrations are at the lower levels of dietary tolerance.

Glucosinolates negatively influenced tADFI, which has also been seen in poultry, swine, and rats (Tripathi and Mishra, 2007; Johnson and Reuber, 1994; Lo and Bell, 1972). While glucosinolates impair growth performance, the best-fit model suggests that this effect is mediated through decreased appetite rather than on growth directly.

Previous studies examining the effect of soluble and insoluble NSP in Nile tilapia reported that feeding 80 g/kg guar gum as a source of soluble fibre reduced feed intake and growth significantly, while feeding 80 g/kg cellulose as a source of insoluble fibre had no effect on growth or feed intake (Amirkolaie et al., 2005). However, purified fibre sources may not be good models for the effects of NSP present in whole ingredients. In the current study, soluble fibre had no effect on tADFI or tSGR. The significant, positive covariances insoluble NSP have with the other four ANF in the model suggests that insoluble NSP interact with or potentiate the activity of the other ANF in the model.

Soybeans contain approximately 192-217 g/kg NSP and approximately two-thirds of these NSP are insoluble (Choct et al., 2010; Bach Knudsen, 1997). SBM reduces lipid digestibility, which has been attributed to its high NSP content (Refstie et al., 1999). Refstie et al. (1998) found a link between SBM and reduced feed intake, whereby reducing the carbohydrates of SBM led to improved nutrient digestibility. Carbohydrates from other plant sources, such as lupins have also been found to reduce nutrient digestibility (Glencross et al., 2003). Glencross (2009) fed soluble and insoluble lupin NSP to rainbow trout at dietary inclusion levels of 100, 200 and 300 g/kg. There was a significant effect of fibre type and level. At all levels, insoluble NSP impaired nutrient digestibility and this effect increased with ingredient inclusion level. Discrepancies between studies with respect to NSP suggest classification beyond soluble and insoluble

is required and they may be better compared on an individual basis. The effect of soluble and insoluble NSP on feed intake and growth are specific to the plant source, and generalizations about their effects based on the six ingredients used in this model may not be appropriate.

An association between SBM and sub-acute enteritis in salmonid fishes was previously thought to be due to the NSP content of SBM. Kraugerud et al. (2007) compared SBM and extracted soy NSP products and found only SBM caused enteritis. This suggests that in SBM is not the culprit for these negative effects, but some other chemical component, such as saponins. Saponins are a diverse group of compounds consisting of a non-sugar aglycone conjugated with a wide variety of sugar chain units (Oleszek, 2002). Due to the difficulty of analyzing the wide variety of compounds contained within the family of saponins, values used in this analysis were based on published values from the literature. Saponins have foaming capabilities in aqueous solution, a bitter taste and hemolyze erythrocytes. They are highly toxic to poikilothermic animals, including fish, and interact with intestinal cell membranes, increasing cell permeability (Sparg et al., 2004; Francis et al., 2002). Depending on the method used, saponins can be relatively resistant to protein concentration and only partially reduced by the process (Drew et al., 2007). Saponins are soluble in aqueous solution and for more thorough extraction, saponins can be extracted by processing with methanol (Balsevich et al., 2009; Heng et al., 2006), such as in the solvent-extraction method used in the production of SPC.

In the best-fit model, the regression coefficients for saponins were significantly negative for both tADFI and tSGR. These coefficients had the highest magnitudes of any

of the other ANF in the model. These negative effects of dietary saponins are in agreement with previously published studies. Saponins and saponin-containing feed ingredients induce sub-acute intestinal enteritis in fish in conjunction with a reduction in growth. This has been noted in common carp, Japanese flounder, Chinook salmon and rainbow trout, although the majority of the research has been conducted in Atlantic salmon (Chikwati et al., 2012; Kortner et al., 2012; Chen et al., 2011; Sørensen et al., 2011; Refstie et al., 2010; Knudsen et al., 2008; Urán et al., 2008; Bureau et al., 1998). This gut damage may have had a negative impact on tSGR due to metabolic and physiological effects. Beyond this, the bitter flavor of the saponins may have reduced the palatability of the diets they were included in, reducing tADFI. Bureau et al. (1998) reported decreases in feed intake in Chinook salmon and rainbow trout due to the bitter flavor of saponins. Saponins were the only ANF present in the ingredients that independently reduced SGR. This suggests that the toxic effects of saponins are mediated by mechanisms beyond feed intake, such as decreased nutrient absorption due to intestinal damage.

The analysis of this experiment using structural equation modeling demonstrates the complex interrelationships between ANF and growth response factors. Using this approach allowed the development of a testable model that assigned direct, numerical values to the effects (negative and positive) of feeding ingredients containing these ANF to rainbow trout. Structural equation modeling with observed and latent variables was chosen, as opposed to only performing path analysis, specifically because it allowed all possible interrelationships and causal networks to be tested (Gan and Yang, 2011; Lamb et al., 2011). The model was selected based on the AIC, which deals with model

selection uncertainty, as a result of the empirical support provided by Burnham and Anderson (2004).

In this experiment, as many ANF were tested as possible, within the financial capabilities of this study. As with any experiment, there were financial limitations on the amount of work we could do and we acknowledge that not all ANF that were possibly present in the ingredients were tested, including sinapine, lectins, glycinin and beta-conglycinin. The effects of ANF not included in the model (as well as other factors that affected growth and feed intake) can be explained by the error terms, although they do not take into account potential covariances between these ANF and other ANF in the model. Excluding important ANF from the model could affect the final outcome of the experiment, providing less than accurate causal relationships. However, this experiment does provide a valid initial model to predict the effects of ANF on rainbow trout and growth. Future experiments can be designed to determine the accuracy of this model. We hope that in the future, more ANF and more ingredients will be added to the model.

Further applications for this model may include investigating diets containing more than one test ingredient to determine if similar results can be obtained based solely on ANF content and if there are ingredient and ANF effects and interactions not accounted for. Examining the effects of additional ingredients, ANF and extending it to other aquaculture species will further strengthen our understanding of the effects of plant ingredients on finfish.

6.6. Conclusion

Dietary formulation can account for ANF that reduce nutrient digestibility, preventing them from impairing growth. The presence or absence of ANF on an individual basis is an unrealistic approach in determining their effect on SGR and ADFI, as they interact with other dietary ANF. The interplay between ANF and nutrients in plant ingredients must be considered when formulating plant-based salmonid diets. This experiment resulted in a structural equation model that can be tested in further studies.

7. DISCUSSION

Plant proteins are used to replace fish meal in salmonid feeds, but these ingredients are not equivalent replacements. At high inclusion levels, plant proteins do not produce the same results as the fish meal they are substituting. Fish meal contains components not present in plant protein sources that have a positive effect on fish growth. These components include certain low molecular weight compounds (Kousoulaki et al., 2009; Aksnes et al., 2006a,b,c), nucleotides (Li and Gatlin, 2006; Burrells et al., 2001a,b), taurine (Lunger et al., 2007; Gaylord et al., 2006), cholesterol (Gómez-Requeni et al., 2004; Kaushik et al., 1995) and essential fatty acids (Steffens, 1997). Aquafeeds containing high levels of plant proteins as a fish meal replacement may be deficient in these compounds, which adds a level of difficulty to interpreting the results of studies involving the replacement of fish meal with plant proteins.

SBM is a highly researched feed ingredient for salmonids, as is CM. PM, PPC, SPC and CPC have been studied to a lesser extent and further studies on these feed ingredient would benefit this field of research. Plant breeding programs have altered these ingredients over time. These plants may possess a number of different qualities that make them more cold-, drought- and pest-tolerant, have improved yields and have a chemical profile that drastically differs from their original nutrient and antinutrient composition. These differences in ingredient composition could have altered their effect on fish performance compared to research that was conducted a quarter of a century ago. Batch variations also exist, depending on crop varieties and growing conditions. There is also great deal of variation in protein concentrate production, thus its chemical composition, particularly for CPC. For example, early studies were conducted by McCurdy and March

(1992), where fibre and phytic acid was removed from CM. However, these studies are not comparable with more recent research, as the canola/rapeseed varieties used at this time were much higher in glucosinolate and euricic acid levels. These differences in feed ingredient composition may be responsible for differences in study results when utilized in salmonid feeds.

The information presented in this thesis provides an expansion of the available information on ingredient and ANF effects on rainbow trout growth and feed intake. Although past research has examined the nutritional consequences of including plant proteins in salmonid diets, differences in study design deter direct comparisons between studies presented in the literature. In many papers, ingredient and dietary chemical composition (both nutrient and antinutrient) is not reported. In any nutritional experiment, the choice of dietary control will have a major impact on the study results and nutritional balance of control and test diets should be given careful consideration. Many studies also fail to provide information on fish meal quality, which is problematic, as it can play a strong role in the outcome of these studies. In plant protein replacement studies, the quality of the fish meal being replaced can affect SGR, thus the interpretation of study results (Mundheim et al., 2004). Brown fish meal, in particular, is a poor quality fish meal source. Oliva-Teles et al. (1994) found replacing brown fish meal with SBM led to improved nutrient digestibility and growth in rainbow trout. In growth studies, a poorly balanced control diet, whether it is due to inadequate protein and amino acid quality, vitamin and mineral composition, protein to energy balance or any other deviation from a properly balanced diet may lead to misinterpretation of experimental results. Unless the goal of a growth experiment is to determine the effect of feeding different levels of

nutrients, digestible nutrient levels must be maintained across treatments. If not, a study involving a test ingredient may inadvertently become a study on the effects of nutrient deficiency, rather than ingredient effects.

Because of differences in experimental parameters and design, nutritional studies examining plant proteins as feed ingredients for salmonid diets are somewhat limited in the level to which direct numerical comparisons can be made among trials. By compiling a composite data set of all available growth data for salmonids fed plant proteins and performing a subsequent meta-analysis of this data, it was possible to compare the information presented by different studies for PM, PPC, SBM, SPC, CM and CPC and determine the effect of the inclusion level of these plant proteins on growth in rainbow trout and Atlantic salmon. By using CD, the SGR of fish fed control diets and diets containing plant protein were compared and this information was used to create regression models to illustrate the effect of the inclusion level of each plant protein on salmonid growth. SGR was chosen as the representative growth indicator for these fish, due to availability of data. Meta-analysis based on ANF composition was not possible as not enough information was available.

In the meta-analysis, an increase in the dietary inclusion level of soy and canola products was associated with a decrease in salmonid SGR. Although it is generally accepted that salmonid growth performance is improved when protein concentrates are fed rather than plant meals, the significantly negative effects on SGR exhibited by SPC and CPC at high inclusion levels indicate that although they may be preferable to their plant meal counterparts, these feed ingredients still cannot be considered suitable fish meal replacements at high inclusion levels. Inclusion level of PM and PPC had no

significant effect on SGR as a result of dietary inclusion levels, which may have been due to lower levels of growth-impairing ANF in the pea products than in the other four plant proteins. The maximum dietary inclusion levels for PM and PPC were 300 and 350 g/kg, respectively. For further insight, it would be beneficial to explore these feed ingredients at higher dietary inclusion levels, as the growth effects of the other plant proteins on salmonids were tested at inclusion levels of 500 g/kg and higher.

Ingredients vary in their nutrient digestibility, thus availability (Burel et al., 2000; Drew et al., 2007a, 2007b, 2005; Øverland et al., 2009). This is dependent on their nutrient and ANF composition and because of this, not all ingredients will behave equally in an aquafeed. The digestibility trial was conducted to determine the digestible nutrient content of all the ingredients that would be used in the following growth studies. Digestibility values for the feed ingredients were within the range of similar feed ingredients reported in the literature (Table 3.1.7) and canola, pea and soybean protein concentrates had higher nutrient digestibilities than their respective meals (Mansfield et al., 1010; Drew et al., 2007; Escaffre et al., 2007). Examination of these plant proteins for nutrient composition and digestibility, as well as antinutrient composition improved the precision with which further stages in this experiment could be planned.

Rather than formulating the diets used in the growth studies on a total nutrient basis, the results determined in the digestibility study were used to formulate these diets to ensure they all had the same digestible nutrient composition. This decision was justified, as the results for the ingredients analyzed in Section 3.1 show these plant proteins varied in their digestible nutrient composition, thus formulation on a total nutrient basis would not have been suitable. As there are few macronutrient digestibility

values published for PPC, CM and CPC in the literature, these digestibility experiments will also contribute to the pool of available information.

In salmonids, ANF research has primarily focused on which ANF negatively affect fish growth performance in cause and effect-type studies. What is lacking is a numeric quantification of these effects. The purpose of the growth trials and ingredient analyses conducted in this experiment was to develop a better understanding of the effect of ANF on fish performance and to determine which ANF in these ingredients are most likely to impair rainbow trout feed intake and growth. Using growth and feed intake data, structural equation modeling was used to ascertain relationships between dietary ANF composition with growth and feed intake of the rainbow trout fed these diets and identify the magnitude of these interactions.

In the meta-analysis, many of the experiments were fish meal replacement studies and involved control diets that contained higher levels of fish meal than the test diets did. For the six growth trials performed in this study, the focus was not on fish meal replacement, but on ingredient effects. To ensure the effects of increasing a specific ingredient and its constituent ANF were being studied, not the effects of decreasing fish meal, variations in fish meal inclusion between test diets within an experiment were kept to a minimum. Rather than replacing fish meal, the experimental ingredients replaced corn gluten meal and meat and bone meal, which have no markedly positive or negative effects on fish growth (Alexis et al., 1985; Bureau et al., 2000; Hardy, 2000; Yu, 2004). This provided a more stable environment to investigate ingredient and subsequent ANF effects on rainbow trout feed intake and growth performance.

For consistency, and to avoid possible consequences of using fish with varying genetic backgrounds, which may affect feed conversion and growth, one lot of fish was purchased prior to the commencement of the growth experiments, and used for all six growth trials with rerandomization between experiments. It was logistically infeasible to conduct all six growth experiments concurrently, due to the number of available tanks. In fish, SGR decreases as body weight increases (Jobling, 1983a, b), which was observed in this series of growth trials. Because of differences in initial weights, SGR and feed intake of fish fed control diets in each trial, it is not possible to compare mean values between experiments. However, within each experiment, all fish were the same age and had the same average initial weight. Additionally, all six trials followed the same experimental protocols with respect to experimental design, diet formulation and statistical calculations. Because of the similarities between these experiments, the resulting regression equations for the effect of each ingredient on rainbow trout growth and feed intake are suitable for comparison with one another.

As the results of this growth trial were published at the time the meta-analysis was performed, these results were included in the meta-analysis data sets. This information was a subcomponent of a series of larger data sets and can still be considered independent. Unlike the results found by the meta-analysis, the SPC and CPC growth trials associated increased inclusion levels of these feed ingredients with a subsequent decrease in SGR. There are studies showing SPC has positive (Vielma et al., 2000; Mambrini et al., 1999) and negative (Brinker and Reiter, 2011; Barrows et al., 2007; Stickney et al., 1996) effects on rainbow trout growth at inclusion levels above 300 g/kg. These discrepancies in results could be due to differences in study design, controls, or

ingredient composition. SPC production methods may have also varied, leading to differences in nutrient and ANF content, which may explain conflicting results between studies.

Growth trial results showed negative associations between SBM, CM and growth, which has been seen in both feed ingredients from levels as low as 14 and 47 g/kg, respectively (Brinker and Reiter, 2011; Alami- Durante et al., 2010; Torstensen et al., 2008; Barrows et al., 2007; Drew et al., 2005; Refstie et al., 2005; De Francesco et al., 2004; Lee et al., 2002; Burel et al., 2000; Satoh et al., 1998; Davies and Morris, 1997; Rumsey et al., 1994; Hilton and Slinger, 1986) and no effect of PM and PPC on growth. In the growth trial conducted for this experiment, feed intake increased with dietary inclusion of PPC, which may warrant further studies on PPC as a feed attractant or palatability enhancer.

To promote life, growth and development, animals do not require ingredients. They require nutrients. These growth trials illustrated the importance of considering additional feed components beyond the commonly considered macro- and micronutrients when feeding plant-based feed ingredients to carnivorous fish. Provided the formulary conditions presented in Section 3.2 are followed, rainbow trout ADFI, SGR, FCR and PER for the same six plant proteins can be predicted using the resulting regression equations. The negative regression (ADFI, SGR, FCR and PER) curves seen for SBM and CM and not for SPC and CPC indicate additional factors beyond nutrients play a role in the impact of these ingredients on growth and feed intake. Digestible nutrients and fish meal levels (thus levels of fish meal-specific nutrients) were maintained across treatments, yet for some plant proteins, growth and feed intake dropped as inclusion

levels rose. This was due to additional dietary components that affect growth, more specifically, ANF. Although dietary nutrient levels were maintained for all diets, while ingredient inclusion levels increased, so did the dietary antinutrients: detrimental, potentially toxic dietary components.

Based on the structural equation model, growth was affected by most ANF in this model via feed intake. Saponins were the only ANF that independently affected growth independently of feed intake. This indicates saponins impaired rainbow trout growth via mechanisms beyond reducing the palatability of the diet, such as decreased nutrient absorption due to gut damage. Previous research has associated feeding saponins to salmonids with sub-acute intestinal enteritis in conjunction with a reduction in growth (Sørensen et al., 2011; Refstie et al., 2010; Knudsen et al., 2008; Bureau et al., 1998). The reduced feed intake due to the saponins was likely a result of the bitter flavour, which has been found previously (Bureau et al., 1998). In the best-fit model, isoflavones were not identified as a significant factor affecting growth or feed intake. As isoflavones are high in SBM, a highly studied plant protein, and are known to affect reproductive traits (Gontier-Latonnelle et al., 2007; Ng et al., 2006; Bennetau-Pelissero et al., 2001) and inflammatory signaling pathways (Dijsselbloem et al., 2004; Kim et al., 1998), it would be worthwhile to use structural equation modeling to gain a deeper understanding of the effects of this ANF.

PM and PPC starch levels were high, but the starch in these six feed ingredients did not impair growth or feed intake. This may have been a result of the starch source. Krogdahl et al. (2004) found starch reduces growth and feed intake in salmonids, but this was using maize starch and effects were more pronounced in Atlantic salmon than in

rainbow trout. Glencross et al. (2012) observed rainbow trout were able to digest pregelatinized wheat starch, but no other dietary polysaccharides included in the diet. Had these experiments been conducted in Atlantic salmon, the starch in these pea products may have had a markedly different impact on growth.

Although insoluble NSP did not directly affect feed intake or growth, they did have significant covariances with the other ANF in the model, suggesting interactions with these ANF. NSP have been associated with increased viscosity and passage time of intestinal contents, reduced feed intake and nutrient digestibility, and alterations of the gastrointestinal tract, including changes in physiology, morphology and gut microflora (Sinha et al., 2011; Owusu-Asiedu et al., 2006; Refstie et al., 1999; Thompson et al., 1987). Amirkolaie et al. (2005) found feeding soluble NSP (guar gum) reduces feed intake and growth, whereas insoluble NSP (cellulose) does not. Glencross et al. (2009) found lupin insoluble NSP impaired nutrient digestibility, whereas soluble lupin NSP did not. Glencross et al. (2012) later investigated the effect of particular polysaccharide classes (cellulose, mannan, lignosulphonate and pectin) on rainbow trout digestibility, these polysaccharides differed in their impact. Lignosulphonate, in particular had the strongest effect, impairing dry matter, gross energy and crude protein digestibility. These discrepancies in results indicate as research advances are made, further research into particular classes of soluble and insoluble NSP would also be warranted.

Tannins can bind to proteins and amino acids, leading to a reduction in digestibility (Mariscal-Landín et al., 2004; Francis et al., 2001), but this did not appear to be the case in this experiment, as high tannin ingredients such as PPC and CM had superior crude protein and amino acid digestibilities to their low tannin counterparts, PM

or CPC, although these differences in digestibility were only significant for PM and PPC. Any possibility that amino acid and protein digestibility would be negatively affected by tannin content was prevented, due to the experimental diets being formulated based on digestible nutrient values. This removed the possibility of impairing fish growth as a result of nutrient deficiencies. However, in this study, tannins increased feed intake and were the only feed component previously assumed to be an ANF that was actually a positive feed component. At the time of this manuscript, there were no published studies exploring the effect of tannins on the feed intake of salmonid fish, although tannins had been found to increase the feed intake of pigs (Grosjean et al., 1991).

Without the use of phytase, protein concentrates are commonly higher in phytic acid than their respective plant meals (Forster et al., 1999; Carnovale et al., 1988), which was seen in this study. Phytic acid was directly associated with decreased feed intake, but not growth. Studies have identified negative associations between inclusion of CPC in the diet with rainbow trout growth (Forster et al., 1999; Stickney et al., 1996), but in these studies, the CPC was not dephytinized. Thiessen et al. (2004) showed dephytinized CPC (low glucosinolate, low euricic acid canola/rapeseed) could be fed to rainbow trout at inclusion levels as high as 490 g/kg without affecting SGR, which is why this same form of CPC was chosen for the growth experiments in this trial. In addition to binding phosphorus and other minerals, phytic acid binds to soluble amino acids, which play a role in rainbow trout olfaction and feeding behaviour (Atema, 1980; Carpio, 1984; Jones, 1990). Future studies could involve studying the effects of phytic acid on feed attractants to verify this hypothesis. In the digestibility experiment, fish fed CPC had a higher phosphorus ADC than those fed CM. Reduced growth in other studies may have been due

to reduced phosphorus digestibility. Denstadli et al. (2006a) reported a reduction in Atlantic salmon growth and feed intake as a result of dietary phytic acid. When Sajjadi and Carter (2004) added phytase to the CM-based diets they fed to Atlantic salmon, phosphorus digestibility also improved. This may have been because when bound as phytic acid, phosphorus became a limiting nutrient, reducing growth. As the diets fed in this experiment were formulated on a digestible nutrient basis, adding dicalcium phosphate when necessary, this did not occur.

Glucosinolates are ANF that are activated during cell breakdown of cruciferous crops, yielding bitter-flavoured end-products, such as thiocyanates and nitriles. They are associated with reduced feed intake and growth in monogastric animals (Tripathi and Mishra, 2007; Grub and Abel, 2006; Pereira et al., 2002; Burel et al., 2001; Johnson and Reuber, 1994; Lo and Bell, 1972). In the best-fit model, glucosinolates directly reduced feed intake, suggesting reduced growth due to glucosinolates is an indirect result of reduced feed intake.

The plant ingredients used in aquafeeds cannot be direct fish meal replacements. They differ in their nutritional profile, where these plant proteins lack some of the nutrients possessed by fish meal. They also contain ANF, which are absent from fish meal. Not all plant proteins will replicate the growth response of fish fed a strictly fish-meal based diet if they contain ANF that inhibit feed intake or growth through another mechanism, even if they have an identical digestible nutrient composition. Formulating aquafeeds on a digestible nutrient basis is more effective than formulating on a total nutrient basis, as it takes into account the ANF that impair nutrient availability. However, depending on the ingredients included in the formulation process, there is no guarantee

these diets will produce the same results as a traditional fish meal diet if these ingredients contain ANF that impair fish performance via alternate mechanisms. As knowledge of these feed ingredients and how they affect fish grows, researchers would do well to include ANF in the diet formulation process.

In experiments where a specific ANF is to be tested by adding it to a synthetic or fish meal diet, if possible, it is best to use purified ANF from that ingredient, rather than obtain it from a source that contains a similar, yet not identical compound as is found in the ingredient of interest. Saponins, for example, can vary from ingredient to ingredient and if jatropha saponins were to be used as a representative for soy saponins, they may elicit very different results. This study has raised questions as to how individual ANF compounds behave and one issue that has been made apparent is that making generalizations about ANF based on their classifications may not always be suitable. For example, NSP include all polysaccharides with the exception of starches (Sinha et al., 2011), yet these fibres differ in their structure and function.

Despite the multiple ANF studies conducted in salmonids, the magnitude of the effects of specific ANF remains unknown. Plant proteins generally contain more than one ANF, which can have the effect of impairing growth performance when included in salmonid diets. Additionally, many ANF can have the same effect which adds a level of complexity when plant proteins containing multiple ANF are fed. If a feed ingredient is added as the ANF source, as was done in this study, or if the diet contains additional ANF-containing ingredients, it is necessary to acknowledge that a complete cocktail of ANF, all of which can affect performance is present in the diet. Structural equation modeling can be used to determine the consequences of feeding an ANF. When including

ANF-containing feed ingredients in a diet, knowledge of the ANF levels these ingredients contain will enable calculation of the level these ANF will be in the diet. Knowledge of the effect these ANF have on fish will also allow diet formulators to know dietary ANF levels that can be included in the diet, thus maximum inclusion levels of ingredients containing these ANF. It may be possible to include these plant proteins in aquafeeds, combining several with complimentary ANF compositions to produce diets below ANF thresholds.

This experiment highlighted the importance of standardizing reported data from fish growth studies, specifically initial weight, final weight, rearing temperature, lighting program and an indication of variation (SD or standard error of the mean) for these values. Preferences may vary between researchers with regards to reporting of growth parameters, such as the use of SGR vs TGC. Regardless of the calculation used, if this information is reported, there is an increased potential for data to be compared among studies. For all fish nutritional studies, it is also necessary to report nutritional information, including diet formulation and the nutritional composition and processing conditions of all experimental diets in addition to data indicating fish meal source (if it is fed) and an indication of its composition and quality. Changes in experimental conditions during an experiment should be noted and may raise a need for additional data. As an example, if there is a change in diet mid-trial, the weights of the fish should be taken prior to feeding the new diet. A certain degree of creative freedom is required for performing scientific research, but it is possible for all researchers to follow the same reporting standards without hampering their creativity. Without this data, it is not always

possible to compare data presented in different manuscripts, which dilutes the amount of information available for the advancement of research in this scientific field.

This research can be expanded in the future to develop feed formulation models. Similar studies could be conducted using additional feed ingredients. With this same dietary and ANF information, structural equation modeling can be used to quantify the effects of ANF on nutrient digestibility, intestinal physiology, morphology and microflora, and fish health and mortality. As new ingredients are added, the model could also be expanded to include additional ANF present in these new ingredients. It could also be developed for other fish species, such Atlantic salmon and Nile tilapia. Similar studies could also be conducted using combinations of these plant proteins to ascertain whether or not the ANF in these ingredients behave the same way when fed simultaneously (if it is strictly an ANF effect) or if there are inter-ingredient interactions and if a level of ANF from one ingredient would have the same effect as the same level of one ANF from multiple ingredients. ANF are classified into specific categories, such as tannins, NSP, saponins and isoflavones, but whether or not these ANF behave the same, independent of the ingredient they are found in remains uncertain, although evidence suggests they do not.

There are specific requirements that must be met by feed ingredients suitable for inclusion in salmonid feeds. Although none can be direct fish meal replacements, it may be possible to combine them with other feed ingredients to create a diet with an appropriate level of available nutrients that promote fish health and growth, and do not contain ANF at a level that will counteract the nutritive value of the feed. A greater understanding of the nutritional needs of these fish and the effects specific ingredients

have on them can also result in more efficient farming practices being employed in aquaculture. The results presented in this manuscript are useful tools in expanding the available information on these ingredients and ANF. They also shared a larger message. For carnivorous fish species, in terms of fish meal replacement, not all plant proteins are equal.

8. CONCLUSIONS

If rainbow trout are to be fed plant-proteins in their diets, these plant-based feed ingredients may first require processing to eliminate or deactivate performance-impairing antinutritive components, so fish fed these diets will perform as well as fish fed a standard fish meal diet. When increasing their dietary inclusion level from 0-300 g/kg in nutritionally balanced diets, SBM and CM will elicit a drop in performance (ADFI, SGR, FCR and PER), which does not occur for SPC or CPC (nor for PM and PPC). The differing factors between these two plant meals and their protein concentrates are their ANF content and composition. This indicates SBM and CM require further processing and including these ingredients in rainbow trout diets requires nutritional considerations beyond their digestible nutrient composition. Provided the nutritional and experimental standards outlined in this experiment are followed, ADFI, SGR, FCR and PER of rainbow trout can be predicted using the resulting equations from this experiment.

Dietary formulation on a digestible nutrient basis takes into account ANF that affect nutrient digestibility. This prevents feeding nutrient-deficient diets, which impair growth. This does not take into account ANF that have effects beyond impairing nutrient digestibility, such as glucosinolates, saponins, phytates, which impair ADFI and in the case of saponins, negatively affect SGR. Interactions between other dietary ANF also require consideration, which may occur when combining more than one plant protein in the diet. The structural equation model depicting the interplay between the ANF in the six tested plant proteins and ADFI and SGR provides a nutritional tool, which can be assessed in future experiments.

Feeding plant proteins does not impair performance for all ingredients (or at all inclusion levels). To replace fish meal with plant proteins in rainbow trout diets, the most effective method will likely be to combine several plant proteins at low inclusion levels. The performance, nutritional and antinutritional information for PM, PPC, SBM, SPC, CM and CPC and the culminating structural equation model presented in this manuscript can be used to assess which ingredients to include in rainbow trout diets and their specific dietary inclusion levels and combinations.

9. REFERENCES

AACC International. 2001. Approved Methods of Analysis. 11th ed. Method 20-20.01. Determination of Isoflavones in Soy and Selected Foods Containing Soy by Extraction, Saponification, and Liquid Chromatography. Approved October 17, 2001. AACC International, St. Paul, USA. doi: 10.1094/AACCIntMethod-20-20.01.

Aarset, B. and Jakobsen, S. 2009. Political regulation and radical institutional change: the case of aquaculture in Norway. Mar. Policy. 33: 280-287.

Abd El-Hady, E.A., Habiba, R.A. 2003. Effect of soaking and extrusion conditions on antinutrients and protein digestibility on legume seeds. Lebensm. Wiss. Technol. 36: 285-293.

Abdou Dade, B., Aguirre, P., Blanc, D., Kaushik, S.J. 1990. Incorporation du colza 00 sous forme de tourteau ou d'amande dans les aliments de la truite arc-en-ciel (*Oncorhynchus mykiss*): Performance zootechnique et digestibilité (Incorporation of rapeseed 00 shape of cattle cake or almond in diets for rainbow trout (*Oncorhynchus mykiss*): Zootechnical performance and digestibility). Bull. Fr. Pêche Piscicult. 317: 50-57.

Abou, Y., Darchambeau, F., Fiogbé, E.D., Micha, J.-C. 2012. Ecology of feeding Nile tilapia under Azolla cover in earthen ponds: an assessment using structural equation modelling. IJB. 2: 105-111.

Aksnes, A., Hjertnes, T., Opstvedt, J. 1996. Comparison of two assay methods for determination of nutrient and energy digestibility in fish. Aquaculture. 140: 343-359.

Aksnes, A., Hope, B., Albrektsen, S. 2006a. Size-fractionated fish hydrolysate as feed ingredient for rainbow trout (*Oncorhynchus mykiss*) fed high plant protein diets. II: flesh quality, absorption, retention and fillet levels of taurine and anserine. Aquaculture. 261: 318-326.

Aksnes, A., Hope, B., Høstmark, Ø., Albrektsen, S. 2006b. Inclusion of size fractionated fish hydrolysate in high plant protein diets for Atlantic cod, *Gadus morhus*. Aquaculture. 261: 1102-1110.

Aksnes, A., Hope, B., Jönsson, E., Björnsson, B.T., Albrektsen, S. 2006c. Size-fractionated fish hydrolysate as feed ingredient for rainbow trout (*Oncorhynchus mykiss*) fed high plant protein diets. I: growth, growth regulation and feed utilisation. Aquaculture. 261: 305-317.

Aksnes, A., Opstvedt, J. 1998. Content of digestible energy in fish feed ingredients determined by the ingredient-substitution method. Aquaculture. 161: 45-53.

Alami-Durante, H., Médale, F., Cluzeaud, M. and Kaushik, S.J. 2010. Skeletal muscle growth dynamics and expression of related genes in white and red muscles of rainbow trout fed diets with graded levels of a mixture of plant protein sources as substitutes for fishmeal. Aquaculture. 303: 50-58.

Alcock, R. E., Behnisch, P. A., Jones, K. C. and Hagenmaier, H. 1998. Dioxin-like PCBs in the environment – human exposure and the significance of sources. Chemosphere. 37: 1457-1472.

Alexis, M.N., Papaparaskeva-Papoutsoglou, E. and Theochari, V. 1985.

Formulation of practical diets for rainbow trout (*Salmo gairdneri*) made by partial or complete substitution of fish meal by poultry by-products and certain plant by-products. Aquaculture. 50: 61-73.

Alipour, D., Rouzbehan, Y. 2010. Effects of several levels of extracted tannin from grape pomace on intestinal digestibility of soybean meal. Livest. Sci. 128: 87-91.

Allameh, S.K., Soofiani, N.M., Pourreza, J. 2007. Determination of digestible and metabolizable energy of fishmeal and soybean meal in rainbow trout with two different sizes (*Oncorhynchus mykiss*). Pak. J. Biol. Sci. 10: 3733-3725.

Amar, E. C., Kiron, V., Satoh, S., Watanable, T. 2001. Influence of various dietary synthetic carotenoids on bio-defense mechanisms in rainbow trout, *Oncorhynchus mykis*s (Walbaum). Aquacult. Res. 32: 162-173.

Amar, E. C., Kiron, V., Satoh, S., Watanabe, T. 2004. Enhancement of innate immunity in rainbow trout (*Oncorhynchus mykiss* Walbaum) associated with dietary intake of carotenoids from natural products. Fish Shellfish Immun. 16: 527-537.

Amirkolaie, A.K., Leenhouwers, J.I., Verreth, J.A.J., Schrama, J.W. 2005. Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile tilapia (*Oreochromis niloticus* L.). Aquacult. Res. 36: 1157-1166.

Ardura, A., Horreo, J.L., Hernandez, E., Jardon, A., Pola, I.G., Martinez, J.L., Garcia-Vazquez, E. 2012. Forensic DNA analysis reveals use of high trophic level marine fish in commercial aquaculture fish meals. Fish. Res. 115-116: 115-120.

Arndt, R.E., Hardy, R.W., Sugiura, S.H., Dong, F.M. 1999. Effects of heat

treatment and substitution level on palatability and nutritional value of soy defatted flour in feeds for Coho salmon, *Oncorhynchus kisutch*. Aquaculture. 180: 129-145.

Aslaksen, M.A., Kraugerud, O.F., Penn, M., Svihus, B., Denstadli, V. Jørgensen, H.Y., Hillestad, M., Krogdahl, Å., Storebakken, T. 2007. Screening of nutrient digestibilities and intestinal pathologies in Atlantic salmon, *Salmo salar*, fed diets with legumes, oilseeds, or cereals. Aquaculture. 272: 541-555.

AOAC (Association of Official Analytical Chemists). 1990. Official Methods of Analysis.15th ed. AOAC, Arlington, VA, USA.

AOAC (Association of Official Analytical Chemists). 1995. Official Methods of Analysis. 16th ed. AOAC, Arlington, VA, USA.

AOCS (American Oil Chemists Society). 1998. Official Methods and Recommended Practices of the American Oil Chemists Society. 4th ed. Method Ak 1-92. Champagne, IL, USA.

Atema, J. 1980. Chemical senses, chemical signals and feeding behavior in fish, in: Bardach, J.E., Mahnusum, J.J., May, R.C., Reinhart, J.M. (Eds.), Fish behavior and its use in the capture and culture of fishes. ICLARM, Manilla, pp. 57-101.

Austreng, E. 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aquaculture. 12: 265-272.

Austreng, E., Storebakken, T., Åsgård, T. 1987. Growth rate estimates for cultured Atlantic salmon and rainbow trout. Aquaculture. 60: 157-160.

Back Knudsen, K.E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Food Sci. Technol. 67: 319-338.

Baeverfjord, G. and Krogdahl, Å. 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. J. Fish Dis. 19: 375-387.

Bakke-McKellep, A.M., Nordrum, S., Krogdahl, Å., Buddington, R.K. 2000. Regional differences in in vitro intestinal uptake of glucose, amino acids, and peptides in Atlantic salmon (*Salmo salar* L.). Fish Physiol. Biochem. 22: 33-44.

Balsevich, J.J., Bishop, G.G., Deibert, L.K. 2009. Use of digitoxin and digoxin as internal standards in HPLC analysis of triterpene saponin-containing extracts. Phytochem. Anal. 20: 38-49.

Barnes, S., Kirk, M., Coward, L. 1994. Isoflavones and their conjugates in soy foods: extraction conditions and analysis by HPLC – mass spectrometry. J. Agric. Food Chem. 42: 2466-2474.

Barrón-Yánez , M.R., Villanueva-Verduzco, C., García-Mateos, M.R, Colinas-León, M.T. 2009. Valor nutricio y contenido de saponinas en germinados de hauauzontle (*Chenopodium nuttalliae* Saff.), calabacita (*Cucurbita pepo* L.), canola (*Brassica napus* L.) y amaranto (*Amaranthys leucocarpus* S. Watson *syn*, *hypochondriacus* L.). Rev. Chapingo Ser. Hortic. 15: 237-243.

Barrows, F.T., Gaylord, T.G., Stone, D.A.J., Smith, C.E. 2007. Effect of protein source and nutrient density on growth efficiency, histology and plasma amino acid concentration of rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquacult. Res. 38: 1747-1758.

Barthet VJ. (2011) Quality of Western Canadian canola 2011. Canadian Grain Commission. Available from: http://www.grainscanada.gc.ca/canola/harvest-recolte/2011/hqc11-qrc11-eng.pdf [accessed 22 Jun 2012].

Bax, L. 2010. MIX 2.0. Professional software for meta-analysis in Excel. Version (2.0.1.2). BiostatXL, http://www.meta-analysis-made-easy.com [accessed 9 Mar 2012].

Bennetau-Pelissero, C., Breton, B., Bennetau, B., Corraze, G., Le Menn, F., Davail-Cuisset, B., Helou, C., Kaushik, S.J. 2001. Effect of genistein-enriched diets on the endocrine process of gametogenesis and on reproduction efficiency of the rainbow trout *Oncorhynchus mykiss*. Gen. Comp. Endocr. 121: 173-187.

Bennett, R.N., Wallsgrove, R.M. 1994. Tansley Review No. 72. Secondary metabolites in plant defense mechanisms. New Phytol. 127, 617-633.

Bergot, F., Breque, J. 1983. Digestibility of starch by rainbow trout: effects of the physical state of starch and of the intake level. Aquaculture. 34, 203-212.

Bisset, N.G. 1989. Arrow and dart poisons. J. Ethnopharmacol. 25: 1-41.

Brasaemle, D. L. 2007. The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis. J. Lipid Res. 48: 2547-2559.

Bravo, L. 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. 56: 317-333.

Brinker, A., Reiter, R. 2011. Fish meal replacement by plant protein substitution and guar gum addition in trout feed, Part I: Effects on feed utilization and fish quality. Aquaculture. 310: 350-360.

Brown, P.B., Wilson, K.A., Jonker, Y. and Nickson, T.E. 2003. Glyphosate tolerant

canola meal is equivalent to the parental line in diets fed to rainbow trout. J Agric. Food Chem. 51: 4268-4272.

Bureau, D.P., Cho, C.Y. 1994 Ingredient quality: an essential factor in the formulation of cost-effective aquaculture diets. *In* Expanding Agriculture Co-product Uses in Aquaculture Feeds Workshop Proceedings. Des Moines, IA, USA.

Bureau, D.P., Cho, C.Y. 1999. Measuring digestibility in fish. UG/OMNR Fish Nutrition Research Laboratory Technical Document, University of Guelph, ON, Canada. pp. 9.

Bureau, D.P., Harris, A.M., Bevan, D.J., Simmons, L.A., Azevedo, P.A., Cho, C.Y. 2000. Feather meals and meat and bone meals from different origins as protein sources in rainbow trout (*Oncorhynchus mykiss*) diets. Aquaculture. 181: 281-291.

Bureau, D.P., Harris, A.M., Cho, C.Y. 1998. The effects of purified alcohol extracts from soy products on feed intake and growth of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 161: 27-43.

Bureau DP, Kaushik SJ, Cho CY. 2002. Bioenergetics. *In* Halver JE, Hardy RW, (Eds.) Fish Nutrition. 3rd ed. Academic Press, San Diego, CA, USA. pp. 1-59.

Burel, C., Boujard, T., Escaffre, A., Kaushik, S.J., Boeuf, G., Mol, K.A., Van der Geyten, S. and Kühn, E.R. 2000. Dietary low-glucosinolate rapeseed meal affects thyroid status and nutrient utilization in rainbow trout (*Oncorhynchus mykiss*). Brit. J. Nutr. 83: 653-664.

Burel, C., Boujard, T., Kaushik, S.J., Boeuf, G., Mol, K.A., Van der Geyten, S., Darras, V.M., Kühn, E.R., Pradet-Balade, B., Quérat, B., Quinsac, A., Krouti, M., Ribaillier, D. 2001. Effects of rapeseed meal-glucosinolates on thyroid metabolism and feed utilization in rainbow trout. Gen. Comp. Endocr. 124: 343-358.

Burel, C., Boujard, T., Kaushik, S.J., Boeuf, G., Van Der Geyten, S., Mol, K.A., Kühn, E.R., Quinsac, A., Krouti, M., Ribaillier, D. 2000a. Potential of plant-protein sources as fish meal substitutes in diets for turbot (*Psetta maxima*): growth, nutrient utilisation and thyroid status. Aquaculture. 188: 363-382.

Burel, C., Boujard, T., Tulli, F., Kaushik, S.J. 2000b. Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Aquaculture. 188: 285-298

Burnham, K.P., Anderson, D.R. 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociol. Meth. Res. 33, 261-304.

Burrells, C., Williams, P.D., Forno, P.F. 2001a. Dietary nucleotides: a novel supplement in fish feeds 1. Effects on resistance to disease in salmonids. Aquaculture. 199: 159-169.

Burrells, C., Williams, P.D., Southgate, P.J., Crampton, V.O. 1999. Immunological, physiological and pathological responses of rainbow trout (*Oncorhynchus mykiss*) to increasing dietary concentrations of soybean proteins. Vet. Immunol. Immunop. 72: 277-288.

Burrells, C., Williams, P.D., Southgate, P.J., Wadsworth, S.L. 2001b. Dietary nucleotides: a novel supplement in fish feeds 2. Effects on vaccination, salt water

transfer on growth rates and physiology of Atlantic salmon (*Salmo salar L.).* Aquaculture. 199: 171-184.

Carnovale, E., Lugaro, E., Lombardi-Boccia, G. 1988. Phytic acid in faba bean and pea: effect on protein availability. Cereal Chem. 65, 114-117.

Carpio, J. 1984. Olfaction and taste in fish, in: Bolis, L., Keynes, R.D., Maddrell, S.H.P. (Eds.), Comparative physiology of sensory systems. Cambridge University Press, Great Britain, pp. 257-284.

Carter, C.H., Hauler, R.C. 2000. Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L. Aquaculture 185, 299-311.

Carter, C.G., Lewis, T.E., Nichols, P.D. 2003. Comparison of cholestane and yttrium oxide as digestibility markers for lipid components in Atlantic salmon (*Salmo salar* L.) diets. Aquaculture. 225: 341-351.

Castell, A.G., Guenter, W., Igbasan, F.A. 1996. Nutritive value of peas for nonruminant diets. Anim. Feed Sci. Technol. 60: 209-227.

CCAC (Canadian Council on Animal Care). 1993. Guide to the care and use of experimental animals. *In* E.D. Olfert, Cross, B. M., McWilliams, A. A. (Eds.) Canadian Council on Animal Care. Ottawa, ON, Canada.

CCAC (Canadian Council on Animal Care). 2005. Guide to the care and use of fish in research teaching and testing, Canadian Council on Animal Care. Ottawa, ON, Canada.

Chavan, U.D., Shahidi, F., Naczk, M. 2001. Extraction of condensed tannins from beach pea (*Lathyrus maritimus* L.) as affected by different solvents. Food Chem. 75: 509-512.

Chen, W., Ai, Q., Mai, K., Xu, W., Liufu, Z., Zhang, W., Cai, Y. 2011. Effects of dietary soybean saponins on feed intake, growth performance, digestibility and intestinal structure in juvenile Japanese flounder (*Paralichthys olivaceus*). Aquaculture. 213: 95-100.

Chibber, B.A.K., Mertz, E.T., Axtell, J.D. 1978. Effects of dehulling on tannin content, protein distribution, and quality of high and low tannin sorghum. J. Agric. Food Chem. 26: 679-683.

Chikwati, E.M., Venold, F.F., Penn, M.H., Rohloff, J., Refstie, S., Guttvik, A., Hillestad, M., Krogdahl, Å. 2012. Interaction of soyasaponins with plant ingredients in diets for Atlantic salmon, *Salmo salar* L. Br. J. Nutr. 107, 1570-1590.

Cho, C.Y. 1992. Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. Aquaculture. 100: 107-123.

Cho, C.Y., Slinger, S.J. 1979. Apparent digestibility measurement in feedstuffs for rainbow trout. *In* J.E. Halver, K. Tiews (Eds.) Finfish Nutrition and Fish feed Technology, Vol. 2. Heenemann gnbH, Berlin, Germany. pp. 239-248.

Cho, C.Y., Slinger, S.J., Bayley, H.S. 1982. Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. Comp. Biochem. Physiol. B. 73: 25-41.

Choct, M., Dersjant-Li, Y., McLeish, J., Peisker, M. 2010. Soy oligosaccharides and soluble non-starch polysaccharides: a review of digestion, nutritive and anti-nutritive effects in pigs and poultry. Asian-Aust. J. Anim. Sci. 23: 1386-1398.

Chung, K., Wei, C., Johnson, M.G. 1998. Are tannins a double-edged sword in

biology and health? Trends Food Sci. Tech. 9: 168-175.

Cohen, J. 1988. Statistical power analysis for the behavioral sciences. 2nd ed. Lawrence Earlbaum Associates, Hillsdale, NJ, USA.

Collins, S.A., Desai, A.R., Mansfield, G.S., Hill, J.E., Van Kessel, A.G., Drew, M.D. 2012. The effect of increasing inclusion rates of soybean, pea and canola meals and their protein concentrates on the growth of rainbow trout: concepts in diet formulation and experimental design for ingredient evaluation. Aquaculture. 344-349: 90-99.

Collins, S.A., Mansfield, G.S., Desai, A.R., Hill, J.E., Van Kessel, A.G, Drew, M.D. 2013. Structural equation modeling of antinutrients in rainbow trout diets and their impact on feed intake and growth. Aquaculture. Accepted Manuscript. doi: 10.1016/j.aquaculture.2013.09.020.

Collins, S.A., Øverland, M., Skrede, A., Drew, M.D. 2013. Effect of plant protein sources on growth rate in salmonids: meta-analysis of dietary inclusion of soybean, pea and canola/rapeseed meals and protein concentrates. Aquaculture. 400-401: 85-100.

Cremades, O., Parrado, J., Alvarez-Ossorio, M., Jover, M., de Terán, L. C., Gutierrez, J. F., Bautista, J. 2003. Isolation and characterization of carotenoproteins from crayfish (*Procambarus clarkii*). Food Chem. 82: 559-566.

Curl, C.L., Price, K.R., Fenwick, G.R. 1985. The quantitative estimation of saponin in pea (*Pisum sativum* L.) and soya (*Glycine max*). Food Chem. 18: 241- 250.

da Silva, J. O., Fernandes, R.S., Ticli, F.K., Oliveira, C.Z., Mazzi, M.V., Franco,

J.J., Giuliatti, S., Pereira, P.S., Soares, A,M., Sampaio, S.V. 2007. Triterpenoid saponins, new metalloprotease snake venom inhibitors isolated from *Pentaclethra macroloba*. Toxicon. 50: 283-291.

Dabrowski, K., Poczyczynski, P., Köck, G., Berger, B.1989. Effect of partially or totally replacing fish meal protein by soybean meal protein on growth, food utilization and proteolytic enzyme activities in rainbow trout (*Salmo gairdneri*). New in vivo test for exocrine pancreatic secretion. Aquaculture. 77: 29-49.

Dadgar, S., Bin Saad, C.R., Alimon, A. R., Kamarudin, M.S., Nafisi Bahabadi, M. 2010. Comparison of soybean meal and cottonseed meal variety pak (CSMP) on growth and feed using in rainbow trout (*Oncorhynchus mykiss*). Iran. J. Fish Sci. 9: 49-60.

Davies, S.J., Morris, P.C. 1997. Influence of multiple amino acid supplementation on the performance of rainbow trout, *Oncorhynchus mykiss* (Walbaum), fed soya based diets. Aquacult. Res. 28: 65-74.

Davies, S.J., Morris, P.C., Baker, R.T.M. 1997. Partial substitution of fish meal and full-fat soya bean meal with wheat gluten and influence of lysine supplementation in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquacult. Res. 28: 317-328.

de Almeida Costa, G.E., da Silva Queiroz-Monici, K., Reis, S.M.P.M., de Oliveria, A.C. 2006. Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. Food Chem. 94: 327-330.

de Francesco, M., Parisi, G., Médale, F., Lupi, P., Kaushik, S.J., Poli, B.M. 2004. Effect of long-term feeding with a plant protein mixture based diet on growth and body/fillet quality traits of large rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 236: 413-429.

de la Higuera, M., García-Gallego, M., Sanz, A., Cardenete, G., Suárez, M.D., Moyano, F.J. 1988. Evaluation of lupin seed meal as an alternative protein source in feeding of rainbow trout (*Salmo gairdneri*). Aquaculture. 71: 37-50.

Deng, J., Mai, K., Ai, Q., Zhang, W., Wang, X., Xu, W., Liufu, Z. 2006. Effects of replacing fish meal with soy protein concentrate on feed intake and growth of juvenile Japanese flounder, *Paralichthys olivaceus*. Aquaculture. 258: 503-513.

Denstadli, V., Skrede, A., Krogdahl, Å., Sahlstrøm, S., Storebakken, T. 2006a. Feed intake, growth, feed conversion, digestibility, enzyme activities and intestinal structure in Atlantic salmon (*Salmo salar* L.) fed graded levels of phytic acid. Aquaculture. 256: 365-376.

Denstadli, V., Storebakken, T., Svijus, B. Skrede, A. 2007. A comparison of online phytase pre-treatment of vegetable feed ingredients and phytase coating in diets for Atlantic salmon (*Salmo salar* L.) reared in cold water. Aquaculture. 269: 414-426.

Denstadli, V., Vestre, R., Svihus, B., Skrede, A., Storebakken, T. 2006b. Phytate degradation in a mixture of ground wheat and ground defatted soybeans during feed processing: effects of temperature, moisture level, and retention time in

small- and medium-scale incubation systems. J. Agric. Food Chem. 54: 5887-5893.

DerSimonian, R., Laird, N. 1986. Meta-analysis in clinical trials. Control. Clin Trials. 7: 177-188.

Desai, A.R., Links, M.G., Collins, S.A., Mansfield, G.S., Drew, M.D., Van Kessel, A.G., Hill, J.E. 2012. Effects of plant-based diets on the distal gut microbiome of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 350-353, 134-142.

Dijsselbloem, N., Vanden Berghe, W., De Naeyer, A., Haegeman, G. 2004. Soy isoflavone phyto-pharmaceuticals in interleukin-6 affections: multi-purpose nutraceuticals at the crossroad of hormone replacement, anti-cancer and anti-inflammatory therapy. Biochem. Pharmacol. 68: 1171-1185.

Drew, M.D., Borgeson, T.L., Thiessen, D.L. 2007. A review of processing of feed ingredients to enhance diet digestibility in finfish. Anim. Feed Sci. Technol. 138: 118-136.

Drew, M.D, Ogunkoya A.E., Janz D.M. and Van Kessel, A.G. 2007. Dietary influence of replacing fish meal and oil with canola protein concentrate and vegetable oils on growth performance, fatty acid composition and organochlorine residues in rainbow trout. Aquaculture. 267: 260-268.

Drew, M.D., Racz, V.J., Gauthier, R. and Thiessen, D.L. 2005. Effect of adding protease to coextruded flax:pea or canola:pea products on nutritional digestibility and growth performance of rainbow trout (*Oncorhynchus mykiss*). Anim. Feed Sci. Technol. 119: 117-128.

Droke, E.A., Hager, K.A., Lerner, M.R., Lightfoot, S.A., Stoecker, B.J., Brackett, D.J., Smith, B.J. 2007. Soy isoflavones avert chronic inflammation-induced bone loss and vascular disease. J. Inflammation. 4, 17-28.

Dumas, A., France, J., Bureau, D. 2009. Modeling growth and body composition in fish nutrition: where have we been and where are we going? Aquacult. Res. 41: 1-21.

Easterbrook PJ, Berlin JA, Gopalan R, Matthews DR. (1991) Publication bias in clinical research. Lancet. 337: 867-872.

Eeckhout, W., De Paepe, M. 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Anim. Feed Sci. Technol. 47: 19-29.

Egounlety, M., Aworth, O.C. 2003. Effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max* Merr.), cowpea (*Vigna unguiculata* L. Walp) and groundbean (*Macrotyloma geocarpa* Harms). J. Food Eng. 56: 249-254.

Ekholm, P., Virkki, L., Ylinen, M., Johansson, L. 2003. The effect of phytic acid and some natural chelating agents on the solubility of mineral elements in oat bran. Food Chem. 80: 165-170.

Eldridge, A.C. 1982a. Determination of isoflavones in soybean flours, protein concentrates, and isolates. J. Agric. Food Chem. 30: 353-355.

Eldridge, A.C. 1982b. High-performance liquid chromatography separation of soybean isoflavones and their glucosides. J. Chromatogr. 234: 494-496.

El-Haroun, E.R., Azvedo, P.A., Bureau, D.P. 2009. High dietary incorporation levels of rendered animal protein ingredients on performance of rainbow trout *Oncorhynchus mykiss* (Walbaum, 1972). Aquaculture. 290: 269-274.

El-Saidy, D.M.S.D., Gaber, M.M.A. 2002. Complete replacement of fish meal by soybean meal with dietary L-lysine supplementation for Nile tilapia *Oreochromis niloticus* (L.) fingerlings. J. World Aquacult. Soc. 33: 297-306.

Enami, H.R. 2011. A review of using canola/rapeseed meal in aquaculture feeding. JFAS. 6: 22-36.

Englyst, H. 1989. Classification and measurement of plant polysaccharides. Anim. Feed Sci. Technol. 23: 27-42.

Englyst, H.N., Hudson, G.J. 1987. Colorimetric method for routine measurement of dietary fibre as non-starch polysaccharides. A comparison with gas-liquid chromatography. Food Chem. 24: 63-76.

Englyst, H.N., Quigley, M.E., Hudson, G.J. 1994. Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. Analyst. 119: 1497-1509.

Escaffre, A.-M., Kaushik, S., Mambrini, M. 2007. Morphometric evaluation of changes in the digestive tract of rainbow trout (*Oncorhynchus mykiss*) due to fish meal replacement with soy protein concentrate. Aquaculture. 273: 127-138.

Fan, M.Z., Sauer, W.C., De Lange, C.F.M. 1995. Amino acid digestibility in soybean meal, extruded soybean and full-fat canola for early-weaned pigs. Anim. Feed Sci. Technol. 52: 189-203.

FAO (Food and Agriculture Organization of the United Nations). 2012. The State of World Aquaculture. Food and Agriculture Organization of the United Nations, Rome, Italy.

Fenwick, G.R., Curtis, R.F. 1980. Rapeseed meal and its use in poultry diets. A review. Anim. Feed. Sci. Technol. 5: 255-298.

Fenwick, G.R., Heaney, R.K. 1983. Glucosinolates and their breakdown products in cruciferous crops, foods and feedingstuffs. Food Chem. 11: 249-271.

Forster, I., Higgs, D.A., Dosanjh, B.S., Rowshandeli, M., Parr, J. 1999. Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss*) held in 11°C fresh water. Aquaculture. 179: 109-125.

Francis, G., Kerem, Z., Makkar, H.P.S., Becker, K. 2002. The biological action of saponins in animal systems: a review. Br. J. Nutr. 88: 587-605.

Francis, G., Makkar, H.P.S., Becker, K. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture. 199: 197-227.

Friedman, M., 1996. Nutritional value of protein from different food sources. J. Agric. Food Chem. 44: 6-29.

Gan, Y., Yang, R. 2011. Advanced statistical tools and their applications. Can. J. Plant. Sci. 91, 599-601.

Gao, Y., Storebakken, T., Shearer, D.K., Penn, M. and Øverland, M. 2011. Supplementation of fishmeal and plant protein-based diets for rainbow trout with a mixture of sodium formate and butyrate. Aquaculture. 311: 233-240.

Gatlin, D. M., Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E. J. Stone, D., Wilson, R. and Wurtele, E. 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquacult. Res. 38: 551-579.

Gaylord, T.G., Teague, A.M., Barrows, F.T. 2006. Taurine supplementation of all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). J. World Aquacult. Soc. 37: 509-517.

Gee, J.M., Wortley, G.M., Johnson, I.T., Price, K.R., Rutten, A.A.J.J.L., Houben, G.F., Penninks, A.H. 1996. Effects of saponins and glycoalkaloids on the permeability and viability of mammalian intestinal cells and on the integrity of tissue preparations *in vitro*. Toxicol. In Vitro. 10: 117-128

Gil, A., Rueda, R., 2002. Interaction of early diet and the development of the immune system. Nutr. Res. Rev. 15: 263-292.

Glencross, B. 2009. The influence of soluble and insoluble lupin non-starch polysaccharides on the digestibility of diets fed to rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 294: 256-261.

Glencross, B.D., Carter, C.G., Duijster, N., Evans, D.R., Dods, K., McCafferty, P., Hawkinds, W.E., Maas, R., Sipsas, S. 2004. A comparison of the digestibility of a range of lupin and soybean protein products when fed to either Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 237: 333-346.

Glencross, B., Evans, D., Dods, K., McCafferty, P., Hawkins, W., Maas, R., Sipsas,

S. 2005. Evaluation of the digestible value of lupin and soybean protein concentrates and isolates when fed to rainbow trout, *Oncorhynchus mykiss*, using either stripping or settlement faecal collection methods. Aquaculture. 245: 211-220.

Gomes, E.F., Corraze, G., Kaushik, S. 1993. Effects of dietary incorporation of a co-extruded plant protein (rapeseed and peas) on growth, nutrient utilization and muscle fatty acid composition of rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 113: 339-353.

Gomes, E.F., Rema, P., Kaushik, S.J. 1995. Replacement of fish meal by plant proteins in the diet of rainbow trout (*Oncorhynchus mykiss*): digestibility and growth performance. Aquaculture. 130: 177-186.

Gómez-Requeni, P., Mingarro, M., Calduch-Giner, J.A., Médale, F., Martin, S.A.M., Houlihan, D.F., Kaushik, S., Pérez-Sánchez, J. 2004. Protein growth performance, amino acid utilization and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). Aquaculture. 232: 493-510.

Gontier-Latonnelle, K., Cravedi, J.P., Laurentie, M., Perdu, E., Lamothe, V., Le Menn, F., Bennetau-Pelissero, C. 2007. Disposition of genistein in rainbow trout (*Oncorhynchus mykiss*) and Siberian sturgeon (*Acipenser baeri*). Gen. Comp. Endocr. 150: 298-308.

Grosjean, F., Bourdon, D., Kiener, T., Castaing, J., Gatel, F. 1991. Valeur alimentaire pour les porcs des pois Français et importés. Journées Rech. Porcine en France. 23: 53-60.

Gross, J. and Budowski, P. 1966. Conversion of carotenoids into vitamins A_1 and A_2 in two species of freshwater fish. Biochem. J. 101: 747-754.

Grove, D.J., Loizides, L.G., Nott, J. 1978. Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. J Fish Biol 12, 507-516.

Grub, C.D., Abel, S. 2006. Glucosinolate metabolism and its control. Trends Plant Sci. 11: 89-100.

Gunawardena, C.K., Zijlstra, R.T., Goonewardene, L.A., Beltranena, E. 2010. Protein and starch concentrates of air-classified field pea and zero-tannin faba bean for weaned pigs. J. Anim. Sci. 88: 2627-2636.

Gwiazda, S., Rutkowski, A. and Kocon, J. 2006. Some functional properties of pea and soy bean protein preparations. Food / Nahrung. 23: 681-686.

Haidar, B., Mott, S., Boucher, B., Lee, C. Y., Marcil, M., Genest, J. 2001. Cellular cholesterol efflux is modulated by phospholipids-derived signaling molecules in familial HDL deficiency/Tangier disease fibroblasts. J. Lipid Res. 42: 249-257.

Hardy, R.W. 1999. Collaborative opportunities between fish nutrition and other disciplines in aquaculture: an overview. Aquaculture. 177: 217-230.

Hardy, R.W. 2000. New developments in aquatic feed ingredients, and potential of enzyme supplements. *In* Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Olvera-Novoa, M.A., Civera-Cerecedo, R. (Eds.) Avances en Nutrición Acuícola V.Memorias del V Simposium Internacional de Nutrición Acuícola, Yucatán, Mexico. pp. 216-226.

Hardy, R.W., Sullivan, C.V. 1983. Canola meal in rainbow trout (*Salmo gairdneri*) production diets. Can. J. Fish Aquat. Sci. 40: 281-286.

Hart, S.D., Bharadwaj, A.S., Brown, P.B. 2010 Soybean lectins and trypsin inhibitors, but not oligosaccharides or the interactions of factors, impact weight gain of rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 306: 310-314.

Hassan, I.A.G., Elzubeir, E.A., El Tinay, A.H. 2003. Growth and apparent absorption of minerals in broiler chicks fed diets with low or high tannin contents. Trop. Anim. Health Pro. 35: 189-196.

Hasanuzzaman, A.F.M., Siddiqui, M.N, Chisty, M.A.H. 2009. Optimum replacement of fishmeal with soybean meal in diet for *Macrobrachium rosenbergii* (De Man 1879) cultured in low saline water. Turk. J. Fish Aquat. Sci. 9: 17-22.

Hays, S. M. and Aylward, L. L. 2003. Dioxin risks in perspective: past, present, and future. Regul. Toxicol. Pharm. 37: 202-217.

Hedges, L.V., Vevea, J.L. 1998. Fixed and random effects models in meta analysis. Psychol. Methods. 3: 486-504.

Heikkinen, J., Vielma, J., Kemilainen, O., Tiirola, M., Eskelinen, P., Kiuru, T., Naviapaldanius, D., Vonwright, A. 2006. Effects of soybean meal based diet on growth performance, gut histopathology and intestinal microbiota of juvenile rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 261: 259-268.

Hemre, G.-I., Sanden, M., Bakke-McKellep, A.M., Sagstad, A. and Krogdahl, Å. 2005. Growth, feed utilization and health of Atlantic salmon *Salmo salar* L. fed genetically modified compared to non-modified commercial hybrid soybeans. Aquacult. Nutr. 11: 157-167.

Heng, L., Vincken, J.P., van Koningsveld, G., Legger, A., Gruppen, H., van Boekel, T., Roozen, J., Voragen, F. 2006. Bitterness of saponins and their content in dry peas. J. Sci. Food Agric. 86: 1225-1231.

Hernandez-Llamas, A. 2009. Conventional and alternative dose-response models to estimate nutrient requirements of aquaculture species. Aquaculture. 292: 207-213.

Hevrøy, E. M., Sandnes, K. and Hemre, G. –I. 2004. Growth, feed utilization, appetite and health in Atlantic salmon (Salmo salar L.) fed a new type of high lipid fish meal, Sea Grain®, processes from various pelagic marine fish species. Aquaculture. 235: 371-392.

Higgs, D.A., Fagerlund, U.H.M., McBride, J.R., Plotnikoff, M.D., Dosanjh, B.S., Markert, J.R., Davidson, J. 1983. Protein quality of altex canola meal for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) considering dietary protein and 3,5,3'-triiodo-L-thyronine content. Aquaculture. 34: 213-238.

Higgs, D.A., McBride, J.R., Markert, J.R., Dosanjh, B.S., Plotnikoff, M.D., Clarke, W.C. 1982. Evaluation of tower and candle rapeseed (canola) meal and bronowski rapeseed protein concentrate as protein supplements in practical dry diets for juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture. 29: 1-31.

Higgins, J.P.T., Green, S. (Eds). 2008. *Cochrane handbook for systematic reviews of interventions.* Version 5.0.0. The Cochrane Collaboration. Available at www.cochrane-handbook.org [accessed 13 Nov 2012].

Hilton, J.W., Slinger, S.J. 1986. Digestibility and utilization of canola meal in

practical-type diets for rainbow trout (*Salmo gairdneri*). Can. J. Fish Aquat. Sci. 43: 1149-1155.

Hopkins, K.D. 1992. Reporting fish growth: a review of the basics. J. World Aquacult. Soc. 23: 173-179.

Igbasan, F.A., Guenter, W. 1996. The feeding value for broiler chickens of pea chips derived from milled peas (*Pisum sativum* L.) during air classification into starch fractions. Anim. Feed. Sci. Technol. 61: 205-217.

Igbasan, F.A., Guenter, W., Slominski, B.A. 1997. Field peas: chemical composition and energy and amino acid availabilities for poultry. Can. J. Anim. Sci. 77: 293-300.

Iji, P.A., Saki, A.A., Tivey, D.R. 2001. Intestinal development and body growth of broiler chicks on diets supplemented with non-starch polysaccharides. Anim. Feed Sci. Technol. 89: 175-188.

Iredale, D. G., York, R. K. 1976. Purging a muddy – earthy flavor taint from rainbow trout (*Salmo gairdneri*) by transferring to artificial and natural holding environments. J. Fish. Res. Board Can. 33: 160-166.

Ireland, P.A., Dziedzic, S.Z., Kearsley, M.W. 1986. Saponin content of soya and some commercial soya products by means of high-performance liquid-chromatography of the sapogenins. J. Sci. Food Agric. 37: 694-698.

Iwama, G.K., Tautz, A. 1981. A simple growth model for salmonids in hatcheries. Can. J. Fish Aquatic. Sci. 38: 649-656.

Iwashita, Y., Suzuki, N., Matsunari, H., Sugita, T., Yamamoto, T. 2009. Influence

of soya saponin, soya lectin, and cholyltaurine supplemented to a casein-based semipurified diet on intestinal morphology and biliary bile status in fingerling rainbow trout *Oncorhynchus mykiss*. Fish. Sci. 75: 1307-1315.

Jobling, M. 1981. Mathematical models of gastric emptying and the estimation of daily rates of food consumption for fish. J. Fish Biol 19: 245-257.

Jobling, M. 1983a. Growth studies with fish–overcoming the problems of size variation. J. Fish Biol. 22: 153-157.

Jobling, M. 1983b. Influence of body weight and temperature on growth rates of Arctic charr, *Salvelinus alpinus* (L.). J. Fish Biol. 22: 471-475.

Jobling, M.J. 1994. Fish Bioenergetics. Chapman and Hall. London, UK.

Jobling M. 2003. The thermal growth coefficient (TGC) model of fish growth: a cautionary note. Aquacult. Res. 34: 581-584.

Johansson, T., Nordvarg, L. 2002. Empirical mass balance models calibrated for freshwater fish farm emissions. Aquaculture. 212: 191-211.

Johnson, I.T., Gee, J.M., Price, K, Curl, C., Fenwick, G.R. 1986. Influence of saponins on gut permeability and active nutrient transport *in vitro*. J. Nutr. 116: 2270-2277.

Jones, K.A. 1990. Chemical requirements of feeding in rainbow trout, *Oncorhynchus mykiss* (Walbaum); palatability studies on amino acids, amides, amines, alcohols, aldehydes, saccharides and other compounds. J Fish Biol. 37, 413-423.

Jongbloed, A.W., Mroz, Z., van der Weij-Jongbloed, R., Kemme, P.A. 2000. The effects of microbial phytase, organic acids and their interaction in diets for growing pigs. Livest. Prod. Sci. 67: 113-122.

Kalinowski, C. T., Izquierdo, M. S., Schuchardt, D. and Robaina, L. E. 2007. Dietary supplementation time with shrimp shell meal on red porgy (*Pagrus pagrus*) skin colour and carotenoid concentration. Aquaculture. 272: 451-457.

Kaushik, S.J., Cravedi, J.P., Lalles, J.P., Sumbper, J., Fauconneau, B., Laroche, M. 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 133: 257-274.

Kaushik, S.J., Seiliez, I. 2010. Protein and amino acid nutrition and metabolism in fish: current knowledge and future needs. Aquacult. Res. 41: 322-332.

Kemme, P.A., Jongbloed, A.W., Mroz, Z., Benyen, A.C. 1998. Diurnal variation of phytic acid by plant phytase in the pig stomach. Livest. Prod. Sci. 54, 33-44.

Kemme, P.A., Jongbloed, A.W., Mroz, Z., Kogut, J., Beynen, A.C. 1999. Digestibility of nutrients in growing-finishing pigs is affected by *Aspergillus niger* phytase, phytate and lactic acid levels: 2. Apparent total tract digestibility of phosphorus, calcium and magnesium and ileal degradation of phytic acid. Livest. Prod. Sci. 58: 119-127.

Khattab, R.Y., Arntfield, S.D. 2009. Nutritional quality of legume seeds as affected by some physical treatments 2. Antinutritional factors. Lebensm. Wiss. Technol. 42: 1113-1118.

Khattab, R., Goldberg, E., Lin, L., Thiyam, U. 2010. Quantitative analysis and free-radical-scavenging activity of chlorophyll, phytic acid, and condensed tannins in canola. Food Chem. 122: 1266-1272.

Kies, A.K., De Jonge, L.H., Kemme, P.A., Jongbloed, A.W. 2006. Interaction between protein, phytate, and microbial phytase. In vitro studies. J. Agric. Food Chem. 54: 1753-1758.

Kim, H., Peterson, T.G., Barnes, S. 1998. Mechanisms of action of the soy isoflavone genistein: emerging role for its effects via transforming growth factor beta signaling pathways. Am. J. Clin. Nutr. 68: 1418S-1425S.

Kleiber, M. (Ed.). 1961. The Fire of Life: an Introduction to Animal Energetics. R.E. Krieger Pub. Co., Huntington, NY, USA. pp. 255-257.

Knudsen, D., Jutfelt, F., Sundh, H., Sundell, K., Koppe, W., Frøkiær, H. 2008. Dietary soya saponins increase gut permeability and play a key role in the onset of soyabean-induced enteritis in Atlantic salmon (*Salmo salar* L.) Br. J. Nutr. 100: 120-129.

Kortner, T.M., Skugor, S., Penn, M.H., Mydland, L.T., Djordjevic, B., Hillestad, M., Krasnov, A., Krogdahl, Å. 2012. Dietary soyasaponin supplementation to pea protein concentrate reveals nutrigenomic interactions underlying enteropathy in Atlantic salmon (*Salmo salar*). BMC Vet. Res. 8, 101.

Kousoulaki, K., Albrektsen, S., Lanhmyhr, E., Olsen, H.J., Campbell, P., Aksnes, A. 2009. The water soluble fraction in fish meal (stickwater) stimulates growth in Atlantic salmon (*Salmo salar* L.) given high plant protein diets. Aquaculture. 289: 74-83.

Kraugerud, O.F., Penn, M., Storebakken, T., Refstie, S., Krogdahl, Å., Svihus, B.

2007. Nutrient digestibilities and gut function in Atlantic salmon (*Salmo salar*) fed diets with cellulose or non-starch polysaccharides from soy. Aquaculture. 273: 96-107.

Krogdahl, Å., Bakke-McKellep, A. M. and Baeverfjord, G. 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). Aquacult. Nutr. 9: 361-371.

Krogdahl, Å., Lea, T.B., Olli, J.J. 1994. Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilities in rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. A. 107: 215-219.

Krogdahl, Å., Penn, M. Thorsen, J., Refstie, S. Bakke, A.M. 2010. Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. Aquacult. Res. 41: 333-344.

Krogdahl, Å., Sundby, A., Olli, J.J. 2004. Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) digest and metabolize nutrients differently. Effects of water salinity and dietary starch level. Aquaculture. 229: 335-360.

Kumar, R., Vaithiyanathan, S. 1990. Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. Anim. Feed Sci. Technol. 30: 21-38.

Ladha, S. 2000. The lipid organisation of the cell membrane. Grasas Aceites. 51: 56-65.

Lamb, E.G., Cahill, J.F. 2008. When competition does not matter: grassland diversity and community composition. Amer. Nat. 171, 777-787.

Lamb, E.G., Shirtliffe, S.J., May, W.E. 2011. Structural equation modeling in the plant

sciences: An example using yield components in oat. Can. J. Plant Sci. 91, 603-619.

Lanno, R.P., Dixon, D.G. 1996. The comparative chronic toxicity of thiocyanate and cyanide to rainbow trout. Aquat. Toxicol. 36: 177-187.

Lauff, R. F. and Wood, C. M. 1996. Respiratory gas exchange, nitrogenous waste excretion, and fuel usage during starvation in juvenile rainbow trout, *Oncorhynchus mykiss*. J. Comp. Physiol. B. 165: 542-551.

Leatherland, J.F., Hilton, J.W. 1988. Thyroidal compensation in rainbow trout (*Salmo gairdneri)* fed canola meal. Fish Physiol. Biochem. 5: 199-207.

Lee, K.-J., Dabrowski, K., Blom, J.H., Bai, S.C. and Stromberg, P.C. 2002. A mixture of cottonseed meal, soybean meal and animal byproduct mixture as a fish meal substitute: growth and tissue gossypol enantiomer in juvenile rainbow trout (*Oncorhynchus mykiss*). J. Anim. Physiol. Anim. Nutr. 86: 201-213.

Lee, K.-J., Rinchard, J., Dabrowski, K., Babiak, I., Ottobre, J.S., Christensen, J.E. 2006. Long-term effects of dietary cottonseed meal on growth and reproductive performance of rainbow trout: three-year study. Anim. Feed Sci. Technol. 126: 93-106.

Lehrfeld, J. 1989. High-performance liquid chromatography analysis of phytic acid on a pH-stable, macroporous polymer column. Cereal. Chem. 6: 510-515.

Lestienne, I., Icard-Vernière, C., Mouquet, C., Picq, C., Trèche, S. 2005. Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. Food Chem. 89: 421-425.

Li, P., Gatlin, D.M. 2006. Nucleotide nutrition in fish: current knowledge and future application. Aquaculture. 251: 141-152.

Li, P., Wang, X., Hardy, R. W. and Gatlin, D. M. 2004. Nutritional value of fisheries by-catch and by-product meals in the diet of red drum (*Scaenops ocellatus*). Aquaculture. 236: 485-496.

Liebert, F., Portz, L. 2005. Nutrient utilization of Nile tilapia *Oreochromis niloticus* fed plant based low phosphorus diets supplemented with graded levels of different sources of microbial phytase. Aquaculture. 248: 111-119.

Lo, M., Bell., J.M. 1972. Effects of various dietary glucosinolates on growth, feed intake, and thyroid function of rats. Can. J. Anim. Sci. 52, 295-302.

Lönnerdal, B., Sandberg, A.S., Sandström, B., Kunz, C. 1989. Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. J. Nutr. 119: 211-214.

Lunger, A.N., McLean, E., Gaylord, T.G., Kuhn, D., Craig, S.R. 2007. Taurine supplementation to alternative dietary proteins used in fish meal replacement enhances growth of juvenile cobia (*Rachycentron canadum*). Aquaculture. 271: 401-410.

Lupatsch, I., Kissil, G. W., Sklan, D. 2003. Comparison of energy and protein efficiency among three fish species gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*) and white grouper (*Epinephelus aeneus*): energy expenditure for protein and lipid deposition. Aquaculture. 225: 175-189.

Maenz, D.D., Engele-Schaan, C.M., Newkirk, R.W., Classen, H.L. 1999. The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-

susceptible forms of phytic acid in solution and in a slurry of canola meal. Anim. Feed Sci. Technol. 18: 177-192.

Mahgoub, S.E.O., Elhag, S.A. 1998. Effect of milling, soaking, malting, heat-treatment and fermentation on phytate level of four Sudanese sorghum cultivars. Food Chem. 61: 77-80.

Mambrini, M., Guillaume, J., 1999. Nutrition protéique. *In* Guillaume, J., Kaushik, S.J., Bergot, P., Métailler, R. (Eds.) Nutrition et alimentation des poissons et des crustacés. Collections Du Labo au Terrain. Éditions INRA, Paris, France. pp. 113-146.

Mambrini, M., Roem, A.J., Carvedi, J.P., Lalles, J.P., Kaushik, S.J. 1999. Effects of replacing fish meal with soy protein concentrate and of DL-methionine supplementation in high-energy, extruded diets on the growth and nutrient utilization of rainbow trout, *Oncorhynchus mykiss*. J. Anim. Sci. 77: 2990-2999.

Mansfield, G.S., Desai, A.R., Nilson, S.A., Van Kessel, A.G., Drew, M.D., Hill, J.E. 2010. Characterization of rainbow trout (*Oncorhynchus mykiss*) intestinal microbiota and inflammatory marker gene expression in a recirculation aquaculture system. Aquaculture. 307: 95-104.

Mariscal-Landín, G., Avellaneda, J.H., Reis de Souza, T.C., Aguilera, A., Borbolla, G.A., Mar, B. Effect of tannins in sorghum on amino acid ileal digestibility and on trypsin (E.C.2.4.21.4) and chymotrypsin (E.C.2.3.21.1) activity on growing pigs. Anim. Feed Sci. Technol. 117: 245-264.

Mattson, F. H. and Volpenhein, R. A. 1964. The digestion and absorption of triglycerides. The Journal of Biological Chemistry. 239: 2772-2777.

Maynard, L.A., Loosli, J.K. 1969. Animal Nutrition. 6th ed. McGraw-Hill, New York, NY, USA.

McCurdy, S.M., March, B.E. 1992. Processing of canola meal for incorporation in trout and salmon diets. JAOCS. 69: 213-220.

McDonald, M. E., Tikkanen, C. A., Axler, R. P., Larsen, C. P., Host, G. 1996. Fish simulation culture model (FIS-C): a bioenergetics based model for aquaculture wasteload application. Aquacult. Eng. 15: 243-259.

Médale, F., Boujard, T., Vallée, F., Blanc, D., Mambrini, M., Roem, A., Kaushik, S.J. 1998. Voluntary feed intake, nitrogen and phosphorus losses in rainbow trout (*Oncorhynchus mykiss*) fed increasing dietary levels of soy protein concentrate. Aquat. Living Resour. 11: 239-246.

Meng, X., Slominski, B.A. 2005. Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a multicarbohydratase preparation of cell wal degrading enzymes. Poultry Sci. 84: 1242-1251.

Merrifield, D.L., Dimitrouglou, A., Bradley, G., Baker, R.T., Davies, S.J. 2009. Soybean meal alters autochthonous microbial populations, microvilli morphology and compromises intestinal enterocyte integrity of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis. 32: 755-766.

Milgate, J. and Roberts, D.C.K. 1995. The nutritional and biological significance of saponins. Nutr. Res. 15: 1223-1249.

Mohamed, J. S., Sivaram, V., Roy, T. S. C., Marian, M. P., Murugadass, S. and Hussain, M. R. 2003. Dietary vitamin A requirement of juvenile greasy grouper (*Epinephelus tauvina*). Aquaculture. 219: 693-701.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G. 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Plos Med. 89: 873-880.

Morales, A.E., Cardenete, G., De la Higuera, M., Sanz, A. 1994. Effects of dietary protein source on growth, feed conversion and energy utilization in rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 124: 117-126.

Morales, G.A., Moyano, F.J., Marquez, L. 2011. *In vitro* assessment of the effects of phytate and phytase on nitrogen and phosphorus bioaccessibility within fish digestive tract. Anim. Feed Sci. Technol. 170: 209-221.

Moreno-Rojas, J.M., Tulli, F., Messina, M., Tibaldi, E., Guillou, C. 2008. Stable isotope ratio analysis as a tool to discriminate between rainbow trout (*O. mykiss*) fed diets based on plant or fish-meal proteins. Rapid Commun. Mass Spectrom. 22: 3706-3710.

Morris, P.C., Gallimore, P., Handley, J., Hide, G., Haughton, P., Black, A. 2005. Full-fat soya for rainbow trout (*Oncorhynchus mykiss*) in freshwater: effects on performance, composition and flesh fatty acid profile in absence of hind-gut enteritis. Aquaculture. 248: 147-161.

Morris, J.G., Rogers, Q.R., Pacioretty, L.M. 1990. Taurine: an essential nutrient for cats. J. Small Anim. Pract. 31: 502-509.

Mundheim, H., Aksnes, A., Hope, B. 2004. Growth, feed efficiency and digestibility in salmon (*Salmo salar* L.) fed different dietary proportions of vegetable protein sources in combination with two fish meal qualities. Aquaculture. 237: 315-331.

Murai, T., Ogata, H., Villaneda, A. and Watanabe, T. 1989. Utilization of soy flour by fingerling rainbow trout having different body size. Nippon Suisan Gakk. 55: 1067-1073.

Mwachireya, S.A., Beames, R.M., Higgs, D.A., Dosanjh, B.S. 1999. Digestibility of canola protein products derived from the physical, enzymatic and chemical processing of commercial canola meal in rainbow trout *Oncorhynchus mykiss* (Walbaum) held in fresh water. Aquacult. Nutr. 5: 73-82.

Naczk, M., Amarowicz, R., Sullivan, A., Shahidi, F. 1998. Current research developments on polyphenolics of rapeseed/canola: a review. Food Chem. 62: 489-502.

Nash, A.M., Eldridge, A.C., Wolf, W.J. 1967. Fractionation and characterization of alcohol extractables associated with soybean proteins. Non-protein components. Agric. Food Chem. 15: 102-108.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M. 2000. Effect of aquaculture on world fish supplies. Nature. 405: 1017-1024.

Newkirk, R.W., Classen, H.L. 1998. In vitro hydrolysis of phytate in canola meal with purified and crude sources of phytase. Anim. Feed Sci. Technol. 72: 315-327.

Newkirk, R.W., Classen, H.L., Edney, M.J. 2003. Effects of prepress-solvent extraction on the nutritional value of canola meal for broiler chickens. Anim. Feed Sci. Technol. 104: 111-119.

NRC (National Research Council). 2011. Nutrient Requirements of Fish and Shrimp. National Academy Press, Washington, DC, USA.

NRC (National Research Council). 1993. Nutrient Requirements of Fish. National Academy Press, Washington, DC, USA.

Ng, Y., Hanson, S., Malison, J.A., Wentworth, B., Barry, T.P. 2006. Genistein and other isoflavones found in soybeans inhibit estrogen metabolism in salmonid fish. Aquaculture. 254: 658-665.

Novak, W.K., Haslberger, A.G. 2000. Substantial equivalence of antinutrients and inherent plant toxins in genetically modified novel foods. Food Chem. Toxicol. 38: 473-483.

Oberleas, D., Muhrer, M.E., O'Dell, B.L. 1962. Effects of phytic acid on zinc availability and parakeratosis in swine. J. Anim. Sci. 21: 57-61.

Oleszek, W.A. 2002. Chromatographic determination of plant saponins. J. Chromatogr. A 967, 147-162.

Oliva-Teles, A., Gouveia, A.J., Gomes, E., Rema, P. 1994. The effect of different processing treatments on soybean meal utilization by rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 124: 343-349.

Olli, J.J., Krogdahl, Å. 2008. Alcohol soluble components of soybeans seem to reduce fat digestibility in fish-meal-based diets for Atlantic salmon, *Salmo salar* L. Aquacult. Res. 26: 831-835.

Önning, G., Wang, Q., Weström, B.R., Asp, N., Karlsson, B.W. 1996. Influence of oat saponins on intestinal permeability *in vitro* and *in vivo* in the rat. Br. J. Nutr. 76: 141-151.

Øverland, M., Sørensen, M., Storebakken, T., Penn, M., Krogdahl, Å., Skrede, A.

2009. Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (*Salmo salar*) – Effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. Aquaculture. 288: 305-311.

Owusu-Asiedu, A., Patience, J.F., Laarveld, B., Van Kessel, A.G., Simmins, P.H., Zijlstra, R.T. 2006. Effects of guar gum and cellulose on digesta passage rate, ileal microbial populations, energy and protein digestibility, and performance of grower pigs. J. Anim. Sci. 84: 843-852.

Palacios, M.C., Haros, M., Sanz, Y., Rosell, C.M. 2008. Selection of lactic acid bacteria with high phytate degrading activity for application in whole wheat breadmaking. LWT – Food Science and Technology. 41: 82-92.

Pallauf, J, Pietsch, M., Rimbach, G. 1998. Dietary phytate reduces magnesium bioavailability in growing rates. Nutr. Res. 18: 1029-1037.

Pauly, D., Christensen, V., Guenette, S., Pitcher, T.J., Sumaila, U.R., Walters, C.J., Watson, R., Zeller, D. 2002. Towards sustainability in world fisheries. Nature. 418: 689-695.

Penn, M.H., Bendiksen, E.Å., Campbell, P., Krogdahl, Å. 2011. High level of dietary pea protein concentrate induces enteropathy in Atlantic salmon (*Salmo salar* L.). Aquaculture. 310: 267-273.

Pereira, O., Rosa, E., Pires, M.A., Fontaínhas-Fernandes, A. 2002. Brassica by-products in diets of rainbow trout (*Oncorhynchus mykiss*) and their effects on performance, body composition, thyroid status and liver histology. Anim. Feed Sci. Technol. 101: 171-182.

Perez-Maldonado, R.A., Mannion, P.F., Farrell. 1999. Optimum inclusion of field peas, faba beans, chick peas and sweet lupins in poultry diets. I. Chemical composition and layer experiments. Brit. Poultry Sci. 40: 667-673.

Pfeffer, E., Beckman-Toussaint, J., Henrichfreise, B., Jansen, H.D. 1991. Effect of extrusion on efficiency of utilization of maize starch by rainbow trout (Oncorhynchus mykiss). Aquaculture. 96: 293-303.

Pierce, L.R., Palti, Y., Silverstein, J.T., Barrows, F.T., Hallerman, E.M and Parsons, J.E. 2008. Family growth response to fishmeal and plant-based diets shows genotype x diet interaction in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 278: 37-42.

Ping, L., Pizzi, A., Guo, Z.D., Brosse, N. 2011. Condensed tannins extraction from grape pomace: characterization and utilization as wood adhesives for wood particleboard. Ind. Crop. Prod. 34: 907-914.

Penn, M.H., Bendiksen, E.A., Campbell, P., Krogdahl, Å. 2011. High level of dietary pea protein concentrate induces enteropathy in Atlantic salmon (*Salmo salar* L.). Aquaculture. 310: 267-273.

Pongmaneerat, J., Watanabe, T. 1993. Effect of extrusion processing on the utilization of soybean meal diets for rainbow trout. Nippon Suisan Gakk. 59: 1407-1414.

Rabanal, H.R. 1988. History of Aquaculture. Food and Agriculture Organization of the United Nations, Rome, Italy.

Raboy, V. 2009. Approaches and challenges to engineering seed phytate and total phosphorus. Plant Sci. 177: 281-296.

Raboy, V., Young, K.A., Dorsch, J.A., Cook, A. 2001. Genetics and breeding of seed phosphorus and phytic acid. J. Plant Physiol. 158: 489-497.

Refstie, S., Baeverfjord, G., Seim, R.R., Elvebø, O. 2010. Effects of dietary yeast cell wall ß-glucans and MOS on performance, gut health, and salmon lice resistance in Atlantic salmon (*Salmo salar*) fed sunflower and soybean meal. Aquaculture. 305: 109-116.

Refstie, S., Helland, S.J., Storebakken, T. 1997. Adaptation to soybean meal in diets for rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 153: 263-272.

Refstie, S., Korsøen, Ø.J., Storebakken, T., Baeverfjord, G., Lein, I., Roem, A.J. 2000. Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Aquaculture. 190: 49-63.

Refstie, S., Oyvind, J. K., Storebakken, T., Baeverfjord, G., Lein, I, Roem, A. J. 2000. Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Aquaculture. 190: 49-63.

Refstie, S., Sahlström, S., Bråthen, E., Baeverfjord, G., Krogedal, P. 2005. Lactic acid fermentation eliminated indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). Aquaculture. 246: 331-345.

Refstie, S., Storebakken, T., Baeverfjord, G., Roem, A.J. 2001. Long-term protein

and lipid growth of Atlantic salmon (*Salmo salar*) fed diets with partial replacement of fish meal by soy protein products at medium or high lipid level. Aquaculture. 193: 91-106.

Refstie, S., Storebakken, T., Roem, A.J. 1998. Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. Aquaculture. 162: 301-312.

Refstie, S., Svihus, B., Shearer, K.D., Storebakken, T. 1999. Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soyabean products. Anim. Feed Sci. Technol. 79: 331-345.

Reveco, F.E., Collins, S.A., Randall, K.M, Drew, M.D. 2011. Aqueous fractionation improves the nutritional value of wheat distillers grains for rainbow trout (*Oncorhynchus mykiss*). Aquacult. Nutr. 18: 202-210.

Romarheim, O.H., Hetland, D.L., Skrede, A., Øverland, M., Mydland, L.T., Landsverk, T. 2013. Prevention of soya-induced enteritis in Atlantic salmon (*Salmo salar*) by bacteria growth on natural gas is dose dependent and related to epithelial MHC II reactivity and CD8 alpha (+) intraepithelial lymphocytes. Br. J. Nutr. 109: 1062-1070.

Romarheim, O.H., Øverland, M., Mydland, L.T., Skrede, A., Landsverk, T. 2011. Soybean meal-induced enteritis in Atlantic salmon. J. Nutr. 256: 354-364.

Romarheim, O.H., Skrede, A., Gao, Y., Krogdahl, Å., Denstadli, V., Lilleeng, E.,

Storebakken, T. 2006. Comparison of white flakes and toasted soybean meal partly replacing fish meal as protein source in extruded feed for rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 256: 354-364.

Roy, P. K., Lall, S. P. 2004. Urinary phosphorus excretion in haddock, *Melanogrammus aeglefinus* (L.) and Atlantic salmon, *Salmo salar* (L.). Aquaculture. 233: 369-382.

Rüfer, C.E., Kulling, S.E. 2006. Antioxidant activity of isoflavones and their major metabolites using different in vitro assays. J. Agric. Food Chem. 54: 2926-2931.

Rumsey, G.L., Siwicki, A.K., Anderson, D.P., Bowser, P.R. 1994. Effect of soybean protein on serological response, non-specific defense mechanisms, growth, and protein utilization in rainbow trout. Vet. Immunol. Immunop. 41: 323-339.

Sajjadi, M., Carter, C.G. 2004. Dietary phytase supplementation and the utilisation of phosphorus by Atlantic salmon (*Salmo salar* L.) fed a canola-meal-based diet. Aquaculture. 240: 417-431.

Sales, J. 2009. The effect of fish meal replacement by soyabean products on fish growth: a meta-analysis. Brit. J. Nutr. 102: 1709-1722.

Sanden, M., Krogdahl, Å., Bakke-McKellep, A.M., Buddington, R.K. Hemre, G.-I. 2006. Growth performance and organ development in Atlantic salmon, *Salmo salar* L. parr fed genetically modified (GM) soybean and maize. Aquacult. Nutr. 12: 1-4.

Sanz, A., Morales, A.E., de la Higuera, M., Cardenete, G. 1994. Sunflower meal

compared with soybean meal as partial substitutes for fish meal in rainbow trout (*Oncorhynchus mykiss*) diets: protein and energy utilization. Aquaculture. 128: 287-300.

Satoh, S. 1998. Effect of extrusion processing on the nutritive value of canola meal for Chinook salmon (*Oncorhynchus tshawytscha*) in seawater. Aquacult. Nutr. 4: 115-122.

Satoh, S., Hernández, A., Tokoro, T., Morishita, Y., Kiron, V., Watanabe, T. 2003. Comparison of phosphorus retention efficiency between rainbow trout (*Oncorhynchus mykiss*) fed a commercial diet and a low fish meal based diet. Aquaculture. 224: 271-282.

Satoh, S., Higgs, D.A., Dosanjh, B.S., Hardy, R.W., Eales, J.G., Deacon, G. 1998. Effect of extrusion processing on the nutritive value of canola meal for Chinook salmon (*Oncorhynchus tshawytscha*) in seawater. Aquacult. Nutr. 4: 115-122.

Schöne, F., Lüdke, H., Hennig, A., Jahreis, G. 1988. Copper and iodine in pig diets with high glucosinolate rapeseed meal. II. Influence of iodine supplements for rations with rapeseed meal untreated or treated with copper ions on performance and thyroid hormone status of growing pigs. Anim. Feed Sci. Technol. 22: 45-59.

Schumacker, R.E., Lomax, R.G. 2010. A Beginner's Guide to Structural Equation Modeling. 3rd ed. Taylor and Francis Group, LLC, New York, NY, USA.

Seegal, R. F., Brosch, K. O. and Okoniewski, R. J. 1997. Effects of *in utero* and lactational exposure of the laboratory rat to 2, 4, 2', 4' – and 3, 4, 3', 4' – tetrachlorobiphenyl on dopamine function. Toxicol. Appl. Pharm. 146: 95-103.

Selden, G.L., Brown, P.B., Ostrowski, A.C., Flores, R.A., Johnson, L.A. 2001. Evaluation of soybean meal-red blood cell coextruded feed ingredient in diets fed to rainbow trout *Oncorhynchus mykiss*. J. World Aquacult. Soc. 32: 409-415.

Shafaeipour, A., Yavari, V., Falahatkar, B., Marammazi, J.Gh., Gorjipour, E. 2008. Effects of canola meal on physiological and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*). Aquacult. Nutr. 14: 110-119.

Shand, P.J., Ya, H., Pietrasik, Z., Wanasundara, P.K.J.P.D. 2007. Physicochemical and textural properties of heat-induced pea protein isolate gels. Food Chem. 102: 1119-1130.

Sharma, R., Derr-Yellin, E. C., House, D. E., Kodavanti, P. R. S. 2000. Age-dependent effects of Aroclor 1254^{R} on calcium uptake by subcellular organelles in selected brain regions of rats. Toxicology. 156: 13-25.

Shepherd, C.J., Pike, I.H., Barlow, S.M. 2005. Sustainable feed resources of marine Origin. EAS Special Publication No. 35. European Aquaculture Society, Oostende, Belgium. pp. 59-66.

Singh, S., Gamlath, S., Wakeling, L. 2007. Nutritional aspects of food extrusion: a review. Int. J. Food Sci. Tech. 42: 916-929.

Singh, K., Garg, S.K., Kalla, A., Bhatnagar, A. 2003. Oilcakes as a protein source in supplementary diets for the growth of *Cirrhinus mrigala* (Ham.) fingerlings: laboratory and field studies. Bioresource Technol. 86: 283-291.

Sinha, A.K., Kuma, V., Makkar, H.P.S., De Boeck, G., Becker, K. 2011. Non-starch polysaccharides and their role in fish nutrition – A review. Food Chem. 127: 1409-1426.

Skonberg, D. I., Yogev, L., Hardy, R. W., Dong, F. M. 1997. Metabolic response to dietary phosphorus intake in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 157: 11-24.

Sokrab, A.M., Ahmed, I.A.M., Babiker, E.E. 2012. Effect of germination on antinutritional factors, total, and extractable minerals of high and low phytate corn (*Zea mays* L.) genotypes. Journal of the Saudi Society of Agricultural Sciences. 11: 123-128.

Sørensen, M., Penn, M., El-Mowafi, A., Storebakken, T., Chunfang, C., Øverland, M., Krogdahl, Å. 2011. Effect of stachyose, raffinose and soya-saponins supplementation on nutrient digestibility, digestive enzymes, gut morphology and growth performance in Atlantic salmon (*Salmo salar*, L). Aquaculture. 314: 145-152.

Sparg, S.G., Light, M.E., van Staden, J. 2004. Biological activities and distribution of plant saponins. J. Ethnopharmacol. 94: 219-243.

Stapleton PP, Charles RP, Redmond HP, Bouchier-Hayes DJ. (1997) Taurine and human nutrition. Clin. Nutr. 16: 103-108.

Steffens, W. 1997. Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish for humans. Aquaculture. 151: 97-119.

Stickney, R.R., Hardy, R.W., Koch, K., Harrold, R., Seawright, D., Massee, K.C.

1996. The effects of substituting selected oilseed protein concentrates for fish meal in rainbow trout *Oncorhynchus mykiss* diets. J. World Aquacult. Soc. 54: 57-63.

Storebakken, T., Shearer, K.D., Roem, A.J. 1998. Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentrate-based diets to Atlantic salmon, *Salmo salar*. Aquaculture. 161: 365-379.

Storebakken, T., Shearer, K.D., Roem, A.J. 2000. Growth, uptake and retention of nitrogen and phosphorus, and absorption of other minerals in Atlantic salmon, *Salmo salar* fed diets with fish meal and soy-protein concentrate as the main sources of protein. Aquacult. Nutr. 6: 103-108.

Sugiura, S.H., Dong, F.M., Rathbone, C.K., Hardy, R.W. 1998. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture. 159: 177-202.

Taherian, A.R., Mondor, M., Labranche, J., Drolet, H., Ippersiel, D., Lamarche, F. 2011. Comparative study of functional properties of commercial and membrane processed yellow pea protein isolates. Food Res. Int. 44: 2505- 2514.

Talbot, C., Hole, R. 1994. Fish diets and the control of eutrophication resulting from aquaculture. J. Appl. Ichthyol. 10: 258-270.

Terrill, T.H., Rowan, A.M., Douglas, G.B., Barry, T.N. 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. J. Sci. Food Agric. 58: 321-329.

Teskeredzic, Z., Higgs, D.A., Dosanjh, B.S., McBride, J.R., Hardy, R.W., Beames,

R.M., Jones, J.D., Simell, M., Vaara, T. and Bridges, R.B. 1995. Assessment of undephytinized and dephytinized rapeseed protein concentrate as sources of dietary protein for juvenile rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 131: 261-277.

Thiessen, D. L., Campbell, G. L., Adelizi, P. D. 2003. Digestibility and growth performance of juvenile rainbow trout (*Oncorhynchus mykiss*) fed with pea and canola products. Aquacult. Nutr. 9: 67-75.

Thiessen, D.L., Maenz, D.D., Newkirk, R.W., Classen, H.L, Drew, M.D. 2004. Replacement of fishmeal by canola protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss*). Aquacult. Nutr. 10: 379-388.

Thompson, D.B., Huang, C., Sieglaff, C. 1987. Rheological behaviour of soluble polysaccharide fractions from soybeans. Food Hydrocolloid. 1, 333-337.

Tocher, D. R., Fonseca-Madrigal, J., Dick, J. R., Ng, W., Bell, J. G., Campbell, P. J. 2004. Effects of water temperature and diets containing palm oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes of rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. B. 137: 49-63.

Topping, D. 2007. Cereal complex carbohydrates and their contribution to human health. J. Cereal Sci. 46: 220-229.

Torstensen, B.E., Espe, M., Sanden, M., Stubhaug, I., Waagbø, R., Hemre, G.-I., Fontanillas, R., Nordgarden, U., Hevrøy, E.M. Olsvik, P. and Berntssen, M.H.G. 2008. Novel production of Atlantic salmon (*Salmo salar*) protein based on combined replacement of fish meal and fish oil with plant meal and vegetable oil blends. Aquaculture. 285: 193-200.

Tran-Duy, A., Smit, B., van Dam A.A. and Schrama, J.W. 2008. Effects of dietary starch and energy levels on maximum feed intake, growth and metabolism of Nile tilapia, *Oreochromis niloticus*. Aquaculture. 277: 213-219.

Tripathi, M.K., Mishra, A.S. 2007. Glucosinolates in animal nutrition: a review. Anim. Feed Sci. Technol. 132: 1-27.

Urán, P.A., Gonçalves, A.A., Taverne-Thiele, J.J., Schrama, J.W., Verreth, J.A.J., Rombout, J.H.W.M. 2008. Soybean meal induces intestinal inflammation in common carp (*Cyprinus carpio* L.). Fish Shellfish Immun. 25: 751-760.

Urbano, G., López-Jurado, M., Aranda, P., Vidal-Valverde, C., Tenorio, E., Porres, J. 2000. The role of phytic acid in legumes: antinutrient or beneficial function? J. Physiol. Biochem. 56: 283-294.

Valaja, J., Plaami, S., Siljander-Rasi, H. 1998. Effect of microbial phytase on digestibility and utilisation of phosphorus and protein in pigs fed wet barley protein with fibre. Anim. Feed Sci. Technol. 72: 221-233.

Van Soest, P.J., Robertson, J.B., Lewis, B.A. 1991. Method for dietary fibre, neutral detergent fibre and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

Vielma, J., Mäkinen, T., Ekholm, P., Koskela, J. 2000. Influence of dietary soy and phytase levels on performance and body composition of large rainbow trout (*Oncorhynchus mykiss*) and algal availability of phosphorus load. Aquaculture. 183: 349-362.

Vielma, J., Ruohonen, K., Peisker, M. 2002. Dephytinization of two soy proteins increases phosphorus and protein utilization by rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 204: 145-156.

Visentainer, J. V., de Souza, N. E., Makoto, M., Hayashi, Franco, M. R. B. 2005. Influence of diets enriched with flaxseed oil on the alpha–linolenic, eicosapentaenoic and docosahexaenoic fatty acid in Nile tilapia (*Oreochromis niloticus*). Food Chem. 90: 557-560.

Vose, J.R., Basterrechea, M.J., Gorin, P.A.J., Finlayson, A.J., Youngs, C.G. 1976. Air classification of field peas and horsebean flours. Chemical studies of starch and protein fractions. Cereal Chem. 53: 928-936.

Wang, X., Warkentin, T.D., Briggs, C.J., Oomah, B.D., Campbell, C.G., Woods, S. 1998. Total phenolics and condensed tannins in field pea (*Pisum sativum* L.) and grass pea (*Lathyrus sativus* L.). Euphytica. 101: 97-102.

Watanabe, T., Pongmaneerat, J., Sato, S., Takeuchi, T. 1993. Replacement of fish meal by alternative protein sources in rainbow trout diets. Nippon Suisan Gakk. 59: 1573-1579.

Willoughby, S. 1999. Manual of Salmonid Farming. Blackwell Science, INC., Oxford, England.

Webster, C.D., Tiu, L.G., Tidwell, J.H., Grizzle, J.M. 1997. Growth and body composition of channel catfish (*Ictalurus punctatus*) fed diets containing various percentages of canola meal. Aquaculture. 150: 103-112.

Yamamoto, T., Shima, T., Furuita, H. and Suzuki, N. 2002. Influence of feeding

diets with and without fish meal by hand and by self-feeders on feed intake, growth and nutrient utilization of juvenile rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 214: 289-305.

Yeh, P-H., Zhy, H., Nicoletti, M.A., Hatch, J.P., Brambilla, P., Soares, J.C. 2010. Structural equation modeling and principal component analysis of gray matter volumes in major depressive and bipolar disorders: Differences in latent volumetric structure. Psychiat. Res-Neuroim. 184, 177-185.

Ytrestoyl, T. and Bjerkeng, B. 2007. Dose response in uptake and deposition of intraperitoneally administered astaxanthin in Atlantic salmon (*Salmo salar* L.) and Atlantic cod (*Gadus morhua* L.). Aquaculture. 263: 179-191.

Yu, Y. 2004. Replacement of fishmeal with poultry byproduct meal and meat and bone meal in shrimp, tilapia and trout diets. *In* Cruz Suárez, L.E., Ricque Marie, D., Nieto López, M.G., Villareal, D., Scholz, U., González, M. (Eds.) Avances en Nutrición Acuicola VII. Memorias del VII Simposium Internacional de Nutrición Acuicola, Hermosillo, Sonora, México. pp. 182- 201.

Zhou, Z., Xie, S., Lei, W., Zhu, X., Yang, Y. 2005. A bioenergetic model to estimate feed requirement of gibel carp, *Carassius auratus gibelio.* Aquaculture. 248: 287-297.

10. APPENDIX A – Additional growth performance and feed intake data from Chapter 5.

10.1. Description

The growth and feed intake data in Chapter 5 is presented using a number of best-fit regression curves. These regression equations provide useful tools for future ingredient evaluation trials, although some researchers may prefer actual numerical values at specific ingredient inclusion levels. Table 10.1.1. provides rainbow trout performance data and hepatosomatic index (HSI) for fish fed SBM, PM, CM, SPC, PPC and CPC at inclusion levels of 0, 75, 150, 225 and 300 g/kg and Table 10.1.2. provides linear and quadratic regression equations for ADFI, SGR, FCR and PER.

Table 10.1.1. Growth performance and feed intake of rainbow trout in SBM, PM, CM, SPC, PPC and CPC growth trials.

	0 g/kg	75 g/kg	150 g/kg	225 g/kg	300 g/kg	SEM
ADG (g / d)						
SBM	3.2	3.7	4.8	3.4	2.0	0.41
PM	4.7	4.8	4.5	5.3	5.6	0.32
CM	5.9[b]	3.9[ab]	4.4[ab]	3.6[ab]	2.9[b]	0.59
SPC	4.8	5.1	5.0	4.0	4.1	0.52
PPC	4.3	4.8	4.5	4.8	4.9	0.28
CPC	4.8	5.1	3.9	3.1	3.2	1.05
SGR (% / d)						
SBM	0.55[b]	0.62[b]	0.67[b]	0.54[b]	0.32[a]	0.039
PM	1.62	1.41	1.32	1.39	1.53	0.051
CM	0.73[b]	0.63[ab]	0.63[ab]	0.49[ab]	0.48[a]	0.038
SPC	0.74	0.72	0.71	0.55	0.60	0.034
PPC	1.25	1.35	1.34	1.34	1.33	0.019
CPC	0.52	0.56	0.54	0.34	0.34	0.055
ADFI (g / d)						
SBM	4.2[a]	4.4[a]	5.7[b]	4.7[ab]	3.4[a]	0.31
PM	5.0	4.2	4.5	5.0	5.1	0.31
CM	6.1	4.2	4.9	5.5	5.2	0.45
SPC	5.6	6.6	5.9	5.2	5.1	0.67
PPC	4.7	4.5	5.0	5.3	5.4	0.30
CPC	8.9	8.6	9.7	8.2	8.5	0.30
FCR (g feed / g gain)						
SBM	1.4	1.2	1.2	1.4	1.8	0.16
PM	1.1	0.9	1.0	1.0	0.9	0.08
CM	1.1	1.1	1.2	1.6	1.9	0.23
SPC	1.2	1.3	1.2	1.3	1.3	0.16
PPC	1.1	0.9	1.1	1.1	1.1	0.06
CPC	2.5	1.7	2.7	3.0	2.8	0.25
PER (g protein / g gain)						
SBM	1.7	1.9	1.9	1.6	1.3	0.14
PM	2.2	2.6	2.2	2.4	2.5	0.17
CM	2.1	2.1	2.0	1.4	1.3	0.30
SPC	1.8	1.8	2.1	1.8	1.9	0.25
PPC	1.2	1.2	1.1	1.2	1.1	0.12
CPC	1.1	1.3	0.9	0.7	0.8	0.09
HSI (%)						
SBM	1.0	0.9	1.0	1.0	1.0	0.05
PM	1.0	1.2	1.1	1.2	1.1	0.06
CM	1.0	1.0	0.8	0.9	1.0	0.06
SPC	1.1	1.0	1.0	1.1	0.9	0.08
PPC	1.2	1.2	1.4	1.2	1.1	0.07
CPC	0.9	0.9	0.7	0.9	0.8	0.05

[ab] Means in the same row with different superscripts are significantly different ($P < 0.05$).
SEM=Pooled standard error of the mean.
ADG=Average daily gain.
SGR=Specific growth rate ([ln final weight – ln initial weight] / time (days) x 100).
ADFI=Average daily feed intake.
FCR=Feed conversion ratio (feed intake / wet weight gain).
PER=Protein efficiency ratio (wet weight gain / protein intake).
HSI=Hepatosomatic index ([wet liver weight / wet body weight] x 100).

Table 10.1.2. Regression analysis (linear and quadratic) of growth performance of rainbow trout in SBM, PM, CM, SPC, PPC and CPC growth trials. Significant *P* - values are presented in bold script.

		Inclusion^2	Inclusion	Constant	r^2	*P* - values
ADFI (g / fish / d)						
SBM	Linear		-0.016	4.739	0.039	0.482
	Quadratic	-0.007	0.185	3.984	0.555	**0.008**
PM	Linear		0.012	4.571	0.058	0.388
	Quadratic	0.002	-0.058	4.836	0.222	0.222
CM	Linear		-0.008	5.277	0.008	0.745
	Quadratic	0.004	-0.118	5.692	0.164	0.342
SPC	Linear		-0.032	6.176	0.100	0.251
	Quadratic	-0.003	0.047	5.878	0.153	0.369
PPC	Linear		0.027	4.544	0.291	**0.038**
	Quadratic	0.001	0.004	4.632	0.309	0.108
CPC	Linear		-0.017	9.036	0.025	0.573
	Quadratic	-0.002	0.036	8.837	0.047	0.750
SGR (% / d)						
SBM	Linear		-0.007	0.643	0.255	0.055
	Quadratic	-0.001	0.022	0.536	0.632	**0.002**
PM	Linear		-0.003	1.491	0.020	0.612
	Quadratic	0.001	-0.035	1.614	0.309	0.109
CM	Linear		-0.088	0.720	0.405	**0.011**
	Quadratic	$2.833x10^{-5}$	-0.009	0.723	0.405	**0.044**
SPC	Linear		-0.006	0.749	0.235	0.067
	Quadratic	$5.022x10^{-6}$	-0.006	0.749	0.235	0.200
PPC	Linear		0.003	1.283	0.183	0.112
	Quadratic	0.000	0.009	1.258	0.265	0.158
CPC	Linear		-0.007	0.574	0.159	0.158
	Quadratic	0.000	0.004	0.532	0.192	0.310
FCR (g feed / g gain)						
SBM	Linear		0.015	1.183	0.246	0.060
	Quadratic	0.002	-0.037	1.378	0.514	**0.013**
PM	Linear		-0.004	1.024	0.090	0.278
	Quadratic	$9.632x10^{-5}$	-0.007	1.034	0.095	0.551
CM	Linear		0.029	0.929	0.450	**0.006**
	Quadratic	0.001	-0.006	1.061	0.506	**0.014**
SPC	Linear		0.003	1.210	0.019	0.628
	Quadratic	0.000	0.009	1.188	0.025	0.860
PPC	Linear		0.002	1.041	0.035	0.507
	Quadratic	0.000	-0.005	1.065	0.076	0.623
CPC	Linear		0.025	2.171	0.090	0.298
	Quadratic	0.001	0.005	2.244	0.095	0.579
PER (g protein / g gain)						
SBM	Linear		-0.014	1.898	0.275	**0.045**
	Quadratic	-0.001	0.030	1.732	0.512	**0.013**
PM	Linear		0.006	2.271	0.057	0.393
	Quadratic	$8.012x10^{-5}$	0.004	2.280	0.057	0.702
CM	Linear		-0.032	2.252	0.360	**0.018**
	Quadratic	-0.001	-0.004	2.147	0.384	0.054
SPC	Linear		0.001	1.858	0.000	0.950
	Quadratic	0.000	0.014	1.809	0.000	0.931
PPC	Linear		-0.004	2.159	0.064	0.362
	Quadratic	0.000	0.005	2.123	0.090	0.567
CPC	Linear		-0.013	1.148	0.187	0.123
	Quadratic	$6.011x10^{-5}$	-0.012	1.141	0.187	0.320

ADFI=Average daily feed intake.
SGR=Specific growth rate ([ln final weight – ln initial weight] / time (days) x 100).
FCR=Feed conversion ratio (feed intake / wet weight gain).
PER=Protein efficiency ratio (wet weight gain / protein intake).