

**Comparing plant protein sources in two Canadian strains of Arctic charr
(*Salvelinus alpinus*) at the juvenile and grower stages**

by

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ABSTRACT

COMPARING PLANT PROTEIN SOURCES IN TWO CANADIAN STRAINS OF ARCTIC CHARR (*SALVELINUS ALPINUS*) AT THE JUVENILE AND GROWER STAGES

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Two feeding trials were performed examining the effects of graded levels of soybean meal and sunflower meal on the performance and health of two genetic strains of Arctic charr (*Salvelinus alpinus*), one diploid and one triploid, at the juvenile and grower stages. Results at the juvenile stage revealed significant difference between strains in terms of feed intake, feed efficiency, nitrogen retention, and carcass lipid content, but similar response in these parameters to increasing plant protein ingredients in both strains. At the grower stage, weight gain, growth rate, and distal intestine villus length and width were significantly reduced with increasing soybean meal, but not sunflower meal inclusion. Histological examination of the distal intestine at the grower stage revealed several of the classical symptoms of non-infectious enteritis pathologies in fish fed soybean meal, but not in the fish fed sunflower meal.

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1 – GENERAL INTRODUCTION

The Arctic charr (*Salvelinus alpinus*) is a cold-water salmonid species native to the Canadian and European Arctic and the Eastern coast of Canada. It is represented by both anadromous (sea-faring) and resident forms, of which several distinct genetic strains have developed since the last glaciation. Arctic charr is currently farmed primarily in Iceland, Sweden, and Canada. Canadian production is small in comparison at about 300 metric tons per annum, representing approximately 1.4 – 1.8 million USD. In Canada, three major strains, and their hybrids are cultured. Start-up production in Canada and the United States has seen a significant number of failures in the last 25 years and has created a stigma associated with the Arctic charr growers. Earlier issues were believed to be a factor of genetic strain, and growth reduction associated with early maturation. However, this is no longer a large issue as current maturation prior to market size of 1kg is estimated at less than 10%. Present issues include both on-site production techniques, processing issues, distance to market, and consumer interest. In many cases, Arctic charr are raised with similar husbandry conditions to rainbow trout, often resulting in sub-optimal performance. Furthermore, Arctic charr are currently unable to be grown in full seawater and require more expensive specialized land-based recirculation systems for culture. Processing issues include farm proximity to processing facilities, a lack of interest by processors in the small quantities of Arctic charr produced, or poor final quality of mechanically-produced fillets. Costs associated with production, processing, the long distance to market, and the inconsistent supply of Arctic charr results in significantly increased final product prices and poorer marketability of the species when compared to other cultured salmonids.

Another significant issue associated with production in Arctic charr and many other aquaculture species is feed costs, which can represent 50-70% of total production cost. Rising prices of fish meals, fish oils and other feed ingredients, has resulted in a significant need for alternate, economical ingredients. As such, there has been a large body of research focused on the nutritive value of plant protein ingredients in diets for cultured carnivorous and omnivorous fish species with general focus on soybean, canola, and corn products. Many of these ingredients represent good sources of digestible protein in feed formulations, their inclusion levels in aquafeed dependent on the ingredient processing techniques, individual species and age class requirements, and tolerances. However, inherent in many plant protein ingredients are indigestible carbohydrates and antinutritional factors (ANF) that have been shown to decrease the performance and nutrient utilization in many fish and livestock species.

Investigation into several ANF originating from soybean ingredients including saponins, lectins, enzyme inhibitors, and antigenic proteins has revealed different negative physiological effects of individual, or combined antinutrients in cultured fish (Storebakken, 1985; Kroghdahl et al., 1994; Olli et al., 1994; Bureau et al., 1998; Burrells et al., 1999; Bennetau-Pelissero et al., 2001; Buyukapar et al., 2011; Sinha et al., 2011; Mugford and Osborne, 2013). When included at high levels in compound feeds, these antinutrients have the capacity to reduce overall growth performance in salmonids, either through a reduction in feed intake (Bureau et al., 1998), depressed nutrient digestibility (Refstie et al., 1998; Kroghdahl et al., 2003; Aslaksen et al., 2007; Burr et al., 2011), and/or may cause alterations in the structure and function of the intestinal mucosa (Olli et al., 1994; Bureau et al., 1998; Kroghdahl et al., 2003). Agronomical conditions, and processing steps such as dehulling, heat treatment techniques, and oil extraction methods are all factors in final ANF and nutrient content (Rosa et al., 2009). The production of value-added plant protein

concentrates and isolates involves additional processing steps which further decrease ANF content, and improves overall ingredient protein content and nutrient digestibility of the feed compared to conventionally-processed plant ingredients, however at a much higher ingredient cost.

Soybean ANF have been linked to the development and progression of sub-acute gastrointestinal enteritis in salmonids, an inflammatory condition in the distal intestine presenting with shortening of intestinal folds, decreased enterocyte supranuclear vacuolization, and infiltration of mixed leukocyte populations into the lamina propria, and submucosal tissues (Van den Inge et al., 1991; Baeverfjord and Kroghdahl, 1996). Feeding solvent-extracted, and full-fat soybean meal have resulted in pathological changes in both Atlantic salmon and rainbow trout (Baeverfjord and Kroghdahl, 1996; Kroghdahl et al., 2003; Heikkinen et al., 2006; Merrifield et al., 2009). Step-wise replacement of SBM in place of fishmeal in diets for Atlantic salmon results in a positive dose-response on the number of fish displaying intestinal alterations, with concomitant decreases in the apparent digestibility of lipid, crude protein, and gross energy (Kroghdahl et al., 2003).

Although past studies in Arctic charr suggest similarities to rainbow trout in terms of specific nutrient requirements (Gurure et al., 1995; Olsen and Ringø, 1998; Simmons et al., 1999), the tolerance of this species to different plant protein ingredients and antinutrients between genetic strains or at different life stages is largely unknown. Research has already indicated significant variations in tolerance towards different plant proteins and their ANF between Atlantic salmon and rainbow trout, with trout showing less severe effects on apparent digestibility and nutrient utilization, and a lower severity of the anomalous histological changes (Refstie et al., 2000). Variation in digestibility and growth performance have also been noted when comparing Arctic charr and Atlantic salmon fed plant-based diets (Burr et al., 2011). Furthermore, strain-specific variation in growth and nutrient digestibility has been observed in salmonid species, including the

rainbow trout when fed diets with high inclusion levels of plant ingredients (Overturf et al, 2012). In Arctic charr, variation in growth rate has also been seen between genetic strains when fed a commercial diet (Nilssen et al., 1993) which may suggest putative differences between strains in sensitivity towards plant proteins and/or antinutrients in this species.

1.1 – Objectives

The general objective of this study was to examine the effects of the addition of plant protein ingredients in the diet of the Arctic charr (*Salvelinus alpinus*). More specifically, this study was aimed at determining and contrasting the effects of increasing levels of soybean meal, sunflower meal, and a novel high protein sunflower meal on growth, nutrient utilization, pro-inflammatory gene expression, and intestinal pathology in two different Canadian strains of Arctic charr at two different life stages. Information from this study may hopefully be used towards a species or strain-specific nutrition programme for Arctic charr in order to benefit Canadian production of this species.

2 – LITERATURE REVIEW

2.1 – Introduction

The Arctic charr (*Salvelinus alpinus*) is a relatively new species to Canadian aquaculture, with a current production volume of approximately 300 metric tons/year (André Dumas, personal communication). In Canada, the species is represented by a set of resident and anadromous genetic strains, or their hybrids originating from watersheds located in Labrador and the Northwest Territories. Very little research has been accomplished regarding nutrient requirements/utilization and dietary tolerances of different strains of this species (Tabachek, 1986; Gurure et al., 1995; Olsen and Ringø, 1998; Simmons et al., 1999). However, studies on Arctic charr and closely related salmonids have indicated that nutrient utilization and tolerance to plant-sourced protein ingredients may vary significantly between genetic strains (Overturf et al., 2003; Rasmussen and Jokumsen, 2009; Overturf et al., 2013).

The cost of feed in intensive aquaculture operations can comprise up to 70% of total production costs. As such, the formulation of feeds and selection of the ingredients requires careful consideration not only to their nutritional value, but also economic considerations such as the price of ingredients. Classically, fishmeal has been implemented as the principal protein source in commercial aquafeeds targeted towards carnivorous salmonid species. However, rising prices have triggered the necessity to increase utilization of less expensive and more readily available plant-sourced ingredients as digestible protein sources in formulated aquafeed to maintain a viable and economical industry.

Co-products of soybean and sunflower processing contain relatively high protein contents and protein digestibility, and have favourable amino acid profiles compared to known salmonid

requirements. However, inherent in these plant protein ingredients are attributes that prevent them from being included at high levels in nutrient-dense feeds targeted for salmonid production. These include limiting levels of the indispensable amino acids methionine and lysine, high levels of indigestible carbohydrates, and a suite of antinutritional factors that have been shown to significantly decrease performance and nutrient utilization in many salmonid species, depending on agronomical factors, ingredient processing techniques, and dietary inclusion level.

An antinutritional factor (ANF) can be defined as an endogenous compound within feeds or foods which can negatively affect the growth performance or health of an organism (NRC, 2011). Several types of ANFs are found in plant feedstuffs commonly used in animal feeds including saponins, lectins, enzyme inhibitors, indigestible carbohydrates, phenolic compounds, estrogenic compounds, and antigenic proteins (Francis et al., 2001). These compounds either alone or combined, have the ability to reduce feed intake, depress growth performance, and alter the physiological structure and function of digestive mucosa in several fish species when included at high levels in compound feeds (Olli et al., 1994; Bureau et al., 1998; Kroghdahl et al., 2003).

Conventionally-processed soybean meal contains high concentrations of saponins, lectins, oligosaccharides, and antigenic proteins compared to other plant protein ingredients, which have been shown to result in the development of an inflammatory condition in the distal intestine called non-infectious subacute gastrointestinal enteritis (Van den Inge et al., 1991; Baeverfjord and Kroghdahl, 1996; Bakke, 2011) as well as to reduce protein and lipid apparent digestibility (Olli et al., 1994; Refstie et al., 1998; Refstie et al., 2000; Kroghdahl et al., 2003). Unlike soybean meal, sunflower meal does not contain high levels of the ANF associated with enteritis. However it does contain relatively high levels of phenolic compounds which have been implemented in growth reduction, lowered protein retention, and reduced availability of the amino acid arginine.

Sunflower meal also presents with high levels of indigestible non-starch polysaccharides (24-31%) which may reduce dietary digestible nutrient density and limit high-level inclusion in nutrient-dense salmonid feeds.

Based on the lack of knowledge of the strain-specific tolerance to different plant ingredients and plant anti-nutrients in Arctic charr, this review will focus on the nutritional limitations, and potential toxicity of select soybean and sunflower ingredients in related salmonid species and outline the effects of processing on ingredient quality and fish performance. It will also investigate the effects of genetics on both tolerance to plant proteins and on nutrient utilization in fish.

2.2 – The Arctic charr

2.2.1 – Life history and rearing

The Arctic charr (*Salvelinus alpinus*) is a cold-water teleost fish, a member of the *Salmoninae* sub-family alongside the more well-known Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). There are four subspecies of *S. alpinus*, together occupying a relatively narrow native range, inhabiting the coastlines of Canada's High Arctic (*S. alpinus erythrinus* and *S. alpinus alpinus*), Greenland, Iceland, Scandinavia (*S. alpinus alpinus*), Russia (*S. alpinus alpinus* and *S. alpinus taranetzi*) above 65° latitude, at water temperatures averaging from 0.5 - 14°C (Jensen, 1994). However some subspecies may be able to thrive in more southern geographies due to cold south-flowing currents, particularly around the coastlines of the Eastern Canadian provinces (*S. alpinus erythrinus* and *S. alpinus oquassa*). Optimum temperature for fecundity, maximum hatch rate, and growth changes with life stage and fluctuate from 2-7°C for broodstock egg production, 1-7°C for ova prior to the eyed stage and less than 12°C during later egg

development, and between 10-15°C from the fry to grow-out stages (Johnston, 2002). In a study by Sutterlin and Stevens (1992) comparing the thermal preference of rainbow trout (1200-2300g) and Arctic charr (470-680g., *S. a. erythrinus*) raised in net pens, the two species displayed similar average temperature preferences of 13.3°C and 13.4°C respectively.

Arctic charr is represented by two forms: an anadromous form with the ability to migrate to the sea for the short periods during the Arctic summer months, and a resident form that remains in land-locked freshwater lakes. The anadromous form differs from other seawater tolerant salmonids such as the Atlantic salmon in that it can only endure full salinity seawater for short periods, and will spend less than 15% its life in the sea. The annual salinity tolerance is initiated through both size and photoperiod-dependant increases in the hypo-osmoregulatory capacity of smoltifying fish via upregulation of Na⁺, K⁺- ATPase in gill epithelial ionocytes, whereby increasing daylight hours and a greater body size while in the freshwater environment coincides with improved capacity (Arnesen et al., 1992). It is also observed that salinity tolerance, estimated by days spent in full seawater, is notably different between subspecies (Delabbio et al., 1990; Gulseth and Nilsson, 2000), and even strains of the same subspecies of Arctic charr (Delabbio et al., 1990). As such, anadromous Arctic charr of many strains have shown poor performance when reared during the fall and winter months in full seawater under natural photoperiod in a commercial grow-out setting (Duston et al., 2007). Post-smolts subjected to abrupt transfer and rearing in 0, 10, 20, and 30 ppt salinity displayed the greatest mortality and up to 75% lower weight gain at the highest salinity investigated, with no evidence of acclimation to the more saline environments with prolonged exposure (Duston et al., 2007). However, both growth and mortality in 10 ppt was similar to freshwater suggesting the possibility of long-term brackish water culture of post-smolts in this species, supported by observations in both experimentally cultured (Dempsen, 1993; Árnasan et

al., 2014; Gunnarsson et al. 2014; Coastal Zones Research Institute, unpublished data) and wild tagged Arctic charr (Jensen and Rikardsen, 2012).

2.2.2 – Aquaculture and economic value

Arctic charr aquaculture is a relatively new venture, beginning with the initial propagation and restocking of this species in Europe in the 1970's as a response to the decimation of lake populations (Johnson, 2002). At this time, knowledge regarding the rearing of other salmonid species was relatively well established, and attempts were made in utilizing already successful culture techniques for both Atlantic salmon and rainbow trout. Early culturing in the 1980's resulted in remarkable growth rates attained at low temperatures at extremely high densities compared to those of other salmonids, with first commercial production levels in Sweden at approximately 50 metric tonnes. However these pioneering enterprises dealt with common issues of first-generation commercial growers including different salinity tolerance, precocious maturation, and non-homogenous growth compared to salmon and trout, ultimately ending many initial ventures. Within Canada, eggs and fingerlings of three strains of Arctic charr sourced from the Fraser River, Labrador NFLD, Nauyuk Lake, NWT, and the Tree River, NWT were distributed to multiple sites with Nauyuk animals showing better growth rates, however not without necessitating multiple labour-intensive size gradings to prevent cannibalism. Since then considerable work has been completed aiming to significantly improve growth rates and size homogeneity, delay maturation via controlled photoperiod regimes (Johnsen et al., 2000; Frantzen et al., 2004; Gunnarssen et al., 2014) and improve salinity tolerance (Ojima et al., 2009) showing very promising results.

Antithetic to the enhancement of key culture qualities in this species, production of Arctic charr remains relatively stagnant and significantly lower than other salmonids in Canada (Table 2.1) primarily as a result of its reputation due to past failures, marketing issues, and continued lack of consumer knowledge and exposure to this species. Production of Arctic charr in Canada currently represents approximately 8% of total global culture of this species and is estimated at between 250 - 300mt annually since 2008, at an approximate value of 1.4 – 1.8 million USD (FAO, FIGIS). Most are reared from private land-based freshwater facilities in the Northern and Eastern coastal provinces and territories, with smaller production in Quebec, Ontario and Manitoba.

Table 2.1 – Aquaculture production statistics of Arctic charr (*S. alpinus*).

Location	Species	Production (MT)			
		2008	2009	2010	2011
Canada	Atlantic salmon ¹	104 070	100 200	101 385	102 064
	Rainbow trout ²	7 932	7 198	6 844	5 600
	Arctic charr ³	300	300	300	300
Iceland	Arctic charr ¹	3160	2405	2427	3021
Total	Arctic charr ¹	4018	3273	3356	3788

¹FAO FIGIS (2015), estimated production.

² Statistics Canada (2014).

³André Dumas, estimated production

2.3 – Soyabean (*Glycine max*) and soybean meal

2.3.1 – World production of soybean and soybean meal

The 2014-2015 world production of soybeans, the highest, and most widely grown oilseed on the planet, is estimated at 314.4 million metric tons (mMT), a 16% increase since 2010. The United States, closely followed by Brazil are the major cultivators of the soybean plant, collectively responsible for approximately 65% of worldwide production. Consequently, these two countries are also the major exporters. Canada presently produces 6 mMT of soybean oilseed, of which over 50% is exported, however the industry has seen a significant 36% growth since 2010. China is the strongest importer of soybeans, holding 65% of worldwide imports at 74 mT in 2014-2015. They are followed by the European Union at 12.8 mMT (USDA, 2015).

Soybean meal is a high protein plant ingredient (44-48% CP) the by-product of soybean oil extraction via mechanical pressing and/or solvent extraction followed by heat treatment. Worldwide production of soybean meal currently sits at 199.5 mMT, dwarfing total fishmeal production (5-6 mMT) and showing a strong 14% growth between 2010 and 2015. China, the United States, Argentina, and Brazil are the four highest producing countries with Argentina, Brazil and the United States also representing the top three exporters. Domestic consumption of approximately 97% of soybean meal production in China, a function of the country's vastly increasing aquaculture and livestock production (Yin et al., 2011) prevents strong export status. Major import countries include those within the European Union, Indonesia, Vietnam, Thailand, Iran, and the Philippines (USDA, 2015).

2.3.2 – Nutritional properties of soybean meal

Historically, fishmeal has been the protein source of choice in nutrient-dense salmonid formulations due to its high crude protein content (65-85%), an amino acid composition that will meet known salmonid requirements with adequate dietary inclusion, its beneficial omega-3 fatty acid content, and high CP apparent digestibility (NRC, 2011). Solvent-extracted soybean meal (SBM) is also highly digestible in Atlantic salmon and rainbow trout (88-89%), however crude protein content is lower at 44-48%. The SBM ingredient is also proportionately low in the amino acids lysine and methionine, and diets with high inclusion may require the addition of exogenous sources of amino acids (crystalline, etc) to meet known salmonid requirements (Table 2.2). SBM also contains relatively high levels of carbohydrates in the form of indigestible oligosaccharides and non-starch polysaccharides, which function to reduce total utilizable nutrient density of the ingredient (Kar-lilienthal et al., 2005; NRC 2011).

Nutrient content of this ingredient is highly dependent on processing steps and conditions, and several agronomical factors. A 2011 survey of soybean cultivar across the United States revealed great variation in seed protein (30-47%) and oil (11-23%) contents (Naeve et al., 2011) and thus specific microclimate and growth conditions result in significant effects of SBM nutrient content. Sampling 55 SBM processing plants at two-week periods showed that total protein, oligosaccharide, and amino acid content of the final meal significantly varied as a result of plant location, as well as with time of harvest (Karr-Lilienthal et al., 2005). Company-specific combinations of processing steps and techniques may also result in vastly different nutrient contents of SBM. Careful consideration of oil extraction techniques and efficiency, as well as heat treatment methods are required. The main oil extraction processes utilized for oilseeds include mechanical screw pressing/extrusion and solvent extraction. Extrusion is the least efficient at oil

removal and will result in partially-defatted meals being expelled with a higher lipid and lower protein content (Fig. 2.1). Heat treatment methods including toasting and steam may inadvertently lower available total protein and individual amino acid content, the severity depending on the temperature and duration of treatment. SBM heat treatment is aimed at balancing the deactivation of trypsin inhibitors, a soybean anti-nutrient, and maintaining acceptable levels of utilizable proteins. However, high temperature treatment necessary for the inactivation of trypsin inhibitors (>100°C) results in the Maillard reaction. This is a non-enzymatic chemical reaction whereby a reducing sugar binds to a lysine residue to form protein adducts or cross-linked proteins which cannot be utilized by the animal. Plant protein sources, particularly those with higher oligosaccharide content such as soybean have an increased propensity for the Maillard reaction to occur with heat treatment.

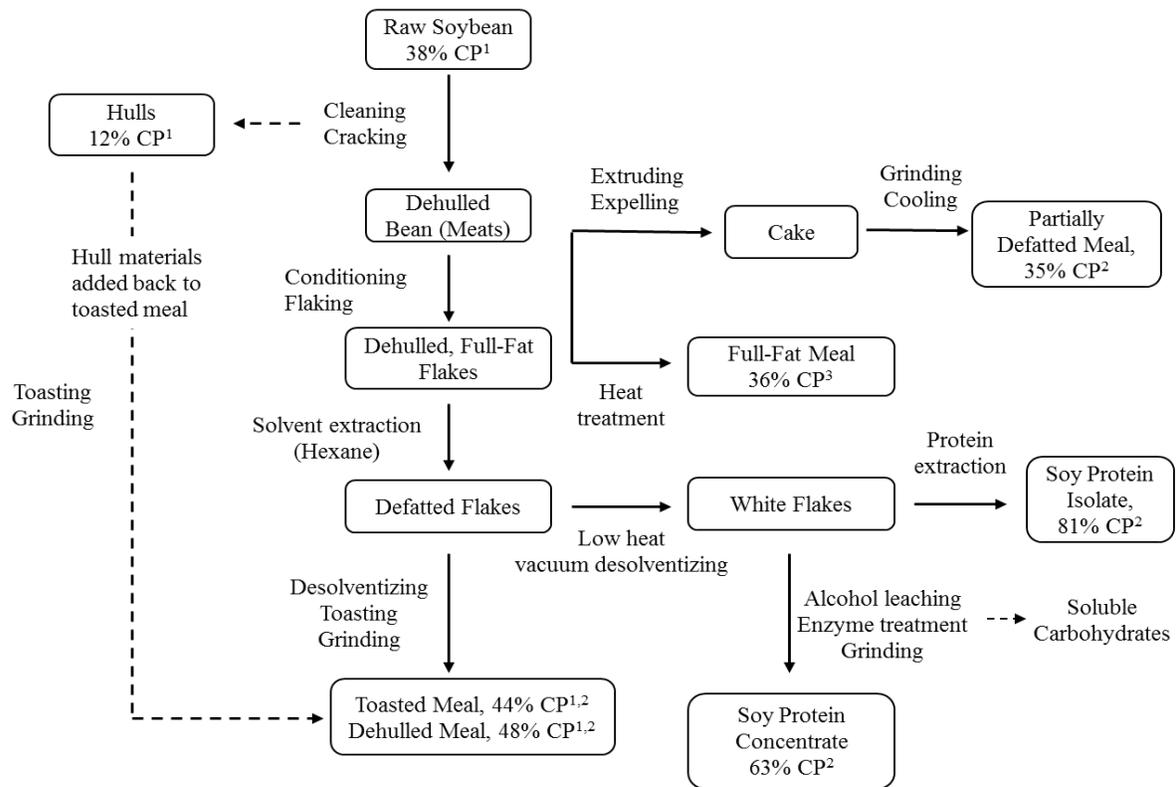


Figure 2.1 – Processing steps of feed-grade soybean protein ingredients

¹United Soybean Board (2015), ²NRC (2011), ³Hertrampf and Piedad-Pascual (2000).

2.3.3 – Utilization in feed formulation

Soybean meal has been utilized in aquafeeds since the early 1940's as a plant protein ingredient which functioned to reduce feed costs, while maintaining similar growth in fish fed slaughter-house by-product based diets at that time (Halver and Hardy, 2002). Currently, commercial soybean meal may be included in high-energy grow-out rainbow trout diets without negative effects on performance, with recommended inclusion rates $\leq 20\%$ (Hertrampf and Piedad-Pascual, 2000; Lim et al., 2008). Experimentation with extremely high inclusion of soybean meal ($\geq 40\%$) and/or genetic manipulation of rainbow trout has indicated the possibility of adaptation to, or total acceptance of the ingredient (Refstie et al., 1997; Sealey et al., 2009; Overturf et al., 2013). However, rainbow trout appear to be more tolerant of dietary soybean meal addition than other salmonid species such as the Atlantic salmon in which significant depressive effects on feed intake or lipid and energy apparent digestibility at inclusion levels ranging from 10-30% are observed (Fowler, 1980; Refstie et al., 2000).

Alternative soybean products with increased protein content including soybean protein concentrates are utilized in fry and fingerling salmonid diets, or in high-energy diets targeted for grow-out operations such as those in the European Union which have strict regulations on the inclusion of livestock-sourced rendered animal proteins in feed formulations (Hardy, 2015). It has been noted that with 32% dietary inclusion, there are significant effects of SPC on growth of rainbow trout (Mambrini et al., 1999).

2.3.4 – Physiological effects of feeding soybean meal in salmonid species

2.3.4.1 – Effects on growth performance and feed conversion

In several cultured salmonid species, increased inclusion of conventionally-processed SBM is shown to be concurrent with reduced weight gain, growth rate, and feed efficiency, depending on ingredient quality and species tolerances (Refstie et al., 1998; Refstie et al., 2000; Krogdahl et al., 2003). In juvenile Atlantic salmon fed diets where 10 – 35% total dietary protein was replaced by SBM (8 – 27% inclusion as-is), linear depression occurs in specific growth and feed efficiency (gain:feed) (Krogdahl et al., 2003). Similar results occur in rainbow trout, however a much higher inclusion level of SBM can be attained before significant reduction in overall weight gain or growth rate are observed. Dietary SBM concentrations of approximately 30% or higher results in notable reduction in specific growth rate and feed conversion in rainbow trout (Burrells et al., 1999; Refstie et al., 2000). Studies have indicated the possible adaptation of rainbow trout to high levels of SBM (Refstie et al., 1997). However, a clear depression in overall weight gain during the adaptation period at the juvenile stage would suggest maintaining lower SBM inclusion levels in this species. Arctic charr perform well fed diets with approximately 12-15% SBM in grower-stage formulations, and present with good growth rates and feed efficiency. However, the response of Arctic charr to higher inclusion levels of dietary SBM are currently unknown.

2.3.4.2 – Soybean meal-induced gastro-intestinal enteritis

Soybean meal-induced sub-acute gastrointestinal enteritis is defined as a non-infectious inflammatory condition, most often presented in the distal intestinal segments of fish with continued exposure to diets containing soybean ingredients. The condition presents with characteristic histopathology including: increased profusion of immune cells into the lamina

propria and submucosal tissues, reduction in enterocyte supranuclear vacuolization, shortening of simple and compound intestinal villi and microvilli, and increased number of goblet cells (Van den Inge et al., 1991; Baeverfjord and Kroghdahl, 1996; Bakke, 2011).

Significant up- or downregulation of specific immune response genes, cytokines, and their respective chemokine and cytokine receptors are also noted with the pathology, indicative of a localized immune response to allergenic ANF (Kortner et al., 2012). These genes include those that code for cluster of differentiation-8 (CD8), nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B), interleukin-1 beta (IL1 β), pregnane-X receptor (PXR), and many others coding for immune system activation and inflammation. CD8 is a transmembrane glycoprotein which functions as a co-receptor molecule for the T-cell receptor on the surface of cytotoxic T-lymphocytes. Together, these membrane proteins recognize foreign antigens presented on the surface of all nucleated cells, and upon binding will trigger the release of cytotoxins resulting in cellular apoptosis. NF κ B is a fast-acting cellular transcription factor present in all differentiated cell types, and is involved in cellular responses to stress, cytokines, and bacterial/viral antigens (Hoffman and Baltimore, 2006). It can be directly activated by cytokines such as IL1 β or tumour necrosis factor alpha (TNF α), and, depending on the host cell, will transcribe genes related to inflammation, apoptosis, cell cycle arrest, differentiation or proliferation (Hoffman and Baltimore, 2006). Similar to NF κ B, PXR is also a transcription factor present in all nucleated cells, but is strictly localized in the nucleus (type II nuclear receptor). It is a general sensing molecule, and upon stimulation will transcribe genes involved in xenobiotic targeting and removal. In rainbow trout, activation of PXR results in downstream production of cytochrome P450 3a (Wassmur et al., 2010), a molecule which oxidizes small foreign biological molecules, and targets them for removal.

The pathological incidence and intensity of the enteritis response is dependent on type and inclusion level of plant protein investigated, as well as fish species (Refstie et al., 2000), or genetic strain studied. However, a more pronounced inflammatory effect is observed with the inclusion of less processed soybean ingredients and soybean alcohol extracts due to their higher ANF/antigen concentrations. Full-fat, and regular, solvent-extracted soybean meal have been shown in many studies to result in this pathology in Atlantic salmon (Baeverfjord and Kroghdahl, 1996; Kroghdahl et al., 2003) and rainbow trout (Heikkinen et al., 2006; Merrifield et al., 2009). Step-wise increase in incorporation of defatted soybean meal (0 - 35% total dietary protein, 5% intervals) in place of fishmeal in diets for Atlantic salmon results in a positive dose-response effect on the number of fish displaying pathological alterations, with concomitant decrease in apparent digestibility of lipid, crude protein, and gross energy observed even at the lowest inclusion level investigated (Kroghdahl et al., 2003). Similar pathologies are present in rainbow trout and Atlantic salmon fed diets with 30% dietary protein supplied by defatted soybean except for the incidence of leukocyte infiltration in the lamina propria and submucosal tissues, which were comparatively few in trout (Refstie et al., 2000). However, the two salmonids investigated often display dissimilar effects of SBM on growth, and nutrient utilization.

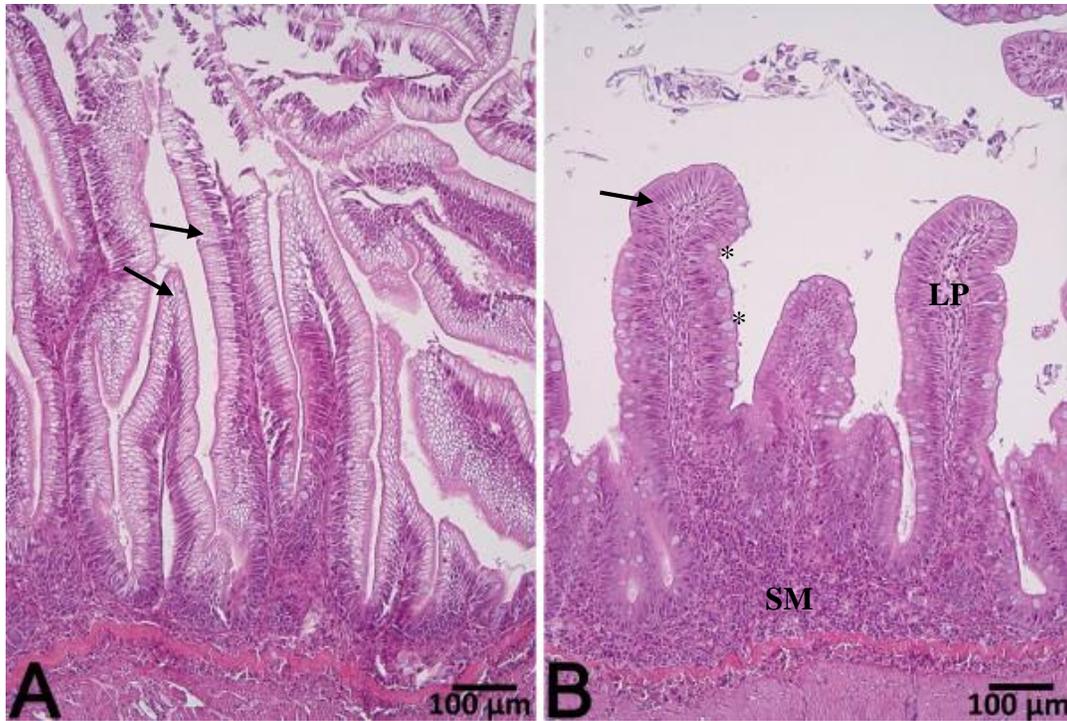


Figure 2.2 - Distal intestine of Atlantic salmon (*Salmo salar*) fed (A) pea protein concentrate, and (B) pea protein concentrate + soyasaponins. Pathophysiology associated with SBM-induced enteritis is shown through villus shortening, lamina propria (LP) and submucosal (SM) swelling, loss of enterocyte vacuolization (arrow), and increased number of mucosal cells (*). Obtained and adapted from Kortner et al. (2012).

2.3.4.3 – Effects on nutrient digestibility and digestive enzyme activity

2.3.4.3.1 – Protein and amino acid digestibility

Unless extremely high levels (>30%, depending on species) or poorly processed SBM high in protease inhibitors are included the inclusion of SBM in salmonid diets does not typically coincide with significantly reduced protein and amino acid digestibility (Olli et al., 1994; Refstie et al., 1998; Refstie et al., 2000; Kroghdahl et al., 2003). Graded levels of SBM up to 27% fed to Atlantic salmon for 60 days results in a significant but only slight reduction in protein digestibility with inclusion (Kroghdahl et al., 2003). Higher SBM inclusion (~30%) in Atlantic salmon and

rainbow trout results in only a marginally greater depression of protein apparent digestibility of approximately ~2-3% (Refstie et al., 1998; Refstie et al., 2000).

The reduction in the apparent digestibility of protein in salmonids as a result of high SBM inclusion has been associated with notable alterations in the expression, activity, and localization of key enzymes and intermediates involved in peptide and specific amino acid digestion and absorption, the most intensely studied of which are pancreatic and intestinal trypsin and chymotrypsin. Trypsin and chymotrypsin are vertebrate serine proteases which cleave carboxyl-terminus peptides of the amino acids lysine and arginine, and tyrosine, tryptophan, and phenylalanine, respectively. Specific proteinase inhibition and proteinase-instigated receptor activation and immune system stimulation are observed in Atlantic salmon and rainbow trout when exposed to protease inhibitors in extracted soybean meal (Krogdahl et al., 1994; Olli et al., 1994; Lilleeng et al., 2007). Alterations in luminal trypsin activity and an increase in distal intestine and fecal trypsin content are noted in salmon with increasing levels of dietary crude trypsin inhibitor (Lilleeng et al., 2007). Although fish are able to compensate in the short term for trypsin losses, presumably through increased pancreatic production and secretion, long term exposure to high levels of trypsin inhibitors ultimately results in a significantly depressed luminal trypsin activity, thus apparent protein digestibility (Olli et al., 1994).

Significantly reduced activity and alterations in the specific localization of leucine aminopeptidase (LAP), a second key enzyme in peptide digestion and amino acid uptake, is noted in the distal intestine enterocyte brush-border of Atlantic salmon and rainbow trout fed diets with 30% and 25% dietary inclusion of SBM, respectively (Bakke-McKellep et al., 2000; Romarheim et al., 2006). Mean mucosal LAP activity in enterocyte homogenates shows step-wise depression with SBM crude protein supplementation as low as 8% in Atlantic salmon (Krogdahl et al., 2003)

and is observed as early as day 2 of exposure (Chickwati et al., 2013). The combined decrease of LAP activity in the distal intestine enterocyte brush border and observed increase in activity within intestinal chyme is suggestive of enterocyte sloughing, a noted result of lectin agglutination (Buttle et al., 2001).

2.3.4.3.2 – Lipid digestibility

Strong effects of SBM on lipid digestibility are observed in Atlantic salmon and rainbow trout, and have been associated with high levels of the soybean ANF lectin. Digestibility is reduced by ~2% with as little as 9% SBM inclusion in Atlantic salmon, and a significantly linear depression in apparent digestibility of lipids is noted when graded levels of up to 27% are fed (Krogdahl et al., 2003). At 30% SBM inclusion, lipid apparent digestibility in Atlantic salmon is reduced by 14% when compared to a fishmeal based diet (Refstie et al., 2000). Contrastingly, rainbow trout do not show significant depression in lipid digestibility, even at high-level inclusion (Refstie et al., 2000; Romarheim et al., 2008) suggesting a reduced sensitivity of this species to the negative effects of soybean ANF. Depression in lipid digestibility has been associated with time-dependant reduction in plasma cholesterol and triacylglyceride concentrations and reduced luminal bile acid concentrations (Romarheim et al., 2008) and is suggestive of a strong hypocholesterolaemic effect of SBM in trout and salmon.

2.3.5 – Major antinutritional factors in soybean meal and their effects in fish

The major types of antinutritional factors and their levels present in select soybean ingredients are listed in Table 2.3.

2.3.5.1 – Saponins

2.3.5.1.1 – Biochemistry

Saponins are a suite of heat stable, alcohol-soluble amphipathic glycosides found in many plant protein sources commonly utilized in animal feed including soybean meal, canola meal, sunflower oil cake, and lupin seed meals. They are composed of a sugar group bound to either a steroid or triterpenoid aglycone. Soya-saponins are triterpene glycosides, and are classed into two major groups: A and B based on the aglycone structure. In defatted soybean meal, levels of soya-saponin range from 0.4-0.8% (Goda et al., 2002, Knudsen et al., 2006). Soyasaponins are indigestible in salmon (Knudsen et al., 2008) and have a strong affinity for cholesterol and bile salts, forming complexes with these two molecules in the intestinal tract. Moreover, an amphipathic charge of soyasaponin allows for intercalation of this ANF into cholesterol-containing membranes of the intestine, forming pores and permitting increased absorption of foreign materials including feed and microflora-borne antigens (Mugford and Osborne, 2013).

The most common processing methods of conventional soybean meal include solvent (hexane)-mediated oil extraction, and heat treatments which neither reduce the activity of soyasaponins nor remove them from the finalized meal. Thus, final concentration of soyasaponin is highly dependent on initial levels in the harvested oilseed, which are tightly regulated by genotype, agronomical factors and their interactions (Seguin et al., 2014; Hu et al., 2002). Examining soyasaponin B concentrations in 20 cultivars of early maturing soybean seeds, levels

varied from 2.3 – 6.6 μ mol/g and were highly dependent on cultivar-specific response to temperature stress (Seguin et al., 2014). Alcohol (usually ethanol) extraction, or enzymatic treatment of oil-extracted soybean flakes, have been seen to effectively remove soyasaponins, as well as many other carbohydrate ANF including oligosaccharides and non-starch polysaccharides. However, the additional processing steps significantly raises the price of soybean concentrate when compared to SBM (Table 2.3), thus may not represent an economical soybean ingredient to feed formulators.

2.3.5.1.2 – Feeding Effects

Soyasaponins have been strongly implicated in feed depression, reduced growth performance the development of soybean meal-induced gastrointestinal enteritis in salmonids. Several studies feeding Coho and Atlantic salmon either soybean meal or aqueous (water)-extracted soy protein isolates generally resulted in poorer fish performance and feed acceptance when compared to the ethanol-extracted soy protein concentrates (Olli et al., 1994; Kaushik et al., 1995). Further investigation of the purified alcohol-soluble fractions of both soybean meal and soy protein isolate, and subsequent addition to a 32% soy protein concentrate-based diet (to represent saponin levels in a 44% SBM diet) revealed significant feed-deterrent effects in Coho salmon, and growth depression in both Coho salmon and rainbow trout (Bureau et al., 1998). Similar effects on growth and feed intake in both species were observed when fed diets with Quillaja bark saponins, including abnormal intestinal histology. The authors concluded that soyasaponins within the alcohol soluble component of soybean meal and aqueous-extracted soy protein isolate were the causative agent behind depressed growth performance.

Further purification of soyasaponins and their inclusion in salmonid feed has revealed inflammatory histopathological effects of these ANF on the distal intestine. Atlantic salmon fed diets with 25% defatted soybean meal or a lupin meal containing graded levels of soya-saponin concentrate (SSC) have shown significant inflammation of the distal intestine (Knudsen et al., 2008). However, these effects were not seen in either a 25% lupin meal diet or an SSC-supplemented fishmeal diet indicating secondary interactions between soyasaponins and other ANF present in legume species are possibly the cause of the inflammatory effects. Decreased intestinal transepithelial resistance and increased apparent permeability of [C14]mannitol were observed with dietary supplementation of SSC in all diets examined, suggesting soyasaponins may be able to induce epithelial disruption (Knudsen et al., 2008). Supplementation of pea protein concentrate with 0.2% soyasaponin resulted in comparable distal intestine inflammation to what was noted in the study by Knudsen et al. (2008) as well as reduced feed intake and apparent digestibility of amino acids and lipids in seawater-raised Atlantic salmon (Chikwati et al., 2012). In the same study when fish were fed sunflower meal, rapeseed meal, or maize gluten meal similarly supplemented with soyasaponin, inflammatory effects were not evident, although there was slight depression in lipid digestibility and faecal dry matter content. The results by Knudsen et al. (2008) and Chickwati et al. (2012) implicate soyasaponins in the development of inflammatory enteritis in salmonids via epithelial disruption, however suggests that the inflammatory effects observed are likely a result of a combination of soyasaponin and other ANF present in legume plant protein ingredients.

Alongside the soyasaponins' gastrointestinal physiological effects, dietary addition of these compounds has been noted to result in reduced lipid, protein, and mineral apparent digestibility in fish (Gu et al., 2014; Chickwati et al., 2012; Bureau et al., 1998). Addition of 0.2%

soyasaponin to a diet with 45% pea protein concentrate fed to Atlantic salmon resulted in hypocholesterolaemia and a 60% reduction in luminal bile salt concentration resulting in a small but significant reduction in overall lipid digestibility (Chickwati et al., 2012). Comparable results including decreased intestinal and plasma bile salt concentrations and concurrent reduction in fatty acid absorption were observed in Atlantic salmon by Gu et al. (2014) when 0.1% soyasaponin was added to both fishmeal and plant meal (lupin, wheat) diets. Additionally Chikwati et al (2012) found that distal intestine brush border leucine amino peptidase activity was dramatically reduced and was associated with a reduction in protein apparent digestibility, while apparent digestibility of both sodium and magnesium was depressed.

2.3.5.2 – Lectins

2.3.5.2.1 – Biochemistry

Lectins (soybean agglutinins, hemagglutinins) are a diverse group of glycoproteins present at levels from 0.01 – 0.87mg/g in conventionally processed SBM (Maenz et al., 1999). Soybean lectin is a tetrameric glycoprotein, with each sub-unit containing a carbohydrate binding site. The glycoprotein shows high affinity binding to N-acetyl-D-galactosamine and its glycosides, and oligosaccharides with terminal N-acetyl-D-galactosamine, with lower affinity towards galactose. (Els et al., 1998). Binding of multiple enterocyte N-acetyl-D-galactosamine and/or galactose glycoconjugates results in cross-linked lattices, and is likely the source of the lectin's cell-cell agglutination capacity. N-acetyl-D-galactosamine-bearing glycoconjugates are present at the highest concentration in the most highly differentiated enterocytes at the villus tips and binding of multiple carbohydrate groups in adjoining cells may result in cell surface receptor activation or enterocyte agglutination and cellular sloughing (Buttle et al., 2001).

With short term steam treatment at 100°C the actively agglutinating soybean lectin content has been shown to be reduced by up to 90%, with an acceptable ingredient protein solubility post-processing (Yo et al., 2003; Qin et al.; 1996) (Table 2.4). However, depending on specific processing plant drying methods (dry vs. moist) and initial lectin content of the raw seed, SBM may retain physiologically functional and significant carbohydrate binding and enterocyte agglutination effects. In an *in vitro* study comparing N-acetyl-D-galactosamine binding and agglutinating capacity of raw soybean to several conventionally processed SBMs in enterocyte micelles formed from broiler chicken small intestine, processed commercial soybean meals retained approximately 10-30% of the glycoconjugate-binding capacity seen in the raw, defatted soy bean (Maenz et al., 1999).

2.3.5.2.2 – Feeding Effects

Soybean lectin is seen in numerous studies to affect the morphology and function of intestinal tissues and of immune function in several species including cultured salmonids (Buttle et al., 2001; Burrells et al., 1999). In general, animals fed either pure agglutinin, or diets with high levels of ingredients with elevated agglutinin content display a reduction in intestinal absorptive surface area and reduced nutrient digestibility and overall growth performance. Supplying soybean lectins through 60% dietary inclusion of dehulled, solvent extracted SBM or in purified extracted form to a fishmeal based diet results in specific binding to enterocyte glycoprotein N-acetyl-D-galactosamine moieties and alterations to the intestinal physiology of both Atlantic salmon and rainbow trout including: increased cellular infiltration of the lamina propria, microvillar sloughing, and formation of large, irregular pinocytotic vesicles at the villus terminus (Buttle et al., 2001). The similarity of response between the SBM and purified lectin diets implicates a role of soybean

lectin in the formation of sub-acute non-infectious enteropathy in cultured salmonids. Nevertheless, other typical histological signs of the pathology such as loss of enterocyte vacuolization were missing and thus designates a combined role of soybean lectin with other ANF to produce the classic results. In a contrasting study by Iwashita et al. (2008), the addition of 0.075% soybean lectin (representing a 50% dietary SBM inclusion) to a casein-based semi-purified diet fed to rainbow trout did not result in any morphological changes signifying the onset of intestinal inflammation. However, when lectin and a mix of other soybean ANF including soya saponin were supplemented, extreme morphological changes were apparent. It is clear through these opposing results that lectins have dissimilar effects between salmonid species, and that further research into the interactive effects of lectin and other ANF are required, particularly when feeding diets with high levels of soybean ingredients.

2.3.5.3 – Protease Inhibitors

2.3.6.5.1 – Biochemistry

Plant protease inhibitors are proteins divided into several classes that bind and inactivate digestive serine proteases in animals and insects. Soybean proteinase inhibitors fall into two different families: the Kunitz family, which exclusively binds trypsin, and the Bowman-Birk family, which have the ability to bind and inactivate trypsin and chymotrypsin simultaneously.

Soybean protease inhibitors are heat-labile molecules, and denature at high temperatures. Steam treatment of raw soybeans at increasing temperatures and time period shows clear depression in trypsin inhibitor activity (TIA) (Qin et al., 1996; Qin et al., 1998) (Table 2.5). Boiling of raw soybeans at 100°C, or autoclaving 121°C can result in up to 91% reduction in TIA. (Arndt

et al., 1999). Conventional methods of SBM processing includes heating steps (toasting, steam treatment, etc) that effectively reduces TIA activity in SBM.

2.3.5.3.2 – Feeding Effects

Addition of increasing levels of soybean trypsin inhibitor to diets for rainbow trout and Atlantic salmon results in depression of trypsin activity in the pyloric caecae, the mid and distal intestine, and the feces, alongside significant reduction of amino acid, crude protein, and lipid apparent digestibility (Krogdahl et al., 1994; Olli et al., 1994). At relatively low trypsin inhibitor activity (approximately 9mg/g dietary protein) Atlantic salmon are able to increase pancreatic trypsin production and compensate for loss by protease inhibition. However at higher inclusion levels pancreatic synthesis is exhausted and results in the significant depression of crude protein and amino acid digestibility and growth (Olli et al., 1994).

2.3.5.4 – Antigenic proteins

2.3.4.5.1 – Biochemistry

The occurrence and efficacy of antigenic proteins in the development of salmonid enteritis remains highly debated in the field of fish nutrition. Glycinin (G) and betaconglycinin (β G) are two of the major seed storage globulins (proteins > 92kDa in molecular weight) present in the soybean. G is a hexameric protein and β G is a trimeric protein with a mix of the three subunits β , α , and α' . Both the quaternary structure and subunits of G and β G have been seen to cause allergic reaction several terrestrial animal species (Taliencio et al., 2014), as well as potentially causing activation of similar non-specific immune response in rainbow trout and Atlantic salmon (Rumsey et al., 1994; Bakke-McKellep et al., 2007).

Several methods have been shown to be able to reduce the antigenicity of SBM. Disassociation or denaturation of the β G trimer to peptides below 20kDa in size through ethanol extraction (55-76% ethanol at 70-80°C), heating (>75°C), bacterial fermentation, and high-pressure treatment (>300 mPa) may reduce soybean antigenicity, however not all of these methods are available or economically viable for processors. Moreover, immunoglobulin inhibition assays of conventionally-processed (hexane extraction, heat treatment) SBM indicates ingredient antigenicity could remain unaltered (Rumsey et al., 1994), potentiating the involvement of G and β G in the noted intestinal inflammatory response.

2.3.5.4.2 – Feeding Effects

Feeding juvenile rainbow trout diets with high-level G and β G soybean meal results in an overall greater depression in growth, feed efficiency, and higher non-specific plasma immunoglobulin levels than a fishmeal or a soy protein concentrate-based diet (Rumsey et al., 1994). Serum immunoglobulin M, an antibody produced by B lymphocytes, is also seen to be elevated in another fish species, the hybrid sea bass (*Morone chrysops* \times *Morone saxatilis*) when exposed to the β -subunit of the trimeric β G protein (Taliercio et al., 2014). Soy-specific antibodies are also seen to be elevated in juvenile rainbow trout with intraperitoneal inoculation of oil-based 2mg/L soybean protein (Burrells et al., 1999) However, feeding trials indicate that even at extremely high levels of SBM inclusion (60-89%), there is no increase in circulating soy protein-specific antibodies, even with increasing incidence of gastro-enteritis pathologies including impaired gastrointestinal epithelia (Burrells et al., 1999), hence no connectivity between the inflammatory response and the dietary antigenic protein concentration.

2.3.5.5 – Oligosaccharides

2.3.5.5.1 – Biochemistry

Oligosaccharides are defined as sugar polymers with greater than three condensed monosaccharides. This grouping is composed of three main α -linked galactooligosaccharides: raffinose, stachyose, and verbascose which are indigestible by intestinal enzymes. In solvent extracted SBM, the levels of galacto-oligosaccharides is approximately 60g/kg (NRC, 2011)), with raffinose and stachyose the most predominant (Table 2.2, 2.6). Much like soyasaponins, levels of soya oligosaccharides are highly dependent on initial seed content, which can differ as a result of varying plant genotypes and agronomical conditions. Surveys of SBM oligosaccharide content from processing plants utilizing crop originating from seven different maturity zones in the Eastern USA have indicated strong variation in both stachyose and raffinose concentrations (Grieshop et al., 2003).

Reduced oligosaccharide content of soy ingredients is seen with additional steps in the processing of soy protein concentrate (SPC). EtOH-extraction or enzymatic treatment are two main methods of reducing SBM oligosaccharide content, and have both been seen to improve ingredient digestibility in rainbow trout (Glencross et al., 2003). EtOH extraction removes a suite of soluble carbohydrate soybean ANF, including oligosaccharides and saponins. Treatment with exogenous α -galactosidase breaks the galacto-oligosaccharide α -galactosidic linkages, reduces them into their monomers and reduces their putative roles in depression on nutrient digestibility (Glencross et al., 2003). Alcohol extraction and/or enzymatic treatments are not typical steps in SBM processing (Figure 2.1) thus, high levels of oligosaccharides are likely to remain in the final ingredient.

2.3.5.5.2 – Feeding Effects

Several studies investigating the effects of oligosaccharides have indicated that they may reduce nutrient digestibility and protein retention in Atlantic salmon and rainbow trout (Grisdale-Helland et al, 2008; Glencross et al., 2003;) and may alter gut microflora. However, many of these studies utilized ingredients with significant levels of other ANF such as soybean meal (Refstie et al., 2005; Refstie et al., 1998) or lupin meal (Glencross et al., 2003) which introduces many confounding factors. In an early study comparing rainbow trout fed diets with lupin meal (12g/kg oligosaccharide) to an enzyme treated (α -galactosidase, 6g/kg oligosaccharide) and an ethanol extracted (6.7g/kg oligosaccharide) meal, apparent digestibility of nitrogen was shown to be the lowest in the untreated lupin meal diet, and the best in EtOH-extracted diet (Glencross et al., 2003). These conclusions seem to indicate a significant role of oligosaccharides on apparent digestibility, however EtOH-extraction of lupin meal also results in the removal of several other carbohydrate ANF, including saponins and other non-starch polysaccharides which may also account for the effects. A later study by Sørensen et al., (2011) revealed that there is no significant effect of industrially-relevant levels of the oligosaccharides stachyose and raffinose on nutrient digestibility, feed efficiency, growth, or intestinal pathologies when added separately, together, or with soyasaponins to a fishmeal-based diet in Atlantic salmon.

2.4 – Sunflower (*Helianthus spp.*) and sunflower meal

2.4.1 – World production of sunflower seed and sunflower meal

2014 world production of sunflower seed was estimated at 40 million tons with approximately 50% occurring in the Ukraine and Russia, and strongest imports from the European Union, and Turkey (USDA, 2015). Canadian production of sunflower seed was 52,000 metric tonnes in 2014, and although a small fraction of worldwide production, is forecasted to increase to 70,000 in the 2015/2016 season. Of the 40 million tons of sunflower seed produced, approximately 55% was utilized in the production of sunflower meals, whereby Russia, the Ukraine, and the EU were highest producers representing 72% of total market (USDA, 2015). The EU also represents the highest import volume (61% of world import) and domestic consumption (99% of EU production and import added, 50% of world consumption).

2.4.2 – Nutritional quality of sunflower meal in salmonid feed

Information on the nutritive value of sunflower products in salmonid feed is relatively sparse. SFM represents a good source of plant-sourced proteins, and has a favorable amino acid profile when compared to salmonid requirements (Table 2.2). Most conventionally-processed SFM has a protein content between 32% and 46% (NRC, 2011) which is highly dependent on seed processing techniques and individual processing plant quality control regulations, and to a smaller extent parental plant genetics and other agronomical factors (Rosa et al., 2009). Studies of two varieties of Brazilian sunflower cultivars revealed a 2% difference in crude protein in the dehulled seeds, which translated into a 4% difference in the dehulled meals (Rosa et al., 2008). Another two-year study utilizing five varieties of African sunflower indicated that location (fertilization regimes, water stress, etc.) had a significant effect on protein, oil, and fibre content of the seeds,

with cultivar type showing less variation (Nel et al., 2000).

SFM is not able to meet total crude protein or specific amino acid requirements of cultured species such as the rainbow trout and Arctic charr with high dietary inclusion whether the ingredient is partially or fully dehulled (Table 2.2). It is deficient in the essential amino acid lysine and potentially arginine. The SFM antinutrient chlorogenic acid is described as a dose-dependent arginase inhibitor, with levels varying in SFM ingredients as a result of varying agronomical factors. Therefore, potential arginine deficiency is conditional to the dietary inclusion level of SFM thus the concentration of chlorogenic acid in diet formulations. In the sunflower meal ingredient total dietary fibre can represent between up to 45% by weight, of which 10 – 30% (deshelled vs. shelled seeds) is crude fibre (Francis et al, 2001), and between 24 – 31% is non-starch polysaccharides (Solsuski and Fleming, 1977; Kocher et al., 2000).

2.4.3. – Utilization in feed formulation

Studies with different sunflower ingredients have given promising results towards its inclusion as an alternative to fishmeal in salmonid diets (Tacon et al., 1984; Sanz et al., 1994; Gill et al., 2006). However, species or age class-specific limitations regarding dietary inclusion levels of SFM in salmonids have not yet been suggested, likely due to the potential variation in quality (nutrient composition) of SFM produced. Similar to SBM, it appears as though dietary tolerance of SFM varies between rainbow trout and Atlantic salmon, either as a result of differences in nutrient digestibility and utilization, or in response to specific sunflower anti-nutrients. However, the increased inclusion of sunflower meal into diets for salmonids necessitates careful examination of the nutritional quality of this ingredient.

2.4.4 – Physiological effects of feeding sunflower meal in salmonid species

2.4.4.1 – Effects on growth and feed conversion

Solvent-extracted sunflower meal (SFM) is generally not associated with significant depression in growth rate in either Atlantic salmon, nor rainbow trout when included in the diet at <40% (Gill et al., 2006; Sanz et al., 1994; Tacon et al., 1984). Early studies have indicated that SFM does not appear to have negative effects on growth or feed efficiency in rainbow trout fingerlings with dietary inclusion up to 22% (Tacon et al., 1984; Stickney et al., 2007), however at ~40% has been shown to cause slight reduction in growth rates, and a significantly reduced feed efficiency (Sanz et al., 1994). In rainbow trout, final body weight is slightly reduced, however feed conversion and protein retention efficiency are not significantly affected with up to 36.5% inclusion (Tacon et al., 1984). Feeding rainbow trout diets with 42% (% DM) SFM inclusion results in slightly reduced growth rates although significantly lower feed efficiency than both a fishmeal or a 32% SBM-based diet (Sanz et al., 1994). In post-smolt Atlantic salmon feed efficiency, growth, and mortality are not significantly affected with up to 27% (dry matter basis) dietary inclusion of dehulled, extruded sunflower meal when compared to a practical diet (Gill, et al., 2006). Reduction in growth and feed efficiency at high levels of sunflower meal inclusion is associated with both decreased dietary nutrient density and elevated levels of sunflower ANFs, including non-starch polysaccharides and phenolic compounds.

2.4.4.2 – Effects on nutrient digestibility and digestive enzyme activity

2.4.4.2.1 – Protein and amino acid digestibility

High level inclusion of sunflower meal seems to result in a less robust depression on protein and amino acid digestibility than SBM, likely due to a lower overall concentration of antinutrients

in SFM than in SBM at comparable dietary inclusion. Related to both a fishmeal and 32% SBM diet, high level inclusion (40%) of sunflower meal in diets for rainbow trout has been shown to result in a similar apparent digestibility of protein (Sanz et al., 1994). In Atlantic salmon 23% SFM addition results in marginally depressed protein and individual amino acid digestibility when compared to a fishmeal diet, but the effect is not as significant as is seen with 20% SBM (Asklaksen et al., 2007).

2.4.4.2.2 – Lipid digestibility

SFM does not appear to have a negative effect on the digestibility of lipids in rainbow trout (42% inclusion) or Atlantic salmon (23% inclusion) when compared to a fishmeal-based diet (Aslaksen et al., 2007; Sanz et al., 1994). This is likely a result of significantly reduced concentrations of the ANF lectin in SFM compared to SBM (Fagbenro et al., 2010) (Table 2.3).

2.4.5 – Major antinutritional factors in sunflower meal and their effects in fish

Common antinutritional factors and their levels in select sunflower ingredients are presented in Table 2.3.

2.4.5.1 – Non-starch polysaccharides

2.4.5.1.1 – Biochemistry

The non-starch polysaccharides (NSP) of plant ingredient sources are commonly fractions of the cell wall material, including celluloses, pectins, and β -glucans. Most are formed as polymers of saccharides other than glucose which are bound with β -glycosidic linkages resistant to enzymatic digestion in many species of animals and fish. They are classed into water soluble, and

water insoluble groups which have different physico-chemical properties including viscosity, the ability to bind water, and the ability to bind both organic and inorganic molecules (NRC, 2011).

Proper mechanical dehulling processes of sunflower seeds are necessary in order to decrease NSP content, because between 22 – 45% of initial seed mass is composed of indigestible cell wall material (cellulose, lignins, etc.) depending on variety of seed processed and agronomical conditions experienced by the parent plant (Rosa et al., 2009). Regardless of the mechanical dehulling process utilized, batches of meal from the same processing plant may show >10% difference in final neutral detergent fibre content (representing indigestible hemicellulose, cellulose, and lignin) and reduced overall utilizable nutrient density and metabolizable energy content of the final meal.

2.4.5.1.2 – Feeding Effects

NSP are largely indigestible to fish, and the inclusion of high levels in diets has been found to have significant effects on digesta viscosity and transit rate, and may lead to alterations in gut physiology and microflora (Sinha et al., 2011). Feeding Atlantic salmon soy protein products (SBM, reduced oligosaccharide SBM, soy protein concentrate, protein isolate) with different NSP levels has been found to result in increased viscosity of digesta and decreased apparent digestibility of lipid and nitrogen (Refstie et al., 1999). Similarly, digestibility of lipids and protein were reduced when rainbow trout were fed diets containing the NSPs guar gum and alginate, with a high level of guar gum addition also negatively affecting growth rate, and body lipid content (Storebakken, 1985). Moreover, increased dietary fibre in the form of α -cellulose is known to depress growth rate and dry matter digestibility in rainbow trout when added at high levels (Hilton et al., 1983), implicating suggested limitations of 10% partially dehulled sunflower meal inclusion

to salmonid diets (Hertrampf and Piedad-Pascual., 2000).

2.4.5.2 – Phenolic Compounds

2.4.5.2.1 – Biochemistry

There are a suite of phenolic compounds present in common plant protein feed ingredients. Of these, the most commonly examined in sunflower products are tannins, chlorogenic acid, and its primary derivative caffeoylquinic acid. Tannins are generally localized in the seed coat of many plant ingredient sources, giving the unprocessed seed a brown colour and a bitter flavour. They are polyhydroxyl phenolic compounds with the ability to precipitate proteins, classified as either hydrolysable or condensed depending on their capacity to be hydrolyzed by digestive enzymes or acids. A second set of phenolic compounds, representing up to 70% of phenolic compounds in partially or non-dehulled SFM is the pseudotannin chlorogenic and caffeoylquinic acid. Chlorogenic acid is the ester of caffeic and quinic acid, with caffeoylquinic acid (5-CQA) as its major derivative.

Several solvent extraction methods aimed at the removal of phenolic compounds have been investigated, including water extraction, ethanol extraction (70%), saline extraction (2% NaCl), and n-butanol and low molarity HCl extraction (Rahma et al., 1981). Each extraction method is shown to significantly reduce chlorogenic acid concentrations and raise crude protein content. Ethanol extraction represents the standard industrial method of removing chlorogenic acid alongside a suite of other carbohydrate-based antinutrients, producing high-protein plant concentrate ingredients.

2.4.5.2.2 – Feeding Effects

Current research into the effects of tannins on nutrient utilization and growth in fish is extremely sparse. Hydrolysable and condensed tannins at levels from 0-25g/kg in diets for Nile tilapia (*Oreochromis niloticus*) resulted in significant reduction of weight gain, protein retention efficiency, and feed intake, and an increase in feed conversion ratio (g fed/g weight gain), whereby hydrolysable tannins had the greatest effects (Buyukapar et al., 2011). In a similar study with common carp (*Cyprinus carpio*), a condensed (quebracho) tannin added to diets at a level of 2%, (representing 100x the concentration observed in SBM) in did not affect weight gain (condensed tannin) however the hydrolysable tannin (tannic acid) source resulted in similar feed rejection as was seen in the tilapia (Becker and Makker, 1999). It is clear that both hydrolysable vs. condensed tannin, as well as fish and/or the chemical form of the tannin investigated likely determine the effectivity of tannins on fish growth and nutrient utilization, and that further research involving economically significant salmonid species should be undertaken.

To date, no studies have investigated the direct effects of chlorogenic or caffeoylquinic acid in salmonids. However, investigations into the chemistry of caffeoylquinic acid has described it as a dose-dependent arginase inhibitor which may act to inhibit the metabolism or synthesis of L-arginine in fish. (NRC, 2011). Limitation of L-arginine may prevent adequate cellular differentiation and/or proliferation in highly proliferative tissues such as the intestinal mucosa through the disruption of polyamine synthesis (NRC, 2011).

Table 2.2 – Essential amino acid profiles of select ingredients used in salmonid aquafeed formulation compared to requirements in rainbow trout and Arctic charr.

Essential AA	Ingredient AA (% as-is) ¹				Requirement (% diet)	
	Herring Meal ^a	SBM ^b	SFM ^c , with hulls	SFM ^d , hulled	Rainbow trout	Arctic charr
Arginine	3.7	3.2	2.9	3.5	1.4 ²	-
Histidine	1.5	1.2	0.9	1.0	0.5-0.6 ³	-
Isoleucine	3.6	2.0	1.4	2.1	0.7-1.4 ³	-
Leucine	4.7	3.4	2.3	2.6	1.1-1.4 ³	-
Lysine	7.3	2.8	1.2	1.7	1.8-2.3 ⁴	-
Methionine	2.2	0.6	0.8	1.5	0.4-0.9 ⁵	0.7 ⁸
Phenylalanine	2.7	2.2	1.7	1.2	0.7 ⁶	-
Threonine	2.5	1.7	1.3	1.5	1.1 ⁷	-
Tryptophan	0.7	0.6	0.4	0.4	0.1-0.2 ⁴	-
Valine	3.3	2.4	1.7	2.3	0.8-1.6 ⁴	-

Data adapted from: ¹(NRC, 2011); ²(Cho et al., 1992); ³(Rodehutscord et al., 1997); ⁴(Encarnação et al., 2004); ⁵(Rodehutscord et al., 1995); ⁶(Kim, 1993); ⁷(Bodin et al., 2008); ⁸(Simmons et al., 1999).

^aFishmeal, 72% CP

^bSoybean meal, solvent extracted, 44% CP (5-04-604).

^cSunflower meal, solvent extracted, 32.3% CP (5-04-737).

^dSunflower meal, solvent extracted, hulls removed, 46% CP (5-04-739).

Table 2.3 – Antinutritional factors and their levels present in commercially processed soybean and sunflower meal

Antinutritional Factor	Soybean Meal	Sunflower Meal
Saponins	0.43 - 0.83% ^{a,b}	N.D.**
Lectins	0.001 - 0.087% ^{c,*}	0.05% ⁱ
Protease Inhibitors		
Trypsin Inhibitor	0.2 – 0.6% ^d	0 – 0.007% ⁱ
Isoflavones (Phytoestrogens)		
Diadzein	.0045% ^e	
Genistein	.0125% ^e	
Phytates	1.0 -15% ^a	
Phenolic Compounds		3.0-3.5% ^j
Tannins	0.3% ^f	
Chlorogenic Acid	1.0% ^g	1.9 – 2.1% ^{j,k}
Caffeic acid		1.6 -1.8% ^k
Non-starch polysaccharides	14 – 18% ^a	24 - 31% ^{l,m}
Oligosaccharides	6.0% ^h	1.7% ^h
Raffinose	1.0 – 2.0% ^a	
Stachyose	50 - 60g/kg ^a	
Verbascose	1.8% ^f	

Data adapted from: ^a(Hart et al.); ^b(Ireland et al., 1986); ^c(Maenz et al., 1999); ^d(Synder and Kwon, 1987); ^e(Kaushik et al., 1995); ^f(Zdunczyk et al., 1999); ^g(Pratt and Birac, 1979); ^h(NRC,2011); ⁱ(Fagbenro et al., 2010), ^j(Gill, 2002), ^k(Solsuski and Fleming, 1977), sunflower flour; ^l(Kocher et al., 2000); ^m(Düsterhöft and Voragen, 1991).

*Actively agglutinating lectin content

**N.D. = Not detected

Table 2.4 – Comparative market prices of select animal and plant protein ingredients

Ingredient	CP (%)	ADC CP (%)	Cost (\$ USD/tonne)	Cost (\$ USD/tonne DP)
Fishmeal, herring	71 ^a	90 ^a	1620 ^h	2535
Meat and bone meal	57 ^b	83 ^b	540 ^h	1141
Poultry by-products meal*	62 ^a	83 ^a	600 ^h	1165
Soybean meal	48 ^c	93 ^c	535 ^h	1198
Soy protein concentrate	71 ^c	96 ^c	1450	2127
Sunflower meal, partially dehulled	32 ^d	81 ^d	267 ⁱ	1030
High protein sunflower meal	46 ^e	96 ^e	561	1270
Rapeseed meal	38 ^f	95 ^g	322 ^j	891

^a(Cheng and Hardy, 2002); ^b(Bureau et al., 1999); ^c(Kaushik et al.,1995);

^d(Aslaksen et al., 2007), data presented is for Atlantic salmon; ^e(Chen et al.,2015), unpublished data; ^f(NRC, 2011); ^g(Cheng and Hardy, 2002b);

^hHammersmith Marketing LTD. Weekly Feed Grain and Protein Report, April 5, 2015. Grain prices FOB bulk grain vessels, container shipments minimum 200m/t, fishmeal FOB North Germany; ⁱNational Sunflower Association (2015). Price converted to USD using April 7, 2015 conversion rate; ^jCanola Council of Canada, average prices for 2014 calendar year.

*Feed-grade

Table 2.5 – Effect of temperature and duration on deactivation of trypsin inhibitors and reduction in the lectin content of raw soybean

Time (min)	Trypsin Inhibitor Activity (mg/100g)			Lectin Content (µg/g)		
	102°C	120°C	134°C	102°C	120°C	134°C
0	23.4	23.4	23.4	7638	7638	7638
2	-	6.0	1.8	-	<0.5	-
5	10.2	2.8	-	-	-	-
10	5.8	1.7	-	1005	-	-
20	2.9	-	-	105	-	-
80	0.8	-	-	-	-	-

Chart adapted from Qin et al. (1996).

Table 2.6 – Carbohydrate ANF content of processed sunflower and soybean meal products

Plant Product	Saponin Content (g/kg)	Total Dietary Fibre (g/kg DM)	Oligosaccharides (g/kg DM)
Soybean, raw	56 ¹	245 ⁷	95 ⁵
Soybean, hulls	20 ¹	758 ⁶	
Soy flour, defatted (solvent extraction)	22 ¹		
Soybean meal, solvent extracted	7 ⁸	233 ³ 242 ⁴	60 ³
Soy protein isolate (solvent extraction, protein isolation)	3-8 ¹	74 ⁴	
Sunflower seed, raw	41 ²		
Sunflower meal, defatted (mechanical pressing)	28 ²	448 ³	17 ³
Sunflower oil cake, partially dehulled		326 ³	19 ³

¹(Fenwick et al., 1981); ²(Fagbenro et al., 2010); ³(NRC, 2011); ⁴(Refstie et al., 1999); ⁵(Han and Baik, 2006); ⁶(Cole et al., 1999), average of reported TDF values; ⁷(Redondo-Cuenca et al., 2008); ⁸(Knudsen et al., 2008).

2.5 –The effect of genetics on nutrient utilization in cultured salmonids

Domestication and intensive production of any cultured animal involves an understanding how specific dietary nutrients are utilized by different genetic species, strains, or even families in order to exploit the phenotypic traits of interest. In salmonid culture, numerous studies have indicated differences in apparent nutrient digestibility, growth rate, feed efficiency and overall body composition between species (Gjedrem and Gunnes, 1978; Refstie et al., 2000; Azevedo et al., 2002; Azevedo et al., 2004; Krogdahl et al., 2004; Grisdale-Helland et al., 2007), as well as differences in growth and/or feed efficiency between strains of the same species (Ming, 1985; Overturf et al., 2003; Rasmussen and Jokumsen, 2009). Genetic manipulation techniques have allowed for the production of transgenic GH (growth hormone) strains of salmon, as well as polyploid strains of rainbow trout and Arctic charr aimed at further improvement of growth rates, and reduction of growth depression associated with precocious maturation.

2.5.1 – Species effects

Early studies investigating growth of Atlantic salmon, Arctic charr, rainbow trout, and pink salmon under farming conditions revealed significantly different growth rates between species at both the juvenile and grower stages (Gjedrem and Gunnes, 1978), suggesting potential differences in nutrient digestibility or utilization. More recent studies have shown that nutrient apparent digestibility is seen to vary between species (Azevedo et al., 2004), and that the effect of plant protein ingredients on apparent digestibility of crude protein and lipid is also variable at the species level (Refstie et al., 2001). Notable differences in nutrient digestibility and utilization between salmonid species have been well-documented (Refstie et al., 2000; Azevedo et al., 2002; Azevedo et al., 2004; Krogdahl et al., 2004; Grisdale-Helland et al., 2007) and has been attributed to species-

specific differences in nutrient utilization. In a study by Azevedo et al. (2004), digestibility, feed efficiency, and nitrogen retention efficiency (nitrogen retained/digestible nitrogen ingested) in diets with graded digestible protein/digestible energy were compared in four species of salmonids. Nutrient digestibility was observed to differ significantly between species. Lowering DP/DE ratios via increased dietary lipid content also resulted in enhanced nitrogen retention in Atlantic salmon and lake trout when compared to rainbow trout. This suggests reduced catabolism of proteins for energy production in salmon and lake trout, but not rainbow trout when exposed to high energy diets. Similar results in species-specific differences in nitrogen retention were observed by Grisdale-Helland et al. (2007) between rainbow trout and Atlantic salmon when fed low and high lipid diets. Enhanced nitrogen and gross energy retention in Atlantic salmon has been associated with an improved capacity to spare nitrogen at the expense of non-protein (usually lipid) sources for energy production (Refstie et al., 2000; Azevedo et al., 2004; Grisdale-Helland et al., 2007). However, enhanced protein and gross energy utilization in Atlantic salmon has also been suggested as one of many factors in the more severe depressive effects of SBM on growth and feed efficiency in this species (Refstie et al., 2000).

2.5.2 – Strain and family effects

Different strains, strain hybrids, and even genetic families of select salmonid species present with variations in macronutrient digestibility (Rasmussen and Jokumsen, 2009), growth rate, feed efficiency and protein retention efficiency (Ming, 1985; Overturf et al., 2003). In juvenile rainbow trout, a 25% difference in feed efficiency and 10% difference in protein retention is seen to occur between five genetic strains when fed fixed rations, which the authors suggested may be a result of strain-specific alterations in protein turnover (Overturf et al., 2003). Selective

hybridization of eight strains of rainbow trout results in a hybrid stock with a dramatic increase in protein retention efficiency (doubled that of lowest-performing parental strain. (Overturf et al., 2013). In the Chinook salmon, variation in the feed efficiency and protein productive value are observed between strains when fed both low and high gelatinized starch diet (Mazur et al., 1992). Moreover, in rainbow trout, apparent digestibility of protein, lipid, and dry matter show significant variations, even at the family level when comparing different individuals within the same genetic strain with no common parentage (Rasmussen and Jokumsen, 2009). Earlier experimentation investigating the effects of Arctic charr strain on growth reveal significant differences in weight gain between strains of the same forms (land-locked or anadromous) (Nilsson, 1993; Naslund and Henricson, 1996). However, true strain-specific effects on nutrient utilization in the Arctic charr remain to be determined.

2.5.3 – Polyploidy and its effects

Production of triploid salmonids, particularly sterile females, has been implemented since the 1970's as a method to circumvent the reduction in growth common to maturing male salmonids, to prevent damage to wild stocks through potential escapees, as well as to produce a fast-growing fish (Cotter et al., 2000; Benfey, 2001). Current methods of triploidy induction include thermal and hydrostatic pressure shock treatment up to one hour after fertilization, processes which interfere with the removal of the second polar body of maternal DNA from the ova, resulting in a fertilized egg with three (triploid) as opposed to two (diploid) sets of chromosomes (Benfey, 2001). Studies comparing nutrient utilization in diploid and triploid salmonids have shown varying results. Tibbetts et al. (2013) revealed that there is little effect of ploidy in Atlantic salmon (100g initial body weight) on growth, nitrogen utilization efficiency, or

overall feed efficiency (weight gain/unit feed consumed). However, contrasting research indicates enhanced weight gain and length in triploid as opposed to diploid salmon when raised in indoor, full seawater tanks (Oppedal et al., 2003). Whether the improved growth in triploids was due to differences in protein utilization or only a result of increased feed intake however, falls into question.

Studies investigating feed restriction in rainbow trout have shown significant effects of ploidy on mechanisms involved in the regulation of protein degradation (Cleveland and Weber, 2013). The rainbow trout preferably utilizes protein (nitrogen) as an energy-yielding nutrient, hence during times of fasting the expression of genes involved in protein catabolism including those coding for select ubiquitin ligases, and those related to autophagy (cathepsin D and L) are upregulated to allow for energy production directly from body protein stores. However, this upregulation is less severe in the skeletal musculature of triploid fish compared to their diploid counterparts, and is assumed to permit less corporeal protein loss to catabolic proteolysis (Cleveland and Weber, 2013). Triploid fish also exhibit a faster return to baseline levels of expression, which results in increased nitrogen retention and specific growth rates during initial refeeding periods (Cleveland and Weber, 2013).

2.6 – Conclusion

Worldwide aquaculture production places heavy reliance on the inclusion of readily-available plant protein ingredients in salmonid feeds, a response to increasing costs and the negative environmental stigmas associated with marine sourced ingredients. Soybean meal represents a plant ingredient that is currently utilized in salmonid diets due to its high global production volume and low cost, and a relatively high and highly digestible crude protein content. However, the occurrence of several antinutritional factors within conventionally-processed soybean ingredients and their negative effects on growth, feed efficiency, and intestinal health tends to limit the inclusion of soybean meal in salmonid feeds. These antinutritional factors have been implicated in the development of gastrointestinal enteritis in salmonid species, as well as reduced macronutrient digestibility in both Atlantic salmon and rainbow trout (Olli et al., 1994; Refstie et al., 2000; Krogdahl et al., 2003; Romarheim et al., 2008). Although not utilized as frequently, sunflower meal also represents a good source of crude protein targeted for salmonid diets, and when compared to soybean meal does not result in as severe depression in growth performance or intestinal health (Sanz et al., 1994). However, inconsistencies in the nutrient density of this ingredient as well as a relatively high indigestible carbohydrate content limits its dietary inclusion in nutrient-dense diets.

Comparative studies on plant protein ingredients have highlighted key differences between species (Refstie et al., 2000; Azevedo et al., 2002; Azevedo et al., 2004; Krogdahl et al., 2004; Grisdale-Helland et al., 2007) and genetic strain (Ming, 1985; Nilsson, 1993; Naslund and Henricson, 1996; Overturf et al., 2003; Rasmussen and Jokumsen, 2009) in overall sensitivity of growth performance or feed efficiency in response to specific plant ingredients and antinutritional factors. However these studies have generally focused on Atlantic salmon and rainbow trout with

no current studies examining the Arctic charr. The Arctic charr is a salmonid species represented by three genetic strains which are currently intensively cultured in land-based facilities across Canada. Current production of 300 metric tonnes represents approximately \$1.8 billion USD. Little is known about the sensitivity of Arctic charr compared to other salmonid species, or between its strains, towards plant protein ingredients such as soybean meal or sunflower meal in terms of growth, feed efficiency, and intestinal health. Thus, there is a need to examine the effects of plant proteins in the Arctic charr, as well as to reveal any differences between strains that are currently cultured in order to promote the expansion of Arctic charr culture in Canada.

3 – THE EFFECTS OF SOYBEAN MEAL AND SUNFLOWER MEAL ON THE PERFORMANCE AND HEALTH OF TWO GENETIC STRAINS OF ARCTIC CHARR (*SALVELINUS ALPINUS*) AT THE JUVENILE STAGE

3.1 – Abstract

The effects of graded levels (0 – 20% total dietary inclusion) of a commercial, solvent-extracted soybean meal (SBM) and expeller sunflower meal (SFM) on growth, nutrient utilization, and intestinal health were examined in two Canadian strains of Arctic charr at the juvenile stage. Feeding diets with increasing inclusion of SBM or SFM for 12 weeks to Arctic charr (initial weight = 9g/fish) resulted in no differences in growth rate (measured as the thermal-unit growth coefficient, TGC), feed efficiency (gain:feed, FE), nitrogen or energy deposition rates, or retention efficiency (NRE, ERE), expression of pro-inflammatory genes, organo-somatic indices, or body composition between genetic strains. Increased inclusion level of plant proteins negatively affected growth rate ($P < 0.05$) and FE ($P < 0.0001$) with effects most commonly observed in tanks fed SBM-based diets. Feed intake ($P < 0.0001$), FE ($P < 0.0001$), NRE and ERE ($P < 0.01$), whole body crude protein, lipid, and gross energy ($P < 0.0001$), and organo-somatic indices ($P < 0.0001$) were significantly affected by genetic strain examined. Strain 2 exhibited the highest FE and carcass crude protein content, and the lowest FI and carcass lipid at all experimental diets. The substantial difference in FE presented between the investigated genetic strains of charr exposed to all dietary treatments in the present study may indicate strain-specific dissimilarities in protein and lipid utilization, with a potential protein-sparing effect of dietary lipid noted in Strain 2.

3.2 – Introduction

With a substantial world-wide projected rise in aquaculture production, and the stagnant marine ingredient supply, there has been significant research efforts into the use of both common and underutilized plant protein and oil sources in formulated aquafeeds. Amongst plant ingredients, legumes (such as peas, and lupin), oilseeds (soybeans, canola, sunflower, linseed), and some grain products are increasingly utilized as a good source of digestible protein in aquafeeds. Of these plant sources, soybean meal has been the classical plant protein ingredient targeted to both terrestrial livestock and aquatic feeds due to its relatively high crude protein content and world-wide production volume. Although much less is produced, sunflower meal has also been shown as a promising plant ingredient for aquaculture feeds (Tacon et al., 1984; Stickney et al., 2007). The dietary inclusion of many plant protein ingredients including soybean and sunflower meals is limited in nutrient-dense feed formulations due to a combination of plant-specific nutrient and anti-nutrient content. Soybean meal and sunflower meal tend to be high in indigestible carbohydrate content, which may reduce overall utilizable dietary nutrient density when the ingredients are added at high levels. Soybean and sunflower meal are also deficient in the essential amino acids methionine, lysine, and potentially arginine. Additionally, the

Plant antinutritional factors are a suite of substances which have the capacity to disrupt normal digestive functioning of the animal intestine and accessory digestive organs (Francis et al., 2001). These compounds can lead to reduction in growth performance, feed efficiency, nutrient utilization and apparent digestibility in different fish, notably salmonid fish species (Baeverfjord and Kroghdahl, 1996; Refstie et al., 1999; Bennetau-Pelissero et al., 2001; Refstie et al., 2001; Glencross et al., 2003; Kroghdahl et al., 2003; Sinha et al., 2011). Prolonged exposure to soybean ingredients with high ANF content or purified ANF extracts from soy have been seen to result in

the development of a non-infectious inflammatory condition in the distal intestine. This condition, termed soybean meal-induced gastro-intestinal enteritis presents with striking alterations to the intestinal tissues of fish including significant reduction in simple and complex fold structure, loss of enterocyte supranuclear vacuolization, and infiltration of mixed leukocyte populations into the lamina propria and submusocal tissues (Van den Inge et al., 1991; Baeverfjord and Kroghdahl, 1996; Bakke, 2011). This inflammatory condition is believed to be closely linked to a combination of the anti-nutrients soyasaponins, lectins, and potentially antigenic proteins. Soyasaponins have been observed to intercalate into the gastrointestinal epithelium and increase intestinal porosity which may permit the absorption of foreign materials, as well as to dramatically decrease overall lipid absorption in salmonids. Lectins have been noted to result in enterocyte sloughing from simple folds, effectively reducing simple fold height (Buttle et al., 2001; Iwashita et al., 2008; Knudsen et al., 2008). Acute instigation of the immune system in animals afflicted with this pathology by plant antigenic proteins, assuming they are absorbed, may promote the upregulation of select genes involved in xenobiotic recognition and removal, T and B lymphocyte maturation and activation, and cytokine-mediated apoptosis signalling cascades (Kortner et al., 2012; Marjara et al., 2012).

Differences in the intestinal histopathologies associated with sub-acute enteritis, as well as growth performance and nutrient digestibility appear between salmonid species when fed conventionally processed soy ingredients. Refstie et al. (2000) investigated the effects of soybean meal in diets formulated for Atlantic salmon and rainbow trout. The researchers found significant depression in growth rate and robust decline in nitrogen retention and macronutrient apparent digestibility in salmon, but not in trout when compared to conspecifics fed a fishmeal-based diet, indicating salmon appear to be much more sensitive to soybean ingredients. Inconsistencies in

performance in salmonids fed plant protein-based diets has likewise been detected at the genetic strain and family level in rainbow trout (Pierce et al., 2008; Venold et al., 2012). Multi-generation selective breeding of rainbow trout has been utilized as a method to create hybrids with increased tolerance and nutrient utilization efficiency with plant protein feeding, which is observed to be a combined result of increased intestinal enterocyte proliferative capacity and nutrient transport in selected fish compared to their non-selected counterparts (Overturf et al., 2012; Venold et al., 2012).

The Arctic charr (*Salvelinus alpinus*) is a member of the Salmoninae sub-family alongside the Atlantic salmon and rainbow trout, with current global production at approximately 3,800 metric tons. The species is separated into four geographically determined subspecies, and subspecies are further delegated into genetic strains which can be of either anadromous (sea-faring) or non-anadromous (land-locked) morphs (Johnston, 2002). Past studies have indicated significant differences in nutrient digestibility and growth performance between Arctic charr and other salmonids (Burr et al., 2011) as well as between anadromous or non-anadromous strains of charr (Nilsson, 1993; Naslund and Henricson, 1996). Although generally unidentified, life-stage specific nutrient requirements of charr are assumed to be similar to those of the rainbow trout, thus charr is currently fed commercially-available trout formulations. However, the tolerance of the Arctic charr towards both commonly and underutilized plant protein ingredients in salmonid aquafeeds is unknown, with no studies investigating the potential effects of individual plant proteins on the performance and overall health of this species. Additionally, similar to the rainbow trout, strain-level differences may have significant effects on digestibility, nutrient utilization, and overall growth performance and feed acceptance in Arctic charr. Thus, the objectives of this study were to investigate and compare the effects of diets containing graded levels of commercially available

solvent-extracted soybean meal and sunflower meal on growth, feed efficiency, feed intake, nutrient retention and utilization, pro-inflammatory mRNA expression, and carcass composition in two Canadian strains of Arctic charr at the juvenile stage.

3.3 – Materials and Methods

3.3.1 – Fish and experimental conditions

Fertilized eggs of Arctic charr (*Salvelinus alpinus*) of diploid and triploid strains (Strain 1 and Strain 2) were obtained from two Canadian Arctic char hatcheries. Groups of 50 fish with initial average body weight 8.8 ± 0.1 g/fish were distributed into 30 tanks in a complete randomized block design according to tank row and experimental diet. Tank was considered the experimental unit. Fish were maintained in a re-circulating system (97%) composed of 100-L fibreglass tanks with an initial flow rate of 4L/min, which was adjusted according to growth. Water temperature was maintained at $12.4 \pm 0.7^\circ\text{C}$ and photoperiod was maintained at 24h light in a windowless laboratory. Fish were kept in accordance with the guidelines of the Canadian Council of Animal Care at the New Brunswick Community College (Caraquet, NB). Fish were acclimated to the experimental conditions for three weeks prior to the start of the study. They were hand fed three times daily to satiation using a commercial salmon feed (EWOS 1.5, Surrey, BC, Canada) during the acclimation period.

At the beginning of the experiment, a pooled sample of 20 fish per strain were taken for determination of initial carcass composition. An additional pooled sample of 25 fish per strain were taken for determination of hepatosomatic and splenic indices. At the end of the 84-day experimental period, 10 randomly selected fish per tank were taken for determination of carcass composition. Five additional fish per tank were sampled for hepatosomatic and splenic indices and

distal intestine sections. Fish to be analyzed for carcass composition were cooked in an autoclave, blended into a homogenous slurry using a food processor, freeze-dried, reground, and stored at -20°C until analyzed.

3.3.2 – Experimental diets and feeding protocol

Five diets were formulated to meet all known nutrient requirements of rainbow trout. Diet A served as a practical trout diet, diets B and C contained 10% and 20% solvent-extracted soybean meal respectively, and diets D and E contained 10% and 20% partially de-hulled sunflower meal respectively on an as-is basis (Table 3.1). All ingredients were mixed using a Hobart mixer (Hobart Ltd, Don Mills, ON, Canada). The five diets were steam pelleted to appropriate size (diameter= 2 mm, length= 2mm) using a laboratory pellet mill (hammer mill, screen size= 2 mm, die thickness= 44.5 mm, California Pellet Mill, San Francisco, CA, USA). Pellets were then dried overnight under forced-air at 60°C (Precision Scientific CO Cat N°: 1054), crumbled and sieved to size according to EWOS suggested pellet size for rainbow trout, and stored at room temperature until used.

Fish were initially hand-fed three times daily to apparent satiation on weekdays and hand-fed twice daily on weekends whereby they were given 80% average daily tank rations, calculated on a weekly basis throughout the duration of the experiment. Upon reaching an average body weight of 20g, feed was withheld one day a week for cleaning of the tanks. Feed intake was recorded on a daily basis.

3.3.3 – Chemical analysis

Dry matter (DM) and ash content of the diets and carcass were analyzed according to methods of AOAC (Association of Official Analytical Chemists) Official Methods (Horwitz,

2006). Crude protein (CP, %N x 6.25) was determined via a Kjeltec™ 8200 auto distillation unit (FOSS, Höganäs, Sweden) and lipid with an Ankom XT20 fat analyzer (Ankon Technology, Macedon, NY, USA). Gross energy (GE) content was calculated using mean GE values of carbohydrates (17.2kJ/g), proteins (23.6kJ/g), and lipids (39.5kJ/g) according to Blaxter (1989).

Table 3.1 – Composition of the experimental diets.

Ingredient (g/100g diet)	Diet				
	A	B	C	D	E
	Practical	10% SBM	20% SBM	10% SFM	20% SFM
Fish meal, herring (71% CP)	35.0	30.4	25.8	28.9	22.8
Poultry by-product meal (67% CP)	19.3	18.6	18.0	18.6	18.0
Soybean meal (46% CP)	-	10.0	20.0	-	-
Sunflower meal (17% CP)	-	-	-	10.0	20.0
Fish oil, herring	12.0	12.6	13.2	12.6	13.1
Corn protein concentrate (76% CP)	9.1	6.5	3.8	7.8	6.6
Wheat gluten (78% CP)	10.0	11.3	12.5	11.3	12.5
Wheat middlings	11.6	7.2	3.0	7.3	2.9
Vitamin premix ^{a1}	0.3	0.3	0.3	0.3	0.3
Mineral premix ^{b1}	0.2	0.2	0.2	0.2	0.2
Vitamin E	0.6	0.6	0.6	0.6	0.6
Lysine (BioLys)	0.1	0.3	0.2	0.3	0.6
DL-Met	0.0	0.1	0.1	0.1	0.2
choline chloride	0.3	0.3	0.3	0.3	0.3
Ca(H ₂ PO ₄) ₂	1.0	1.1	1.3	1.1	1.3
NaCl	0.3	0.3	0.3	0.3	0.3
Rovimixstay-C (25%)	0.3	0.3	0.3	0.3	0.3
Total	100.0	100.0	100.0	100.0	100.0
Analyzed Composition (dry matter basis)					
Dry Matter (%)	94.8	94.8	95.2	95.0	95.3
Crude Protein (%)	56.1	55.3	53.8	51.7	49.1
Lipid (%)	19.7	19.3	19.2	21.6	24.1
Carbohydrate (%)	14.1	15.2	17.2	16.5	17.2
Ash (%)	10.1	10.2	9.8	10.2	9.7
GE (KJ/g)	23.6	23.4	23.4	23.7	24.2

^aVitamin premix. Provided per kg of diet: retinyl acetate (vitamin A), 75 mg; cholecalciferol (vitamin D₃), 60 mg; menadione Na-bisulfate (vitamin K), 1.5 mg; cyanocobalamine (vitamin B₁₂), D-biotin, 210 mg; choline chloride, 3448 mg; folic acid, 1.5 mg; niacin, 15 mg; calcium-D-pantothenate, 33 mg; pyridoxine –HCl, 7.5 mg; riboflavin, 9 mg; thiamin-HCl, 1.5 mg.

^bMineral premix. Provided per kg of diet: sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; potassium iodine (KI, 24%K, 76%I), 10.5 mg; ferrous sulphate (FeSO₄, 7H₂O, 20%Fe), 65 mg; manganese sulphate (MnSO₄, 36%Mn) 88.9 mg; zinc sulphate (ZnSO₄.7H₂O, 40%Zn), 150 mg; copper sulphate (CuSO₄.5H₂O, 25%Cu), 28 mg; sodium Selenite (Na₂SeO₃, 45.66% Se), 0.7 mg.

¹Martin Mills Inc., Elmira, ON

3.3.4 – Genetic analysis

0.5-1cm sections of distal intestine tissue and whole spleens were excised, placed in a 1.5mL tube of RNALater, and immediately frozen at -20°C for genetic analysis. Samples were shipped to Dr. Ken Overturf at the Hagerman Fish Culture Experiment Station, University of Idaho, USA. Targeted gene products included cluster of differentiation 8 (CD-8), nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), pregnane X receptor (PXR), and interleukin-I beta (IL1β).

Total RNA was isolated from each sample using TRIzol® Reagent (Invitrogen) per the manufacturer's protocol and analyzed for quantity on a spectrophotometer (Nanodrop 2000, Wilmington, DE) and quality using the Agilent (Santa Clara, CA) 2100 Bioanalyzer Nano Kit. Potential DNA contamination was then removed using Amplification Grade DNase I (Invitrogen). To detect the level of gene expression, real-time quantitative RT-PCR was carried out using an ABI Prism 7900HT Sequence Detection System and the TaqMan One-Step RT-PCR Master Mix Reagents kit, according to the protocol provided by ABI (Foster City, CA). The final concentration of each reaction was: Master Mix, 1x (contains AmpliTaq Gold enzyme, dNTPs including dUTP, a passive reference, and buffer components); MultiScribe reverse transcriptase, 0.25 U/μl; RNase inhibitor mix, 0.4 U/μl; forward primer 600 nM; reverse primer 600 nM; probe, 250 nM; total RNA, 75 ng. Probes and primer sequences are listed in Table 3.2. Cycling conditions for genes tested were as follows: 30 m at 480C, 10 m at 95C, then 40 cycles of PCR consisting of 15 s at 95C followed by 1 m at 60C. For each gene, assays were run in triplicate on RNA samples isolated from individual fish. Raw cycle threshold (Ct) values were extracted from each run and normalized to *β-actin* mRNA expression using the delta-delta Ct method (Pfaffl, 2001).

Table 3.2 – Gene names, accession numbers and sequences.

Gene	Genebank accession no.	Primer/probe sequence (listed 5'-3')
<i>β-actin</i>	AF254414	BactinF: CCCTCTTCCAGCCCTCCTT BactinR: AGTTGTAGGTGGTCTCGTGGATA BactinMGB: 6FAM-CCGCAAGACTCCATACCGA-NFQ
<i>PXR</i>	EF517132	PXRF: GCAGATCCGCTTCAACATGATG PXRR: CGTCGTCCATGCAGTATGTCA PXRMGB: 6FAM- CACTCTCCAGATGCC -NFQ
<i>NFκβ</i>	CA356763	NFKB: TGACAAAGGCATCTGCATCACA NFKB: CATGGAGGATGCCCAGGTT NFKBMGB: 6FAM-CTGATGCTGGAGTCTTT-NFQ
<i>IL-1β</i>	Aj004821	IL1BF: GCAGTAAAGGAGAGTACGAGTGTAG IL1BR: GCTCCATAGACCCTTCGTTGAG IL1BMGB: 6FAM- CCCAGCACTTGTCTC-NFQ
<i>CXCL-8</i>	AJ279069	CD8F: ACACCAATGACCACAACCATAGAG CD8R: GCTGTAACCCAGGACTCAATGTG CD8MGB: 6FAM-ACCAGCTCTACAACTGCCAAGTCG TGC -NFQ

3.3.5 – Calculations

Growth rate was measured as a function of the thermal-unit growth coefficient (TGC).

$$TGC = 100 \times [(FBW^{1/3} - IBW^{1/3})/\Sigma(T \times D)]$$

Where: FBW = final average body weight (g/fish); IBW = initial body weight (g/fish);

$\Sigma(T \times D)$ = sum of temperature (°C) x days.

Feed efficiency (FE), the ratio of weight gain:feed intake was calculated for each tank as:

$$FE = \text{live weight gain (g)/dry feed intake(g)}$$

Where: live weight gain = (tank FBW(g)/number of fish – tank IBW(g)/number of fish); dry feed

intake (g) = tank dry feed intake (g)/number of fish.

Hepatosomatic Index (HSI) and spleen somatic index (SSI) were calculated as:

$$HSI = \text{liver wet weight (g)/live body weight (g)}$$

$$SSI = \text{spleen wet weight (g)/live body weight (g)}$$

Where: liver wet weight included full gall bladders in weight measurement.

Nitrogen deposition rate (NDR) and energy deposition rate (EDR) were calculated as:

$$\text{NDR} = (\text{N content}_{\text{final}} - \text{N content}_{\text{initial}}) / \Sigma(\text{T} \times \text{D})$$

$$\text{EDR} = (\text{GE content}_{\text{final}} - \text{GE content}_{\text{initial}}) / \Sigma(\text{T} \times \text{D})$$

Where: $\text{N content}_{\text{final}}$ = final carcass nitrogen (mg/fish); $\text{N content}_{\text{initial}}$ = initial carcass nitrogen (mg/fish).

GE_{final} = final carcass energy (kJ/fish); $\text{GE content}_{\text{initial}}$ = initial carcass energy (kJ/fish)

Nitrogen retention efficiency (NRE) and Energy retention efficiency (ERE) were calculated as:

$$\text{NRE (\% IN)} = 100 \times [(\text{FBW} \times \text{N content}_{\text{final}}) - (\text{IBW} \times \text{N content}_{\text{initial}})] / \text{IN}$$

$$\text{ERE (\% IE)} = 100 \times [(\text{FBW} \times \text{GE content}_{\text{final}}) - (\text{IBW} \times \text{GE content}_{\text{initial}})] / \text{IE}$$

Where: $\text{N content}_{\text{final}}$ = nitrogen content (%) of the final carcass sample; $\text{N content}_{\text{initial}}$ = nitrogen content (%) of the initial carcass sample; $\text{GE content}_{\text{final}}$ = GE content (kJ/g fish) of the final carcass sample; $\text{GE content}_{\text{initial}}$ = GE content (kJ/g fish) of the initial carcass sample;

IN = nitrogen ingested (g/fish); IE = energy ingested (KJ/fish).

Fold-change expression of inflammatory gene mRNA was calculated through the relative CT method:

$$F = 2^{-\Delta\Delta\text{CT}} \quad \Delta\Delta\text{CT} = \Delta\text{CT}_{\text{test sample}} - \Delta\text{CT}_{\text{calibrator sample}}$$

Where: F = fold-change; $\Delta\Delta\text{CT}$ = difference in CT between the test and control sample of the examined gene normalized to a housekeeping gene; $\Delta\text{CT}_{\text{test sample}}$ = difference in CT between the test and control sample of the examined gene; $\Delta\text{CT}_{\text{calibrator sample}}$ = difference in CT between the test and control sample of the housekeeping gene; CT = number of cycles required to meet threshold fluorescence.

3.3.6 – Statistical analysis

All data were analyzed as a complete randomized block design according to tank row and experimental diet using the MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Data on weight gain (BWG), growth rate (TGC), feed efficiency (FE), feed intake, nitrogen deposition rate (NDR), energy deposition rate (EDR), nitrogen retention efficiency (NRE), energy retention efficiency (ERE), hepatosomatic index (HSI), spleen somatic index (SSI), mRNA expression of select genes, and body proximate conditions were analyzed using linear and quadratic orthogonal polynomial contrasts. Contrasts were assigned to compare the effects of graded levels of either solvent-extracted soybean meal or solvent-extracted sunflower meal on the aforementioned parameters between two genetic strains of Arctic charr. For applicable variables, a second set of contrasts were assigned to uncover any linear or quadratic effects at graded levels of either plant protein ingredient within genetic strain. The significance level was $P < 0.05$, with variables displaying significance level of $P < 0.1$ designated as trends. Data are presented as tank means \pm 1SEM (N) (N=tank average at experimental diet).

3.4 – Results

3.4.1 – Growth performance

3.4.1.1 – Between Strains

Growth data for body weight gain (BWG), feed efficiency (FE), growth rate (TGC), and feed intake are shown in Table 3.3 with associated growth curves for Strain 1 and Strain 2 Arctic charr shown in Figure 3.1. There was not a significant effect of strain, ingredient, level, or any interactions on BWG (Table 3.3). There were also no significant differences seen in the linear or quadratic contrasts between strains with increasing inclusion levels of SBM or SFM. Growth curves show remarkably similar trajectories when comparing experimental diets within genetic strain, and when comparing trends seen between Strain 1 and Strain 2 (Figure 3.1). Although no differences were observed in contrasts analysis between strains for the TGC, there was a significant effect ($P < 0.05$) of inclusion level of plant ingredients, whereby higher plant protein inclusion level on average resulted in a depressed growth rate. The effect of increasing dietary SBM or SFM inclusion level on feed intake and feed efficiency (FE) did not differ either linearly or quadratically between strains. However, both of these parameters were significantly affected by strain ($P < 0.0001$), with Strain 1 displaying a pointedly higher feed intake paired with a lower FE than Strain 2 at all diets examined. FE also exhibited notable difference ($P < 0.0001$) with dietary level of SBM or SFM whereby a higher inclusion level generally resulted in less efficient feed conversion.

3.4.1.2 – Within Strain

Strong linear depression in FE with increasing inclusion level of both SFM and SBM ingredients were noted in both Strain 1 ($P < 0.001$ and $P < 0.01$ respectively) and Strain 2 ($P < 0.01$). Strain 1 exhibited negative linear trends in TGC for SBM ($P = 0.08$) and both linear and quadratic

trends in SFM diets ($P=0.06$) with increasing inclusion. Strain 2 did not display any linear or quadratic trends with level in either ingredient investigated. Size variation between conspecifics fed all experimental diets was noted in Strain 2, however not in Strain 1 and is noted in

3.4.2 – Nutrient deposition and retention

3.4.2.1 – Between Strains

The results of nitrogen and energy deposition rate (NDR, EDR) and retention efficiency (NRE, ERE) are shown in Table 3.4. There were no significant differences in the linear or quadratic contrasts with increasing dietary SBM or SFM inclusion between genetic strains. On average, NDR was negatively affected ($P<0.05$) by increasing the inclusion level of the examined plant ingredients. The energy deposition rate was significantly higher in Strain 1 than Strain 2 ($P<0.01$), while both the NRE and ERE were on average greater in Strain 2 than in Strain 1 ($P<0.001$). The NRE and ERE were also notably affected by the ingredient investigated ($P<0.01$). On average, Strain 2 retained a higher percentage of ingested nitrogen and energy than Strain 1, while fish fed diets with SFM retained a higher percentage of nitrogen and energy at any specified dietary inclusion level than those fed diets with SBM.

3.4.2.2 – Within Strain

Within Strain 1, tanks fed increasing inclusion of SBM demonstrated no effect on NRE, although a significantly linear reduction in ERE ($P<0.05$). A notable linear increase in NRE ($P<0.05$) was also observed in Strain 1 tanks fed SFM diets. Strain 2 presented no significant effects, however there was a linear trend ($P=0.06$) for increasing NRE with SFM inclusion.

3.4.3 – Organosomatic Indices

3.4.3.1 – Between Strains

Table 3.5 outlines the data for the hepatosomatic and the spleen somatic indices. Both the HSI and SSI were significantly affected ($P < 0.0001$) by genetic strain, with Strain 1 showing the highest average HSI and lowest average SSI. Fish fed diets with SFM had significantly higher HSI than those fed diets with SBM ($P < 0.01$). SSI was also significantly affected by plant ingredient inclusion level ($P < 0.0001$) with 10% inclusion resulting in the highest indices. All tanks fed diets with plant ingredient inclusion resulted in higher SSI values than were seen in tanks fed the control diet. Neither the HSI nor SSI showed noteworthy linear or quadratic differences between strains at increasing inclusion levels of either SBM or SFM ingredients.

3.4.3.2 – Within Strain

There were no noted significant linear or quadratic patterns in HSI Strain 1 Arctic charr fed either SBM or SFM experimental diets. SSI showed a significantly quadratic pattern ($P < 0.05$) in tanks fed SFM ingredients. Within Strain 2 charr, HSI was affected linearly by inclusion of SFM ($P < 0.05$) and displayed a similar increasing trend in SBM-fed tanks ($P = 0.05$). There was a notable linear rise in SSI within both SBM and SFM-based experimental diets in Strain 2 ($P < 0.05$) as well as quadratic trends for both ingredients ($P = 0.06$ and $P = 0.05$ respectively).

3.4.4 – Genetic Analysis

RT-qPCR-derived fold change data for select genes involved in the enteritic response within distal intestine and spleen tissues are shown in Tables 3.6 and 3.7 respectively. There was no significant effect of the linear or quadratic contrasts noted between strains within any gene examined in either tissue. In the distal intestine samples, there was a notable effect of ingredient

($P < 0.05$) on fold-change expression of PXR mRNA, where on average SFM diets resulted in reduced expression compared to SBM. Within spleen tissues, there appeared to be a significant effect of inclusion level ($P < 0.05$) on fold-change expression of IL-1 β , particularly in Strain 1 fish.

3.4.5 – Carcass Composition

3.4.5.1 – Between Strains

The linear or quadratic responses for all carcass proximate parameters did not show significant difference between strains in diets with either SBM or SFM ingredients at increasing inclusion levels (Table 3.8). Neither nitrogen nor ash content differed significantly by strain, ingredient, or inclusion level investigated. Moisture, lipid content, and GE content showed significant difference ($P < 0.0001$) between genetic strains. On average, Strain 1 fish had 1-2% greater lipid content, 1-2% lesser moisture content, and a notably higher GE content than Strain 2 fish. Moisture, lipid, and GE were also affected by the plant ingredient investigated and dietary inclusion level. Moisture was lowest ($P < 0.05$) in fish fed diets with SFM compared to SBM, while the opposite trend was seen in lipid content ($P < 0.01$). Following the pattern in lipid content, calculated GE was also significantly higher ($P < 0.01$) in tanks fed SFM diets than in those fed SBM diets. On average, inclusion level of plant proteins within the experimental diets also significantly affected whole body moisture ($P < 0.05$), lipid ($P < 0.01$), and energy ($P < 0.01$) content in the Arctic charr.

3.4.5.2 – Within Strain

Strain 1 Arctic charr showed a negative linear pattern in corporeal moisture content ($P < 0.05$) as well as a linear trend for elevated lipid ($P = 0.09$) with increasing levels of dietary SFM. Strain 2 displayed significantly reduced moisture ($P < 0.01$) and energy content ($P = 0.05$), as well

as increased lipid content ($P < 0.01$) with inclusion level in SFM-fed tanks. There were no significant effects of SBM inclusion on carcass parameters in Strain 2 charr.

Table 3.3 – Growth, feed intake and feed efficiency (FE) ratio of two strains of Arctic charr (initial average weight = 8.8g/fish) fed the experimental diets for 84 days, T_{avg}=12.4°C.

	Gain g/fish	TGC^a g^{1/3}/°C*day	Feed Intake^b g/fish	FE gain/feed
<i>Strain 1</i>				
Diet A, Control	53.1	0.182	44.0	1.17
Diet B, 10% SBM	54.4	0.181	47.0	1.12
Diet C, 20% SBM	50.5	0.177	46.5	1.08
Diet D, 10% SFM	50.1	0.175	45.1	1.11
Diet E, 20% SFM	50.8	0.177	45.7	1.11
<i>Significance^c</i>				
Linear SBM	N.S. ^e	P=0.08	N.S.	P<0.001
Quadratic SBM	N.S.	N.S.	N.S.	N.S.
S.E.M. ^d	1.8	0.002	1.8	0.01
Linear SFM	P=0.06	P=0.06	N.S.	P<0.01
Quadratic SFM	P=0.08	P=0.06	N.S.	P<0.05
S.E.M.	0.7	0.001	1.7	0.01
<i>Strain 2</i>				
Diet A, Control	52.6	0.181	39.6	1.34
Diet B, 10% SBM	50.6	0.177	40.4	1.25
Diet C, 20% SBM	47.5	0.169	38.8	1.21
Diet D, 10% SFM	47.0	0.169	38.1	1.23
Diet E, 20% SFM	50.9	0.178	40.9	1.25
<i>Significance^c</i>				
Linear SBM	N.S.	N.S.	N.S.	P<0.01
Quadratic SBM	N.S.	N.S.	N.S.	N.S.
S.E.M.	2.1	0.004	1.7	0.02
Linear SFM	N.S.	N.S.	N.S.	P<0.01
Quadratic SFM	N.S.	N.S.	N.S.	P<0.05
S.E.M.	2.8	0.006	2.2	0.01
<i>Between Strains</i>				
<i>Significance^f</i>				
Linear	N.S.	N.S.	N.S.	N.S.
Quadratic	N.S.	N.S.	N.S.	N.S.
S.E.M. ^d	1.9	0.004	1.7	0.01
<i>Effects of</i>				
Strain	N.S.	N.S.	P<0.0001	P<0.0001
Ingredient	N.S.	N.S.	N.S.	N.S.
Level	N.S.	P<0.05	N.S.	P<0.0001
Level*Ingredient	N.S.	N.S.	N.S.	N.S.

Strain*Level	N.S.	N.S.	N.S.	P<0.05
Strain*Ingredient*Level	N.S.	N.S.	N.S.	N.S.

^a TGC = thermal-unit growth coefficient.

^b Feed intake measured in g dry matter.

^c Significance = significance of the orthogonal linear and quadratic contrasts of dependent variables across inclusion level

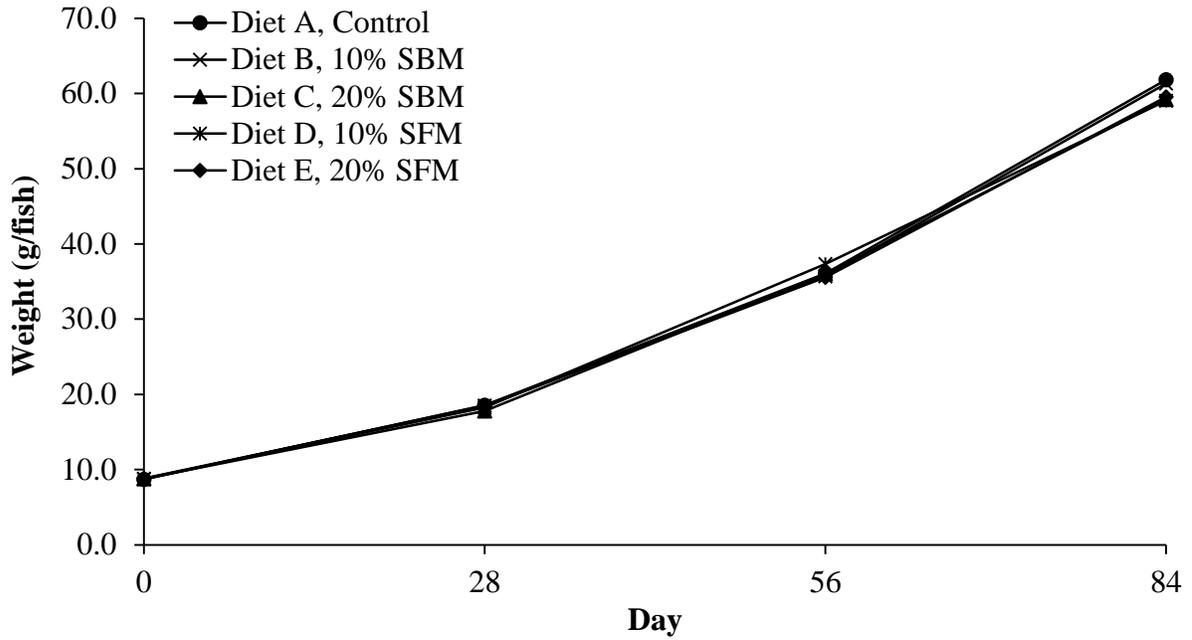
Strain*Ingredient*Level interaction.

^d S.E.M. = Standard error of the mean.

^e N.S. = Not statistically significant ($P \geq 0.05$).

^f Significance of the orthogonal linear and quadratic contrasts of dependent variables across the Strain*Ingredient*Level interaction

A.



B.

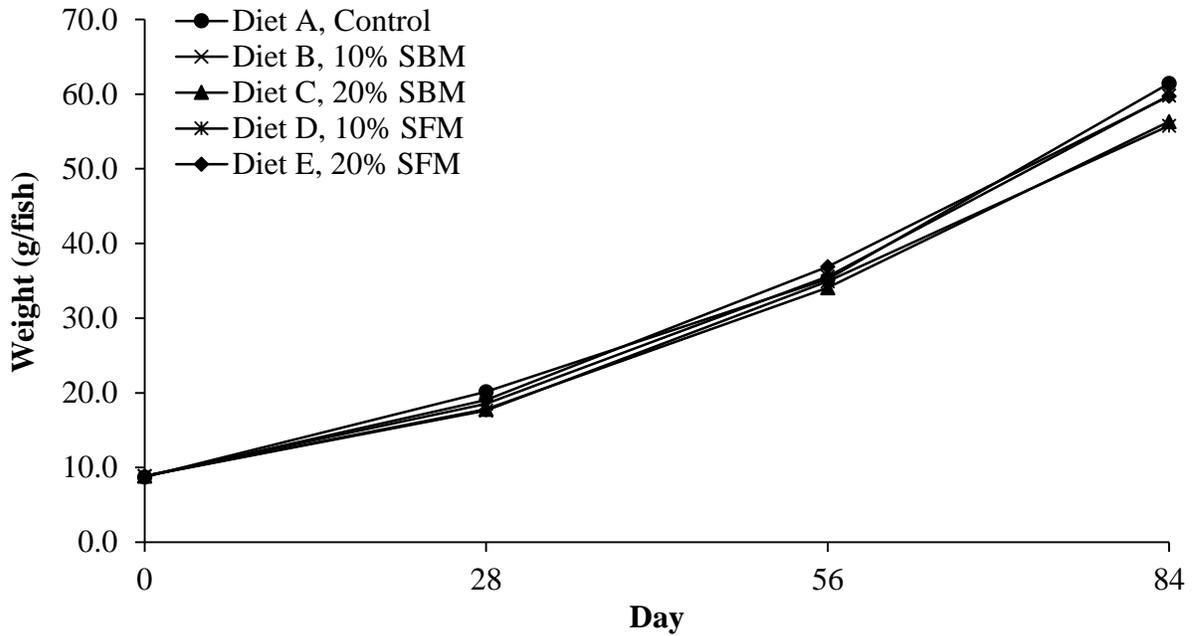


Figure 3.1- Growth curves of Strain 1 (initial average weight = 8.7g/fish) (A) and Strain 2 (B) Arctic charr (initial average weight = 8.8g/fish) fed the experimental diets (n=3 for each diet).

Table 3.4 – Nitrogen deposition rate (NDR), energy deposition rate (EDR), nitrogen retention efficiency (NRE), and energy retention efficiency (ERE) of two strains of Arctic charr (initial average weight = 8.8g/fish) fed the experimental diets for 84 days, T_{avg}=12.4°C.

	NDR <i>mg/fish/°C*day</i>	EDR <i>KJ/fish/°C*day</i>	NRE <i>% intake</i>	ERE <i>% intake</i>
<i>Strain 1</i>				
Diet A, Control	1.33	0.46	33.6	42.0
Diet B, 10% SBM	1.29	0.45	32.5	41.7
Diet C, 20% SBM	1.29	0.43	33.4	39.8
Diet D, 10% SFM	1.26	0.45	35.2	42.1
Diet E, 20% SFM	1.24	0.46	35.8	41.6
<i>Significance^a</i>				
Linear SBM	N.S. ^c	P=0.09	N.S.	P<0.05
Quadratic SBM	N.S.	N.S.	P=0.05	N.S.
S.E.M. ^b	0.02	0.01	0.3	0.4
Linear SFM	P=0.06	N.S.	P<0.05	N.S.
Quadratic SFM	N.S.	N.S.	N.S.	N.S.
S.E.M.	0.02	0.01	0.5	0.8
<i>Strain 2</i>				
Diet A, Control	1.33	0.41	39.2	45.1
Diet B, 10% SBM	1.28	0.41	37.1	43.8
Diet C, 20% SBM	1.23	0.39	37.9	42.7
Diet D, 10% SFM	1.18	0.41	38.8	45.3
Diet E, 20% SFM	1.25	0.45	40.7	46.0
<i>Significance^a</i>				
Linear SBM	N.S.	N.S.	N.S.	N.S.
Quadratic SBM	N.S.	N.S.	N.S.	N.S.
S.E.M.	0.06	0.02	0.8	0.8
Linear SFM	N.S.	N.S.	P=0.06	N.S.
Quadratic SFM	N.S.	N.S.	N.S.	N.S.
S.E.M.	0.06	0.03	0.4	0.7
<i>Between Strains</i>				
<i>Significance^d</i>				
Linear	N.S.	N.S.	N.S.	N.S.
Quadratic	N.S.	N.S.	N.S.	N.S.
S.E.M. ^f	0.04	0.02	0.6	0.6
<i>Effects of</i>				
Strain	N.S.	P<0.01	P<0.001	P<0.001
Ingredient	N.S.	N.S.	P<0.01	P<0.01

Level	P<0.05	N.S.	N.S.	N.S.
Level*Ingredient	N.S.	N.S.	P<0.05	P<0.05
Strain*Level	N.S.	N.S.	N.S.	N.S.
Strain*Ingredient*Level	N.S.	N.S.	N.S.	N.S.

^a Significance = significance of the orthogonal linear and quadratic contrasts of dependent variables across inclusion level

^b S.E.M. = Standard error of the mean.

^c N.S. = Not statistically significant or a trend ($P \geq 0.1$).

^d Significance = significance of the orthogonal linear and quadratic contrasts of dependent variables across the Strain*Ingredient*Level interaction.

Table 3.5 – Hepatosomatic index (HSI) and spleen somatic index (SSI) in two Arctic charr strains (initial average weight = 8.8g/fish) fed the experimental diets for 84 days, T_{avg}=12.4°C.

	HSI %BW^a	SSI %BW
<i>Strain 1</i>		
Initial	2.36	
Diet A, Control	2.03	0.16
Diet B, 10% SBM	2.05	0.20
Diet C, 20% SBM	1.97	0.19
Diet D, 10% SFM	2.06	0.21
Diet E, 20% SFM	1.99	0.18
<i>Significance^b</i>		
Linear SBM	N.S. ^d	N.S.
Quadratic SBM	N.S.	N.S.
S.E.M ^c	0.04	0.02
Linear SFM	N.S.	N.S.
Quadratic SFM	N.S.	P<0.05
S.E.M.	0.06	0.01
<i>Strain 2</i>		
Initial	2.12	
Diet A, Control	1.79	0.18
Diet B, 10% SBM	1.87	0.24
Diet C, 20% SBM	1.91	0.23
Diet D, 10% SFM	1.96	0.25
Diet E, 20% SFM	2.05	0.24
<i>Significance^b</i>		
Linear SBM	P=0.05	P<0.05
Quadratic SBM	N.S.	P=0.06
S.E.M.	0.03	0.01
Linear SFM	P<0.05	P<0.05
Quadratic SFM	N.S.	P=0.05
S.E.M.	0.04	0.01
<i>Between Strains</i>		
<i>Significance^e</i>		
Linear	N.S	N.S.
Quadratic	N.S.	N.S.
S.E.M. ^c	0.05	0.01
<i>Effects of</i>		
Strain	P<0.0001	P<0.0001

Level	N.S.	P<0.0001
Ingredient	P<0.05	N.S.
Level*Ingredient	N.S.	N.S.
Strain*Level	P<0.01	N.S.
Strain*Ingredient*Level	N.S.	N.S.

^aBW = wet weight (g)

^bSignificance = significance of the orthogonal linear and quadratic contrasts of dependent variables across inclusion level.

^cS.E.M. = Standard error of the mean.

^d N.S. = Not statistically significant or a trend ($P \geq 0.1$).

^e Significance = significance of the orthogonal linear and quadratic contrasts of dependent variables across the Strain*Ingredient*Level interaction.

Table 3.6 – Fold-change expression of pregnane X receptor (PXR) and interleukin –I beta (IL1 β) mRNA in the distal intestine of in two genetic strains of Arctic charr exposed to the experimental diets.

	PXR	IL1 β
<i>Strain 1</i>		
Diet A, Control	1	1
Diet B, 10% SBM	0.9	1.3
Diet C, 20% SBM	0.8	1.1
Diet D, 10% SFM	0.8	0.6
Diet E, 20% SFM	1.0	0.7
<i>Strain 2</i>		
Diet A, Control	1	1
Diet B, 10% SBM	1.5	1.0
Diet C, 20% SBM	1.2	1.3
Diet D, 10% SFM	0.9	1.3
Diet E, 20% SFM	1.2	1.3
<i>Significance^a</i>		
Linear	N.S. ^c	N.S.
Quadratic	N.S.	N.S.
S.E.M. ^b	0.2	0.2
<i>Effects of</i>		
Strain	N.S.	N.S.
Level	N.S.	N.S.
Ingredient	P<0.05	N.S.
Level*Ingredient	N.S.	N.S.
Strain*Level	N.S.	N.S.
Strain*Ingredient*Level	N.S.	N.S.

^aSignificance = significance of the orthogonal linear and quadratic contrasts of dependent variables across the Strain*Ingredient*Level interaction.

^bS.E.M. = Standard error of the mean.

^cN.S. = Not statistically significant or a trend (P \geq 0.1).

Table 3.7 – Fold-change expression of interleukin –I beta (IL1 β), nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ β) and cluster of differentiation 8 (CD8) mRNA in the spleen of two genetic strains of Arctic charr exposed to the experimental diets.

	IL1 β	NF- κ β	CD8
<i>Strain 1</i>			
Diet A, Control	1	1	1
Diet B, 10% SBM	1.7	0.9	1.1
Diet C, 20% SBM	2.3	1.0	0.9
Diet D, 10% SFM	1.3	0.9	1.0
Diet E, 20% SFM	1.5	1.0	1.1
<i>Strain 2</i>			
Diet A, Control	1	1	1
Diet B, 10% SBM	1.2	1.0	1.0
Diet C, 20% SBM	1.2	0.9	1.2
Diet D, 10% SFM	1.4	0.9	1.2
Diet E, 20% SFM	1.5	1.0	1.0
<i>Significance^a</i>			
Linear	N.S. ^c	N.S.	N.S.
Quadratic	N.S.	N.S.	N.S.
S.E.M. ^b	0.4	0.2	0.1
<i>Effects of</i>			
Strain	N.S.	N.S.	N.S.
Level	P<0.05	N.S.	N.S.
Ingredient	N.S.	N.S.	N.S.
Level*Ingredient	N.S.	N.S.	N.S.
Strain*Level	N.S.	N.S.	N.S.
Strain*Ingredient*Level	N.S.	N.S.	N.S.

^aSignificance = significance of the orthogonal linear and quadratic contrasts of dependent variables across the Strain*Ingredient*Level interaction.

^bS.E.M. = Standard error of the mean.

^c N.S. = Not statistically significant or a trend ($P \geq 0.1$).

Table 3.8 – Chemical body composition of the whole carcass of two strains of Arctic charr (initial average weight = 8.8g/fish) fed the experimental diets for 84 days, $T_{avg} = 12.4^{\circ}\text{C}$.

	H₂O %	CP^a %	Lipid %	Ash %	GE^b KJ/g
<i>Strain 1</i>					
Diet A, Control	69.2	16.2	12.3	2.5	8.6
Diet B, 10% SBM	69.1	15.9	12.4	2.5	8.7
Diet C, 20% SBM	69.1	16.4	12.0	2.6	8.6
Diet D, 10% SFM	68.6	16.1	12.8	2.4	8.9
Diet E, 20% SFM	68.4	15.8	13.2	2.4	9.0
<i>Significance^c</i>					
Linear SBM	N.S. ^e	N.S.	N.S.	N.S.	N.S.
Quadratic SBM	N.S.	P=0.09	N.S.	N.S.	N.S.
S.E.M. ^d	0.3	0.2	0.2	0.1	0.1
Linear SFM	N.S.	N.S.	P=0.09	N.S.	N.S.
Quadratic SFM	N.S.	N.S.	N.S.	P=0.07	N.S.
S.E.M.	0.5	0.3	0.3	0.0	0.2
<i>Strain 2</i>					
Diet A, Control	71.0	16.1	10.3	2.5	7.9
Diet B, 10% SBM	70.3	16.2	11.0	2.4	8.2
Diet C, 20% SBM	70.5	16.4	10.8	2.5	8.1
Diet D, 10% SFM	69.4	16.1	12.1	2.4	8.6
Diet E, 20% SFM	69.2	15.8	12.6	2.4	8.7
<i>Significance^c</i>					
Linear SBM	N.S.	N.S.	N.S.	N.S.	N.S.
Quadratic SBM	N.S.	N.S.	N.S.	N.S.	N.S.
S.E.M.	0.4	0.2	0.4	0.1	0.1
Linear SFM	P<0.05	N.S.	P<0.01	N.S.	P<0.01
Quadratic SFM	N.S.	N.S.	N.S.	N.S.	N.S.
S.E.M.	0.4	0.1	0.3	0.0	0.1
<i>Between Strains</i>					
<i>Significance^f</i>					
Linear	N.S.	N.S.	N.S.	N.S.	N.S.
Quadratic	N.S.	N.S.	N.S.	N.S.	N.S.
S.E.M.	0.4	0.2	0.4	0.1	0.1
<i>Effects of</i>					
Strain	P<0.0001	N.S.	P<0.0001	N.S.	P<0.0001
Ingredient	P<0.05	N.S.	P<0.01	N.S.	P<0.01
Level	P<0.05	N.S.	P<0.01	N.S.	P<0.01
Level*Ingredient	N.S.	P<0.05	P<0.05	N.S.	N.S.

Strain*Level	N.S.	N.S.	N.S.	N.S.	N.S.
Strain*Ingredient*Level	N.S.	N.S.	N.S.	N.S.	N.S.

^a CP = Crude protein.

^b GE =Gross energy.

^cSignificance = significance of the orthogonal linear and quadratic contrasts of dependent variables across inclusion level.

^d S.E.M. = Standard error of the mean.

^e N.S. = Not statistically significant or a trend ($P \geq 0.1$).

^fSignificance = significance of the orthogonal linear and quadratic contrasts of dependent variables across the Strain*Ingredient*Level interaction.

3.5 – Discussion

3.5.1 – Growth performance

No difference in final body weights within both genetic strains of Arctic charr with SBM or SFM inclusion suggests that Arctic charr juveniles are tolerant towards relatively high (20%) inclusion levels of these plant proteins in nutrient-dense diets. This is in agreement with studies in Atlantic salmon fed diets with similar macronutrient specs (50% CP, 20% lipid) as well as in rainbow trout, whereby inclusions of 30% and 45% of solvent-extracted SBM had no statistically significant effects on final body weight or specific growth rate respectively when compared to a control diet (Heikinnin et al, 2006; Weeks et al., 2010).

Overall average growth rate, measured as the thermal-unit growth coefficient was between 0.181 (practical diet) and 0.169 (20% SBM) within the present study. The growth rate from the practical diet is notably lower than those obtained by Simmons et al. (1999), as well as the CZRI (André Dumas, personal communication) in Canadian Arctic charr strains at a similar size, which were 0.212 and 0.230 respectively. The comparatively low TGC may have been a function of initial stress-induced and/or feed-borne saprolegniasis infection of experimental tanks, which occurred in the first month of the growth trial. However, there was no noted improvement in TGC value with successful treatment of the fungus. Tank density is a known constraint on growth in Arctic charr (Metusalech et al., 1999; Brännäs and Linnér, 2000;) with suggested stocking density of fingerlings between 40 – 150kg/m³ in effort to prevent stress due to conspecific aggression and cannibalism (Johnston, 2002). Initial average tank density in the present study was quite low (4.4kg/m³), approximately one half of that in the study by Simmons et al. (11kg/m³).

Although there were no differences in feed efficiency, feed intake, or body proximate composition between Strain 1 and Strain 2 Arctic charr with increasing levels of plant protein,

nearly all parameters were highly affected by the genetic strain examined. Several earlier studies have indicated that genetic strain has a potent effect on growth performance, and nutrient utilization, in salmonid fish species including the rainbow trout (Burton et al., 1983; Smith et al., 1988; Overturf et al., 2003) and the Arctic charr (Nilsson, 1993; Naslund and Henricson, 1996).

Feed efficiency was on average 12% greater in Strain 2 charr, however BWG was similar between strains, signifying the potential for a greater capacity of nutrient utilization in Strain 2 fish. In a similar study comparing five strains of juvenile rainbow trout fed a typical trout diet to satiation, there was significant variation in FE with genetic strain examined, ranging from 0.87 – 1.10 (Overturf et al., 2003). The discrepancy in FE in the rainbow trout resulted in noteworthy differences in weight gain between strains. In the current study, increased FE in Strain 2 fish had no bearing on BWG between strains because feed (N) intake was significantly lower in Strain 2. Instead, an elevated FE combined with a 2% lower carcass lipid content in Strain 2 compared to Strain 1 fish may point towards the process of protein sparing at the expense of lipids. The process of protein sparing by lipids is commonly seen in Atlantic salmon fed relatively low digestible protein:digestible energy diets as a method to reduce amino acid catabolism for metabolizable energy, and spare them for growth (Hillestad and Johnson, 1994; Azevedo et al, 2002; Azevedo et al., 2004). This process of protein sparing may have allowed for similar growth between strains even though overall nitrogen intake was much lower in Strain 2 fish. The diets within this study were formulated to meet the known nutrient requirements of juvenile rainbow trout according to the NRC (2011) with >50% CP and ~20% lipid and have DP:DE ratios of approximately 24g/MJ. However, poor composition of the SFM ingredient resulted in elevated dietary lipid and reduced protein content with additive inclusion of the ingredient, possibly reducing DP:DE ratios in SFM-

based diets. Investigation into the effects of reduced dietary DPDE ratios in Strain 2 Arctic charr is suggested to determine the strain's capacity for protein sparing.

Linear depression of FE and similar trends in TGC and BWG within ingredient investigated was noted with increasing inclusion level in both strains of Arctic charr. This is in complete agreement with several other studies outlining the well-known depressive effects of less processed plant proteins on growth performance in juvenile salmonids (Refstie et al., 1998; Francis et al., 2001; Mundheim et al., 2004). Relative to weight gain and growth rate however, Strain 1 seemed to be more tolerant of dietary plant protein addition than Strain 2, while exhibiting a similar linear decrease in FE when exposed to both ingredients. This is likely a result of individual variation in BWG (data not shown) skewing tank weight in Strain 2 and resulting in greater standard errors among experimental tanks. BWG variation between domesticated (10 generation) and undomesticated families has been examined in Atlantic salmon, whereby reduced genetic variation in relation to weight gain was noted in the domesticated families (Solberg et al., 2013). Additionally, these researchers noted that the more domesticated fish also presented with less sensitivity towards environmental stress. As such, it may be that lesser domestication of Strain 2 fish resulted in the noted BWG variation amongst conspecifics.

Soybean meal contains relatively high levels of plant antinutritional factors (ANF) compared to sunflower meal, of which have the ability to impede nutrient digestibility and directly affect intestinal physiology in salmonid fish (Francis et al., 2001; Mugford and Osborne, 2013). Research indicates that soybean meal elicits the strongest effects on growth performance in salmonid fish, and promotes ANF-induced intestinal inflammatory pathologies (Baeverfjord and Kroghdahl, 1996; Kroghdahl et al., 2003; Bakke, 2011). However, the present experiment presents that dietary addition of SBM and SFM ingredient resulted in similar effects on growth

performance, which may not be a result of ANF alone. In a comparable study examining the effect of increasing levels of solvent-extracted sunflower meal on fingerling rainbow trout, there appeared to be no significant effect on final body weight in diets with up to 36% SFM (32% CP) (Tacon et al., 1984). Poor composition of the sunflower meal in the present study (18% CP, 25% lipid, 58% total carbohydrate) compared to that utilized by Tacon et al (1984) resulted in reduced dietary CP and elevated carbohydrate content in SFM diets (Table 3.1). Cumulative increase in indigestible carbohydrate fractions (fibres, non-starch polysaccharides) and polyphenol concentration likely reduced specific AA digestibility. Thus diets with elevated SFM concentration may not have met the formulated specific AA requirements of juvenile rainbow trout which was utilized in this trial.

3.5.2 – Nutrient retention and deposition

Although Strain 1 and Strain 2 reacted similarly towards addition of SBM and SFM ingredients in terms of all nutrient parameters investigated, Strain 2 displayed on average a ~4% lower energy deposition rate (MJ/fish/°C*day), an approximate 5% higher nitrogen retention and 3% higher energy retention efficiency (% intake). Within salmonid species, nitrogen retention efficiency can differ dramatically, and is closely linked with dietary nitrogen content, overall intake and digestibility, and species or strain-specific capacity for alternate nutrients to be catabolized for energy directed towards metabolism. The presented NRE of Arctic charr grown from 9 – 200g fed a practical diet appears to be between that illustrated in Atlantic salmon (47%) and rainbow trout (30%) fed fishmeal-based diets (Resftie et al., 2000), with Strain 2 fish showing an NRE more similar to that of salmon. In contrast, the highest SBM addition in this study (20%) only resulted in very minor reduction (~2%) in NRE, more similar to that experienced by rainbow

trout in a study by Refstie et al. (2000). However, it is important to note that the study by Refstie et al. included 30% dietary SBM, while the current study investigated maximum 20% and this likely had a strong effect on the noted NRE in Atlantic salmon.

It is well-known that Atlantic salmon are more sensitive to the addition of dietary plant protein ingredients and their associated ANF contents than rainbow trout, displaying greater depression in apparent nutrient digestibility and retention and presenting with a more pronounced enteritis histopathology. As such, Arctic charr juveniles appear to be less sensitive towards the addition of SBM than Atlantic salmon. Whether the decreased sensitivity of NRE towards SBM addition in the Arctic charr is a result of enhanced nitrogen digestibility, or reduced enteritic pathology remains to be determined.

Comparable nitrogen (feed) intake between Strain 1 and Strain 2 tanks in this study combined with a lower energy deposition rate and body lipid content, suggests that the improved NRE in Strain 2 might be due to a protein sparing effect of lipid.

3.5.3 – Organo-somatic indices

The application of increased levels of carbohydrates in feeds targeted towards carnivorous fish species has been seen to result in an increase of liver size and weight as well as to induce increased hepatic lipogenesis (Kim and Kaushik, 1992; Alvarez et al., 2000; Kamalam et al., 2012). An increased HSI may be indicative of elevated hepatic lipogenic activity or of increased glycogen synthesis and deposition, thus elevated liver lipid or glycogen stores. In juvenile rainbow trout, upregulation of key enzymes in de novo lipid synthesis including glucose-6-phosphate dehydrogenase and fatty acid synthase is observed with increasing dietary carbohydrate fractions (Gélineau et al., 2002). Similarly in the present study, Strain 2 tanks fed SFM diets presented with

a significant increase in HSI and those fed SBM showed a similar trend with inclusion level, indicative of a pronounced effect of dietary indigestible carbohydrates on HSI. In a contrasting study with juvenile Atlantic salmon, SBM inclusion up to 30% in nutrient-dense diets did not elicit any significant changes in HSI (Weeks et al., 2010). These researchers believed that a constant HSI with high SBM inclusion was the result of protein sparing at the expense of the digestible carbohydrate fraction (gelatinized starch content). Unlike Atlantic salmon, Strain 2 Arctic charr may be unable to efficiently utilize the digestible carbohydrate fraction to spare proteins in neither SBM nor SFM-based diets. Interestingly, and much like the study by Weeks et al. (2010), the HSI and body lipid stores of Strain 1 fish did not change with inclusion of dietary SBM, proposing an enhanced ability of Strain 1 charr to utilize dietary carbohydrates. Strain 2 fish present as more susceptible to the either the plant carbohydrate-mediated increase in lipogenic activity or to enhanced hepatic glycogen storage than Strain 1. However, Strain 2 fish also possess a slight, but significantly lower average HSI than Strain 1 at all experimental diets. This may indicate either a reduced capacity for hepatic lipogenesis, or an increased capacity or efficiency of fatty acid catabolism (β -oxidation) in Strain 2 compared to Strain 1 charr, which is in line with the proposed protein sparing by lipids in this strain.

Spleen somatic index (ratio of spleen size to body size) can be an indication of relative immune function in teleost fish, and will generally increase with exposure to blood-borne bacterial pathogens (Hadidi et al., 2013). Non-infectious subacute enteritis in salmonids results in significant pathological changes to the distal intestine as a result of plant ANF, including, but not limited to a reduction in epithelial tissue integrity. Increased porosity, as well as disassociation of epithelial enterocytes can result in increased transcellular and paracellular infiltration of normally unabsorbed items such as intestinal bacteria, and may ultimately affect spleen size. Increased

exposure to plant proteins, particularly in Strain 2 charr, resulted in linear increase in SSI. Similarly, elevated SSI in rainbow trout exposed to *F. psychrophilum* infection has been seen to be inversely correlated to mortality (Hadidi et al., 2013). However, splenectomy studies with trout infected with Bacterial Coldwater Disease have indicated that a reduction in spleen size does not necessarily result in associated reduction in survival and is more an indirect indicator of immune status (Weins et al., 2015). In the present study, mortality was below 3% in all diets with SFM and SBM diets in both strains eliciting similar mortality amongst tanks with no distinguishable pattern by means of increased inclusion (data not shown). Increased spleen size in Strain 2 charr had no bearing on mortality, as no differences were noted between strains. Thus, this may point toward an elevated baseline production of immunological cells in Strain 2 compared to Strain 1 fish, likely a result of multi-generational selection pressure. Increased sensitivity of Strain 2 fish towards plant proteins could possibly be a result of a greater effect of plant ANF on intestinal integrity compared to Strain 1, or of greater immune response towards similar levels of blood-borne pathogens.

3.5.4 – Inflammatory mRNA expression

Within both spleen and distal intestine tissues, there was no indication of differential expression of pro-inflammatory mRNA between strains of charr after 84 days of exposure to SBM or SFM based diets. In contrast, in a similar study with Atlantic salmon (500-600g) fed 20% dietary SBM, rt-qPCR analysis of distal intestine tissue showed significant upregulation of genes involved in apoptosis, cellular repair and differentiation, and stress response, including a precursor to NF- κ B (Sahlmann et al., 2013). In a second study investigating 270g Atlantic salmon fed diets with soyasaponin supplementation representing a level comparable to 20-30% SBM, mRNA expression of the P100 subunit of NF- κ B was elevated twofold (Kortner et al., 2012). Since select

immunological response towards dietary SBM and/or antinutrient content appears to be significantly elevated in Atlantic salmon, and to increase in severity in juvenile fish, it is interesting to see that charr examined in the present study show no alteration in expression, even at the highest inclusion level. Additionally, the extremely short time of observed upregulation in the study by Sahlmann et al. (7 days post-SBM introduction) and the continued increased expression at day 54 in the study by Kortner et al. indicates a chronic condition that would permit observable upregulation given the experimental time period of 84 days in the present study. However, as there was no observable alteration in expression it appears as though Arctic charr again show less sensitivity towards SBM ingredients compared to Atlantic salmon.

3.6 – Conclusions

Graded levels of soybean meal and sunflower meal from 0 – 20% fed to two genetic strains of Canadian Arctic charr resulted in no differences in linear or quadratic patterns in growth, feed efficiency, nutrient utilization, viscero-somatic indices, pro-inflammatory mRNA expression, or body composition with dietary inclusion. Although the contrasts were not significant, strain-level differences in feed efficiency, body composition, nutrient retention, and viscero-somatic indices were apparent. Strain 2 charr had significantly greater feed efficiency and nitrogen retention efficiency as well as lowered body lipid content, suggestive of protein sparing via an enhanced role of lipid utilization for energy in this strain. Furthermore, SBM ingredient addition produced enhanced deleterious effects compared to SFM, the majority of which were delegated primarily to Strain 2 fish.

Overall, the findings of this study indicate for the first time significant strain-level differences in the utilization of dietary macronutrients for growth in Canadian strains of Arctic

charr. Reduced HSI in Strain 2 compared to Strain 1 charr suggests a potential role of elevated hepatic β -oxidation in energy contribution towards metabolism in Strain 2, however this remains to be investigated. An elevated FE as a result of increased lipid utilization in Strain 2 charr may allow for future formulations of more economical diets targeted for Arctic charr. Given that Strain 2 presents with a significantly higher FE than Strain 1, and that Strain 1 appears to be more tolerant to plant protein ingredients than Strain 2, it is also suggested that a breeding program be implemented. Strain 2 represents a sterile hybrid of two commercial strains currently grown in Canada. Investigations into the parental strains comprising Strain 2 is necessary to determine which may possess genetic markers associated with the observed increase in FE and potential increase in lipid utilization observed in this study. If successful, future hybridization between Strain 1 and the parental strain aimed at combining their beneficial traits may produce a new, more economical strain for Canadian culture.

4 – THE EFFECTS OF SOYBEAN MEAL AND HIGH PROTEIN SUNFLOWER MEAL ON THE PERFORMANCE AND HEALTH OF A CANADIAN STRAIN OF ARCTIC CHARR (*SALVELINUS ALPINUS*) AT THE GROWER STAGE

4.1 – Abstract

The effects of graded levels (0-25% total dietary inclusion) of a typical solvent-extracted soybean meal (SBM) and high-protein sunflower meal (HPSFM) were examined in a Canadian strain of anadromous Arctic charr (*Salvelinus alpinus*) at the grower stage. Feeding diets with increasing inclusion of SBM or HPSFM for 12 weeks to charr (initial weight =330g/fish) resulted in no difference between SBM or HPSFM diets in body weight gain, growth rate (measured as the thermal-unit growth coefficient, TGC), feed efficiency, feed intake, or pro-inflammatory mRNA expression with increasing dietary inclusion. Significant differences in quadratic contrasts for nitrogen deposition rate (NDR) and nitrogen retention efficiency (NRE, % intake) ($P<0.05$) were observed, whereby HPSFM diets elicited the strongest positive response. Histological measurements of distal intestine simple fold lengths and widths revealed significantly linear differences ($P<0.001$ and $P<0.01$ respectively) between SBM and HPSFM fed fish. SBM resulted in notable reduction in both measurements ($P<0.0001$) with step-wise increases in dietary inclusion, and dietary HPSFM had no effect. Additionally, histological observations of individual villi indicate several, however not all classical symptoms of non-infectious subacute gastrointestinal enteritis in tanks fed SBM, many of which were not present in tanks fed HPSFM.

4.2 – Introduction

Within cultured salmonids, several legume, oilseed and grain species have been investigated and are increasingly utilized in nutrient-dense diets. Solvent-extracted soybean meal has been used in fish feeds since the mid-20th century, a result of availability, a consistent supply and quality, and a comparatively lower price point than marine-sourced protein ingredients. Soybean meal also has a good amino acid profile, a relatively high overall crude protein content, as well as a high apparent digestibility of crude protein in several salmonid species.

Certain plant protein ingredients including soybean meal contain chemical compounds often called antinutritional factors (ANF) which may result in significant reduction in overall growth performance, nutrient digestibility, and feed efficiency in salmonids, and thus limits dietary inclusion levels of select plant protein ingredients in feed formulations (Baeverfjord and Koghdahl, 1996; Francis et al., 2001; Refstie et al, 2001; Glencross et al., 2003; Kroghdahl et al., 2003; Sinha et al., 2011).

Gastrointestinal enteritis is an acute inflammatory condition which occurs primarily in the distal intestine of cultured salmonids fed diets with high levels of conventionally processed soybean ingredients. The condition is associated with several physiological changes to intestinal tissue, whereby classical pathophysiology includes shortening of intestinal villi, loss of enterocyte supranuclear absorptive vacuolization, mixed leukocyte infiltration of the lamina propria and submucosal tissues, and an increased proportion of endothelial mucosal cells (Van den Inge et al., 1991; Baeverfjord and Kroghdahl, 1996; Bakke, 2011). This physiology is paired with concurrent up or down-regulation of specific immune response genes, comprising those coding for general xenobiotic sensing molecules, pro-inflammatory or apoptotic cytokines and their receptors, inflammatory pathway transcription factors, and immune cell differentiation and maturation

(Hoffman and Baltimore, 2006; Wassmur et al., 2010; Kortner et al., 2012). Atlantic salmon and rainbow trout fed enhanced levels of full-fat or defatted soybean meal have displayed this pathology, which were closely associated with negative effects on growth performance and nutrient digestibility (Baeverfjord and Kroghdahl, 1996; Kroghdahl et al., 2003; Heikkinen et al., 2006; Merrifield et al., 2009).

Although certain ANF have been associated with the onset of the enteritis pathology, proper processing and value-added processing steps of plant ingredients have been shown to significantly reduce ingredient ANF content, and provide a more utilizable source of required nutrients in diets for cultured salmonids (Kaankuka et al., 1996; Arndt et al., 1999). Selection of initial dehulling, fat-extraction, and heat treatment processes have a significant effect on end-product ANF content and macronutrient specs, and may result in reduced growth performance depending on the species cultured, life stage, the initial ingredient ANF content, and its inclusion level (Rosa et al., 2009; Saez et al., 2015). Many plant proteins, including soybean and sunflower products can be further processed into protein concentrates and isolates, which improves their protein content and lowers their indigestible carbohydrate fraction, however also results in an ingredient with a much higher price point than those which are conventionally processed. In juvenile rainbow trout, soy protein concentrate (SPC) (>60% CP) with lysine and methionine supplementation replacing 75% of fishmeal protein did not negatively affect growth performance, or protein retention in a study by Kaushik et al. (1995). In a similar study in trout investigating complete step-wise replacement of fishmeal by SPC, >50% replacement of fishmeal resulted in reduced lipid digestibility and poor growth performance (Médale et al., 1998; Mambrini et al., 1999). Differences in the performance between studies may be attributed to many factors unrelated

to feed composition, however overall the higher degree of processing in SPC production allows for increased inclusion in salmonid diets.

The Arctic charr (*Salvelinus alpinus*) is a teleost fish species similar to the Atlantic salmon and rainbow trout, currently cultured mostly in Canada, Iceland and Sweden. Canadian production of this species is approximately 250 - 350 metric tonnes annually, representing 1.4 – 1.8 million USD. Although generally unidentified, life-stage specific nutrient requirements of charr are assumed to be similar to those of the rainbow trout and charr is currently fed commercially-available trout diets. However, the tolerance of grower stage Arctic charr towards both conventionally and additionally processed plant protein ingredients in salmonid aquafeeds is unknown, with little research investigating the potential effects of individual plant proteins or additionally processed ingredients on the performance and overall health of this species (Burr et al., 2011). As feed represents up to 60% of total production cost of Arctic charr, investigation into nutrient requirements and specific ingredient tolerance may represent a significant financial factor in the continuation of Canadian Arctic charr culture. Additionally, past studies have indicated significant differences in nutrient digestibility and growth performance between Arctic charr and other salmonids (Nilsson, 1993; Burr et al., 2011). Thus, the objectives of this study were to investigate and compare the effects of diets containing graded levels of commercially available solvent-extracted soybean meal and a further processed high-protein sunflower meal on growth, feed efficiency, feed intake, apparent digestibility, nutrient retention and utilization, pro-inflammatory mRNA expression, intestinal health, and carcass composition in a Canadian domesticated strain of Arctic charr at the grow-out stage.

4.3 – Materials and Methods

4.3.1 – Fish and experimental conditions

Fertilized eggs Arctic charr (*Salvelinus alpinus*) of diploid and triploid strains (Strain 1 and Strain 2) were obtained from Canadian Arctic charr hatcheries in January 2013. The fish were grown until February 2014 and thereafter distributed into 15 tanks of Strain 1 (average weight 330.1 ± 8.9 g) and 15 tanks of Strain 2 (average weight 290.2 ± 5.4 g) fish in a completely randomized block design according to tank row and experimental diet. Tank was considered the experimental unit. Fish were maintained in a re-circulating system (95% recirculated) composed of 850 L self-cleaning fiberglass tanks with a flow rate of 10L/min, with water temperature maintained at 10.0 ± 0.8 °C. Photoperiod was 12h light:12 h dark in a windowless laboratory setting. Fish were acclimated to the experimental conditions for one week prior to the start of the study. They were hand fed two times daily to satiation using a commercial salmon feed (Skretting 3.0mm, St. Andrews, NB) during the acclimation period.

Prior to the start of the experimental period, 20 fish of each strain were euthanized using a lethal dose of Tricaine methane sulfonate (200g/L water) and taken for hepatosomatic analysis. An additional 20 fish of each strain were pooled and taken for carcass composition and stored at -20°C until analysis. All protocols were accepted by the Canadian Council of Animal Care of the New Brunswick Community College (Caraquet, NB).

Every 28 days, fish were anaesthetized in 60mg/L MS-222, counted, and tank biomass recorded. Any animal with noted deformities during the weighing periods over the course of the experiment was removed and euthanized using a lethal dose of MS-222. Strain 2 was removed from the experimental conditions at day 56 due to poor growth performance as a result of inadequate initial tank density, and not included in the analysis. At day 84, 4 fish/tank of Strain 1

were taken for carcass analysis and 2 fish/tank for hepatosomatic and intestinal indices. These 2 fish were additionally sampled for distal intestine sections destined for molecular analysis. Animals for carcass analysis were stored at -20°C. Distal intestine tissues destined for genetic analysis were stored in RNAlater at -20°C.

4.3.2 – Experimental diets and feeding protocol

Five diets were formulated at the University of Guelph, ON. Diet A served as a practical trout diet, diets B and C contained 12.5% and 25% solvent extracted soybean meal, and diets D and E contained 12.5% and 25% high protein sunflower meal on an as-is basis (Table 3.1). All ingredients were mixed using a Hobart mixer (Hobart Ltd, Don Mills, ON, Canada). The five diets were steam pelleted to appropriate size (diameter= 2 mm, length= 2mm) using a laboratory pellet mill (hammer mill, screen size= 2 mm, die thickness= 44.5 mm, California Pellet Mill, San Francisco, CA, USA). Pellets were then dried overnight under forced-air at 60°C (Precision Scientific CO Cat N°: 1054), sieved to appropriate size, and stored at room temperature until used.

For the duration of the feeding trial, fish were hand fed twice daily at 8:00am and 4:00pm once a week, and feed distributed per tank calculated. This amount of feed was placed on 12-hr electronic feeding belts for the remaining 6 days of the week. Midway through the growth trial, a two-week digestibility trial was implemented whereby fish were hand fed once daily to apparent satiation at 8:00am.

Table 4.1 – Composition of the experimental diets.

Ingredients	Diet				
	A	B	C	D	E
	Practical Diet	12.5% SBM	25% SBM	12.5% HPSFM	25% HPSFM
Fish meal, herring (71% CP)	17.0	14.0	11.0	14.0	11.0
Poultry by-product meal (67% CP)	21.0	19.5	18.0	20.0	19.0
Feather meal (82% CP)	4.0	4.0	4.0	4.0	4.0
Soybean meal (46% CP)	-	12.5	25.0	-	-
High protein sunflower meal (47% CP)	-	-	-	12.5	25.0
Corn gluten meal (60% CP)	17.0	12.0	7.0	13.0	9.0
Fish oil	8.0	8.5	9.0	8.5	9.0
Canola oil	6.0	6.0	6.0	6.0	6.0
Wheat middlings	15.0	12.0	9.0	10.5	6.0
Arbocel (cellulose)	2.1	1.6	1.1	1.6	1.1
Vitamin premix	1.0	1.0	1.0	1.0	1.0
Mineral premix	1.0	1.0	1.0	1.0	1.0
Vitamin E	0.6	0.6	0.6	0.6	0.6
Lysine (BioLys)	2.1	2.1	2.0	2.0	1.9
DL-Met	0.03	0.09	0.15	0.10	0.17
choline chloride	0.3	0.3	0.3	0.3	0.3
Ca(H ₂ PO ₄) ₂	1	1.1	1.3	1.1	1.3
NaCl	2.0	2.0	2.0	2.0	2.0
Rovimixstay-C (25%)	0.3	0.3	0.3	0.3	0.3
Yttrium oxide	0.01	0.01	0.01	0.01	0.01
Total	100	100	100	100	100
Analyzed composition (dry matter basis)					
Dry matter (%)	96.7	96.7	96.7	96.0	95.9
Crude protein (%)	42.7	42.6	42.0	41.7	41.4
Lipid (%)	19.7	19.9	20.1	20.0	19.6
Carbohydrate (%)	24.1	24.4	25.2	24.6	25.5
Ash (%)	13.4	13.1	13.1	13.6	13.6
GE (kJ/g)	22.2	22.3	22.2	22.2	22.1

^aVitamin premix. Provided per kg of diet: retinyl acetate (vitamin A), 75 mg; cholecalciferol (vitamin D₃), 60 mg; menadione Na-bisulfate (vitamin K), 1.5 mg; cyanocobalamine (vitamin B₁₂), D-biotin, 210 mg; choline chloride, 3448 mg; folic acid, 1.5 mg; niacin, 15 mg; calcium-D-pantothenate, 33 mg; pyridoxine –HCl, 7.5 mg; riboflavin, 9 mg; thiamin-HCl, 1.5 mg.

^bMineral premix. Provided per kg of diet: sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; potassium iodine (KI, 24%K, 76%I), 10.5 mg; ferrous sulphate (FeSO₄, 7H₂O, 20%Fe), 65 mg; manganese sulphate (MnSO₄, 36%Mn) 88.9 mg; zinc sulphate (ZnSO₄.7H₂O, 40%Zn), 150 mg; copper sulphate (CuSO₄.5H₂O, 25%Cu), 28 mg; sodium Selenite (Na₂SeO₃, 45.66% Se), 0.7 mg.

4.3.3 – Chemical analysis

Dry matter (DM) and ash content were analyzed according to methods of AOAC (Association of Official Analytical Chemists) Official Methods (Horwitz, 2006). Crude protein (CP, %N x 6.25) was determined via a Kjeltec™ 8200 auto distillation unit (FOSS, Höganäs, Sweden) and lipid with an Ankom XT20 fat analyzer (Ankom Technology, Macedon, NY, USA). Gross energy (GE) content was calculated using mean GE values of carbohydrates (17.2kJ/g), proteins (23.6kJ/g), and lipids (39.5kJ/g) according to Blaxter (1989).

4.3.4 – Histological examination

At the end of the growth trial, 2 fish/tank were dissected and clinical examinations were performed. The mid-intestine (from the last pyloric caecae to the mid-distal intestine junction) and the distal intestine (from the mid-distal intestine junction to the anus) were excised, flushed with 10% formalin solution, freed of visceral fat and weighed. After weighing, a 1-2cm section of both the mid intestine and distal intestine were taken, immediately fixed in 10% formalin and stored at room temperature. The samples of distal intestine were later processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) by the Animal Health Laboratory of the Ontario Veterinary College (University of Guelph, ON, Canada) for examination.

Distal intestine segments were viewed under light microscopy at 400x magnification to determine villi measurements via OpenLab software. Villus length was measured from the stratum compactum, following the lamina propria to the villus terminus. Villus width was measured perpendicular to the lamina propria at the widest point of the villus. Twenty-four villi per experimental diet were measured with only undamaged simple villi selected for analysis.

4.3.5 – Genetic analysis

After weighing the distal intestine, a 0.5-1cm section of tissue was excised, placed in a 1.5mL tube of RNALater, and immediately frozen at -20°C for genetic analysis. Samples were shipped to Dr. Ken Overturf at the Hagerman Fish Culture Experiment Station, University of Idaho, USA. Tentative targeted gene products include cluster of differentiation 8 (CD-8), nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB), pregnane X receptor (PXR), and interleukin-I beta (IL1 β).

Total RNA was isolated from each sample using TRIzol® Reagent (Invitrogen) per the manufacturer's protocol and analyzed for quantity on a spectrophotometer (Nanodrop 2000, Wilmington, DE) and quality using the Agilent (Santa Clara, CA) 2100 Bioanalyzer Nano Kit. Potential DNA contamination was then removed using Amplification Grade DNase I (Invitrogen). To detect the level of gene expression, real-time quantitative RT-PCR was carried out using an ABI Prism 7900HT Sequence Detection System and the TaqMan One-Step RT-PCR Master Mix Reagents kit, according to the protocol provided by ABI (Foster City, CA). The final concentration of each reaction was: Master Mix, 1x (contains AmpliTaq Gold enzyme, dNTPs including dUTP, a passive reference, and buffer components); MultiScribe reverse transcriptase, 0.25 U/ μ l; RNase inhibitor mix, 0.4 U/ μ l; forward primer 600 nM; reverse primer 600 nM; probe, 250 nM; total RNA, 75 ng. Probes and primer sequences are listed in Table 4.2. Cycling conditions for genes tested were as follows: 30 m at 480C, 10 m at 95C, then 40 cycles of PCR consisting of 15 s at 95C followed by 1 m at 60C. For each gene, assays were run in triplicate on RNA samples isolated from individual fish. Raw cycle threshold (Ct) values were extracted from each run and normalized to *β -actin* using the delta-delta Ct method (Pfaffl, 2001).

Table 4.2 – Gene names, accession numbers and sequences.

Gene	Genebank accession no.	Primer/probe sequence (listed 5'-3')
<i>β-actin</i>	AF254414	BactinF: CCCTCTTCCAGCCCTCCTT BactinR: AGTTGTAGGTGGTCTCGTGGATA BactinMGB: 6FAM-CCGCAAGACTCCATACCGA-NFQ
<i>PXR</i>	EF517132	PXRF: GCAGATCCGCTTCAACATGATG PXRR: CGTCGTCCATGCAGTATGTCA PXRMGB: 6FAM- CACTCCCAGATGCC -NFQ
<i>NFκβ</i>	CA356763	NFKB: TGACAAAGGCATCTGCATCACA NFKB: CATGGAGGATGCCCAGGTT NFKBMGB: 6FAM-CTGATGCTGGAGTCTTT-NFQ

4.3.6 – Calculations

Growth rate was measured as a function of the thermal-unit growth coefficient (TGC).

$$TGC = 100 \times [(FBW^{1/3} - IBW^{1/3})/\Sigma(T \times D)]$$

Where: FBW = final average body weight (g/fish); IBW = initial body weight (g/fish);

$\Sigma(T \times D)$ = sum of temperature (°C) x days.

Feed efficiency (FE) was calculated for each tank as:

$$FE = \text{live weight gain (g)/dry feed intake(g)}$$

Where: live weight gain = (tank FBW(g)/number of fish – tank IBW(g)/number of fish); dry feed intake (g) = tank dry feed intake (g)/number of fish.

Hepatosomatic Index (HSI) and intestinal somatic index (ISI), the ratio of distal intestine weight: total body weight were calculated as

$$HSI = \text{liver wet weight (g)/live body weight (g)}$$

$$ISI = \text{distal intestine wet weight (g)/live body weight (g)}$$

Where: liver wet weight included full gall bladders in weight measurement.

Nitrogen deposition rate (NDR) and energy deposition rate (EDR) were calculated as:

$$\text{NDR} = (\text{N content}_{\text{final}} - \text{N content}_{\text{initial}}) / (\text{T} \times \text{D})$$

$$\text{EDR} = (\text{GE content}_{\text{final}} - \text{GE content}_{\text{initial}}) / (\text{T} \times \text{D})$$

Where: $\text{N content}_{\text{final}}$ = final carcass nitrogen (mg/fish); $\text{N content}_{\text{initial}}$ = initial carcass nitrogen (mg/fish); D = number of days; T = average temperature ($^{\circ}\text{C}$).

GE_{final} = final carcass energy (kJ/fish); $\text{GE content}_{\text{initial}}$ = initial carcass energy (kJ/fish)

Nitrogen retention efficiency (NRE) and Energy retention efficiency (ERE) were calculated as:

$$\text{NRE (\% IN)} = 100 \times [(\text{FBW} \times \text{N content}_{\text{final}}) - (\text{IBW} \times \text{N content}_{\text{initial}}) / \text{IN}]$$

$$\text{ERE (\% IE)} = 100 \times [(\text{FBW} \times \text{GE content}_{\text{final}}) - (\text{IBW} \times \text{GE content}_{\text{initial}}) / \text{IE}]$$

Where: $\text{N content}_{\text{final}}$ = nitrogen content (%) of the final carcass sample; $\text{N content}_{\text{initial}}$ = nitrogen content (%) of the initial carcass sample; $\text{GE content}_{\text{final}}$ = final carcass gross energy content (kJ/g fish); $\text{GE content}_{\text{initial}}$ = initial carcass gross energy content (kJ/g fish); IN = nitrogen ingested (g/fish); IE = energy ingested (KJ/fish).

4.3.7 – Statistical analysis

All data were analyzed as a completely randomized block design using the MIXED procedure of SAS (SAS version 9.4, SAS Institute Inc., Cary, NC, USA). Pre-planned contrasts were used to compare the responses in Arctic charr by different parameters to graded levels of either solvent extracted soybean meal (SBM) or a novel high protein sunflower meal (HPSFM) inclusion. Tank means of weight gain, thermal-unit growth coefficient (TGC), feed intake, feed efficiency (FE), nitrogen deposition rate (NDR), energy deposition rate (EDR), nitrogen retention efficiency (NRE), energy retention efficiency (ERE), body composition, hepatosomatic index (HSI), intestinal somatic index (ISI), villus length and width, pro-inflammatory mRNA expression,

and chemical body composition for tanks fed all diets were analyzed using linear and quadratic orthogonal polynomial contrasts. The significance level was $P < 0.05$, with variables displaying $P < 0.1$ designated as trends. Data are presented as tank means $\pm 1\text{SEM}$ (N) (N=tank average).

4.4 – Results

4.4.1 – Growth performance

Table 4.3 outlines the growth data for Strain 1 Arctic charr. The associated growth curve is shown in Figure 4.1. Feeding Diet B (12.5% SBM), C (25% SBM), and E(25% HPSFM) resulted in a 40g lower average weight gain per fish than either Diet A (control) or Diet E (Fig. 4.1). There were no significant differences in the linear or quadratic response in any of the examined growth parameters with increasing levels of dietary inclusion comparing SBM to HPSFM diets (Table 4.3). However, on average the tanks fed diets with HPSFM had significantly higher growth rates ($P<0.05$). As well as this, on average BWG and TGC were seen to be significantly depressed with increasing dietary inclusion of plant protein ingredients ($P<0.05$) except in tanks fed diets with 12.5% HPSFM inclusion.

4.4.2 – Nutrient deposition and retention

Data for nitrogen and energy deposition rates and retention efficiencies are shown in Table 4.4. Quadratic contrasts comparing trends in NDR and NRE (% digestible nitrogen intake) with increasing dietary inclusion of SBM and HPSFM were significant ($P<0.05$). Fish fed HPSFM diets showed significantly higher NRE than those fed SBM ($P<0.05$). NRE was also affected by inclusion level, and the ingredient*level interaction ($P<0.05$) in which a 12.5% inclusion level of HPSFM appeared to markedly enhance nitrogen retention compared to both examined inclusion levels of SBM or 25% HPSFM. In accordance with these results, additional contrast analysis for HPSFM diets determined that both NRE (% intake) and NDR showed clear quadratic trends with increasing dietary inclusion ($P<0.05$).

4.4.3 – Organo-somatic indices and histological examination

The organosomatic indices and distal intestine villi measurements are shown in Table 4.5 and 4.6 respectively. Liver indices were not affected by type of plant protein examined, however tended to be higher in HPSFM diets (D, E) than in SBM diets (B, C). Additionally, HSI appeared to be significantly affected by inclusion level of plant-sourced ingredients ($P < 0.01$). Linear and quadratic contrasts did not indicate any dissimilarities in HSI between HPSFM and SBM ingredients at increasing dietary levels.

Distal intestine (DI) data indicated significant linear difference in organ weight (%BW) between tanks fed SBM and HPSFM-based diets with increasing inclusion level ($P < 0.01$) (Fig. 4.3). Contrast analysis for tanks fed SBM diets showed significantly linear decrease ($P < 0.001$) in DI ratios with increasing dietary inclusion, while those fed HPSFM diets were unaffected. Significant effects were also observed with plant ingredient examined ($P < 0.001$), whereby, on average, fish fed HPSFM had markedly higher DI ratios. Secondary contrasts showed a significantly linear depression ($P < 0.0001$) with elevated inclusion level in tanks fed SBM diets, however the same trend was not observed with HPSFM diets.

Villi length and width measurements (Table 4.6) also indicate clearly significant linear contrasts ($P < 0.001$, $P < 0.01$ respectively) between the two investigated ingredients at increasing dietary inclusion. Tanks fed diets with stepwise addition of SBM exhibited notably decreasing villi lengths and widths, which are indicated by significantly linear contrasts ($P < 0.0001$) compared to those fed HPSFM diets (Figure 4.4). Significant effects of ingredient and inclusion ($P < 0.0001$) were both apparent, whereby on average fish fed SBM diets, and those fed the highest inclusion levels displayed decreased villi width and length measurements (Table 4.8).

Images of distal intestine villi representing fish fed Diets A – E are displayed in Figures 4.2 – 4.6. Notable reduction in the number of enterocyte supranuclear absorptive vacuoles, slightly increased villus infiltration of polymorphonuclear leukocytes (PMN), and observable reduction in villus length was seen in distal intestine samples of tanks fed increasing levels of SBM. In tanks fed HPSFM diets, PMN were not visibly elevated however the formation of enlarged absorptive vacuoles, was noted at villi termini, particularly at the highest inclusion level (25%).

4.4.4 – Genetic analysis

Fold-changes relative to β -actin in the distal intestine of Strain 1 Arctic charr are shown in Table 4.7. There were no significant differences in the linear or quadratic orthogonal contrasts of PXR or NF- κ B between SBM or HPSFM ingredients at increasing inclusion level. However, NF- κ B data indicated a significant increase ($P < 0.05$) in expression with inclusion level of plant protein ingredients, a pattern which was not observed with PXR.

4.4.5 – Carcass composition

Carcass dry matter, crude protein, lipid, ash and gross energy values are given in Table 4.8. There was no significance associated to the linear or quadratic contrasts comparing trends in carcass nutrient composition between tanks fed SBM and HPSFM diets. Neither the plant protein, the dietary inclusion level, nor the interaction seemed to cause any marked variation in carcass proximate composition in Strain 1 Arctic charr.

Table 4.3 – Growth, feed intake (FI) and feed efficiency (FE) of Strain 1 Arctic charr (initial average weight = 330±9g /fish) fed the experimental diets for 84 days at T_{avg} = 10°C.

	Gain <i>g/fish</i>	TGC^a <i>g^{1/3}/°C*day</i>	FI^b <i>g/fish</i>	FE <i>gain/feed</i>
Diet A, Control	402	0.253	425	0.95
Diet B, 12.5% SBM	371	0.239	391	0.93
Diet C, 25% SBM	360	0.233	419	0.85
Diet D, 12.5% HPSFM	402	0.249	421	0.98
Diet E, 25% HPSFM	371	0.242	397	0.94
<i>Significance^c</i>				
Linear SBM	P<0.05	P<0.01	N.S.	P=0.09
Quadratic SBM	N.S. ^f	N.S.	N.S.	N.S.
S.E.M.	10	0.003	23	0.03
<i>Significance^d</i>				
Linear HPSFM	N.S.	N.S.	N.S.	N.S.
Quadratic HPSFM	N.S.	N.S.	N.S.	N.S.
S.E.M.	11	0.006	16	0.03
<i>Significance^d</i>				
Linear	N.S.	N.S.	N.S.	N.S.
Quadratic	N.S.	N.S.	N.S.	N.S.
S.E.M. ^e	10	0.005	19.3	0.03
<i>Effects of</i>				
Ingredient	N.S.	P<0.05	N.S.	N.S.
Level	P<0.05	P<0.05	N.S.	N.S.
Level*Ingredient	N.S.	N.S.	N.S.	N.S.

^a TGC = thermal unit growth coefficient.

^b Feed intake measured in g dry matter.

^c Significance= significance of the orthogonal linear and quadratic contrasts of dependent variables across inclusion level.

^d Significance= significance of the orthogonal linear and quadratic contrasts of dependent variables across the Ingredient*Level interaction.

^e S.E.M.= Standard error of the mean.

^f N.S.=Not statistically significant (P≥0.05).

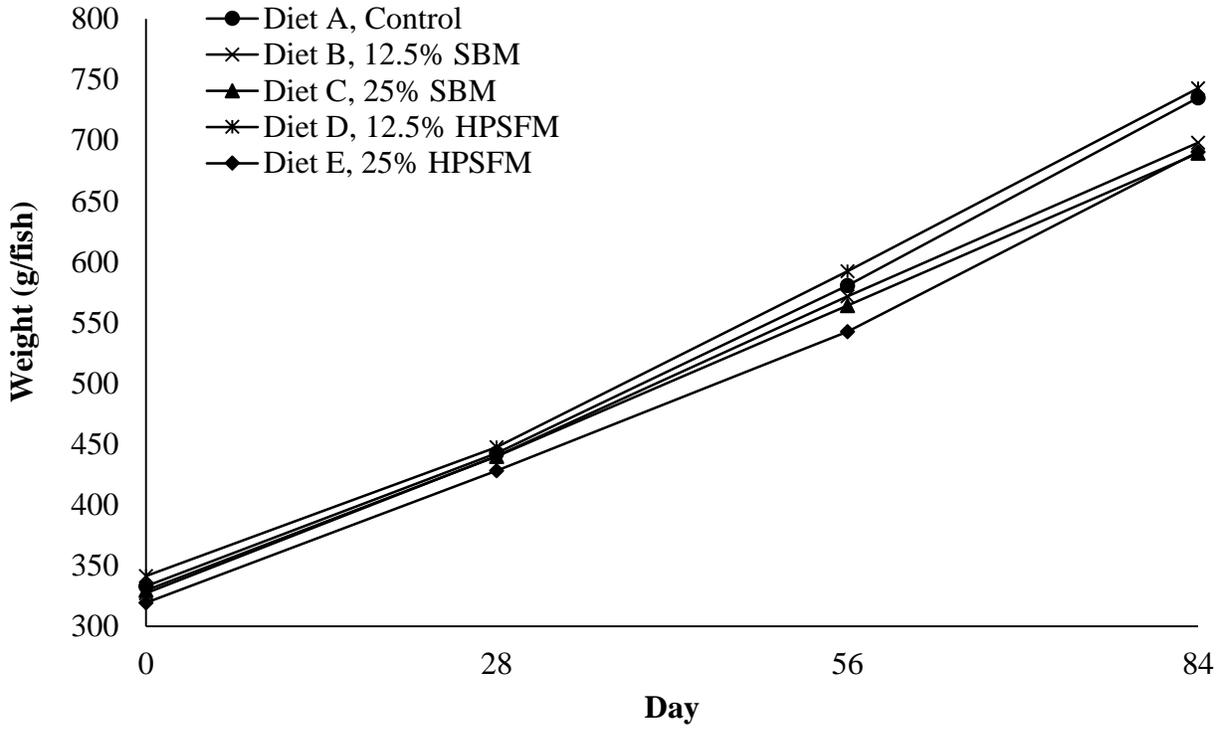


Figure 4.1 – Growth curves of Strain 1 Arctic charr (initial average weight = 330.1±8.9g /fish) fed the experimental diets for 84 days (n=3 for each diet).

Table 4.4 – Nitrogen deposition rate (NDR), energy deposition rate (EDR), nitrogen retention efficiency (NRE), and energy retention efficiency (ERE) of Strain 1 Arctic charr (initial average weight = 330.1±8.9g/fish) fed the experimental diets for 84 days, T_{avg} = 10°C.

	NDR <i>mg/fish/°C*day</i>	EDR <i>kJ/fish/°C*day</i>	NRE <i>% intake</i>	ERE <i>% intake</i>
Diet A, Control	10.3	4.2	30.0	36.5
Diet B, 12.5% SBM	10.4	4.5	33.2	42.8
Diet C, 25% SBM	11.2	4.2	33.7	37.3
Diet D, 12.5% HPSFM	12.4	4.9	38.8	44.1
Diet E, 25% HPSFM	10.5	4.4	34.4	41.9
<i>Significance^a</i>				
Linear SBM	N.S. ^d	N.S.	N.S.	N.S.
Quadratic SBM	N.S.	N.S.	N.S.	N.S.
S.E.M ^b	0.7	0.3	0.7	2.2
Linear HPSFM	N.S.	N.S.	N.S.	N.S.
Quadratic HPSFM	P<0.01	N.S.	P<0.05	N.S.
S.E.M	0.3	0.3	1.3	1.6
<i>Significance^c</i>				
Linear	N.S.	N.S.	N.S.	N.S.
Quadratic	P<0.05	N.S.	P<0.05	N.S.
S.E.M.	0.5	0.3	1.1	1.7
<i>Effects of</i>				
Ingredient	N.S.	N.S.	P<0.05	N.S.
Level	N.S.	N.S.	P<0.05	N.S.
Level*Ingredient	N.S.	N.S.	P<0.05	N.S.

^a Significance = significance of the orthogonal quadratic contrasts of the dependent variables across inclusion level.

^b S.E.M. = Standard error of the mean.

^c Significance = significance of the orthogonal linear and quadratic contrasts of dependent variables across the Ingredient*Level interaction.

^d N.S. = Not statistically significant or a trend (P_≥ 0.1).

Table 4.5 – Hepatosomatic index (HSI) and distal intestine index in Strain 1 Arctic charr fed the experimental diets for 84 days, T_{avg} = 10°C.

	HSI %BW^a	Distal intestine %BW
Diet A, Control	1.64	0.60
Diet B, 12.5% SBM	1.38	0.44
Diet C, 25% SBM	1.33	0.36
Diet D, 12.5% HPSFM	1.43	0.65
Diet E, 25% HPSFM	1.55	0.65
<i>Significance^b</i>		
Linear SBM	P=0.08	P<0.001
Quadratic SBM	N.S. ^e	N.S.
S.E.M. ^d	0.09	0.02
Linear HPSFM	N.S.	N.S.
Quadratic HPSFM	N.S.	N.S.
S.E.M.	0.06	0.05
<i>Significance^c</i>		
Linear	N.S.	P<0.01
Quadratic	N.S.	N.S.
S.E.M.	0.07	0.04
<i>Effects of</i>		
Ingredient	N.S.	P<0.001
Level	P<0.01	N.S.
Level*Ingredient	N.S.	P<0.01

^aBW = Wet weight.

^bSignificance = significance of the orthogonal linear and quadratic contrasts of dependent variables across inclusion level.

^c Significance = significance of the orthogonal linear and quadratic contrasts of dependent variables across the Ingredient*Level interaction.

^d S.E.M. = Standard error of the mean.

^eN.S. = Not statistically significant or a trend (P ≥ 0.1).

Table 4.6 – Length and width measurements of simple villi within the distal intestine of Strain 1 Arctic charr fed the experimental diets for 84 days, $T_{avg} = 10^{\circ}\text{C}$.

	Villus Length	Villus Width
	μm	μm
Diet A, Control	1058	181
Diet B, 12.5% SBM	843	138
Diet C, 25% SBM	480	106
Diet D, 12.5% HPSFM	1060	170
Diet E, 25% HPSFM	964	160
<i>Significance^a</i>		
Linear SBM	P<0.0001	P<0.0001
Quadratic SBM	N.S. ^d	N.S.
S.E.M.	52	7
Linear HPSFM	N.S.	P=0.08
Quadratic HPSFM	N.S.	N.S.
S.E.M.	71	10
<i>Significance^b</i>		
Linear	P<0.001	P<0.01
Quadratic	N.S.	N.S.
S.E.M. ^c	62	8
<i>Effects of</i>		
Ingredient	P<0.0001	P<0.0001
Level	P<0.0001	P<0.0001
Level*Ingredient	P<0.01	P<0.01

^a Significance = significance of the orthogonal linear contrasts of the dependent variables across inclusion levels.

^b Significance = significance of the orthogonal linear and quadratic contrasts of dependent variables across the Ingredient*Level interaction

^c S.E.M. = Standard error of the mean.

^d N.S. = Not statistically significant or a trend ($P \geq 0.1$).

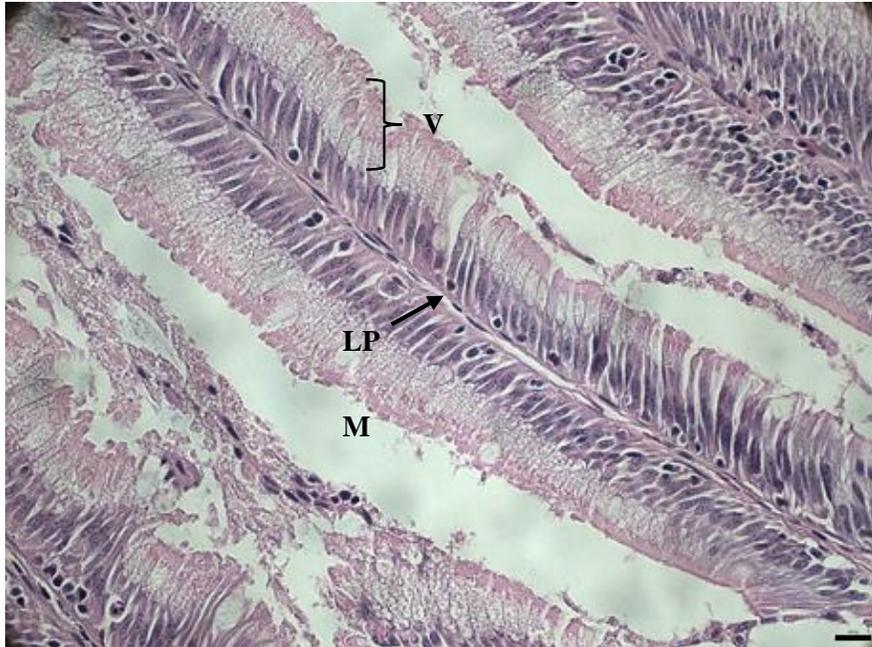


Figure 4.2 – Villus in the distal intestine of Strain 1 Arctic charr fed Diet A (control diet) exhibiting normal basolateral arrangement of enterocyte nuclei, lamina propria (LP) thickness, supranuclear vacuolization (V), and microvilli (M) (bar length = 35µm).

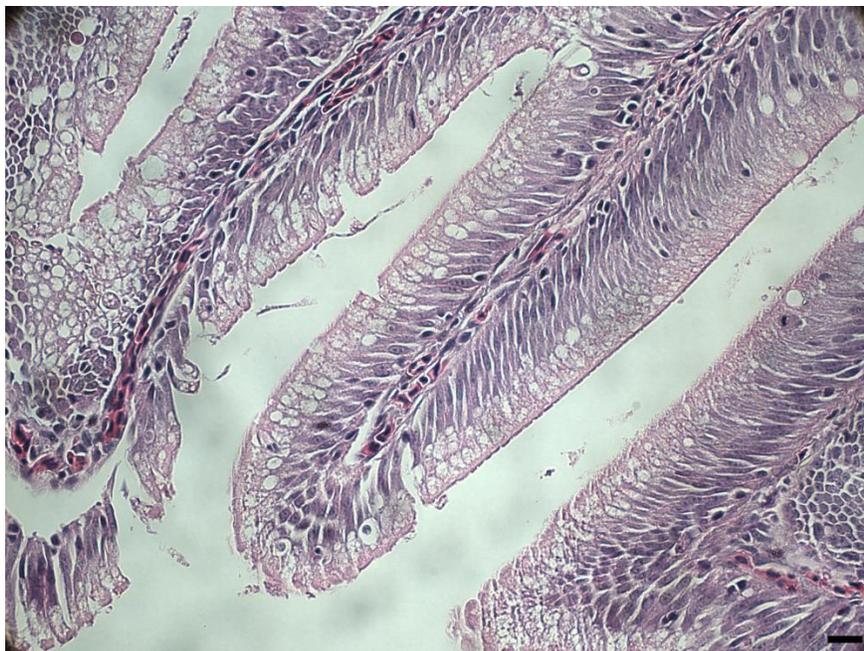


Figure 4.3 – Villus in distal intestine of Strain 1 Arctic charr fed Diet B (12.5% SBM) exhibiting loss of enterocyte vacuolization, increase in vacuolar size, and slight thickening of the lamina propria (bar length=35µm)

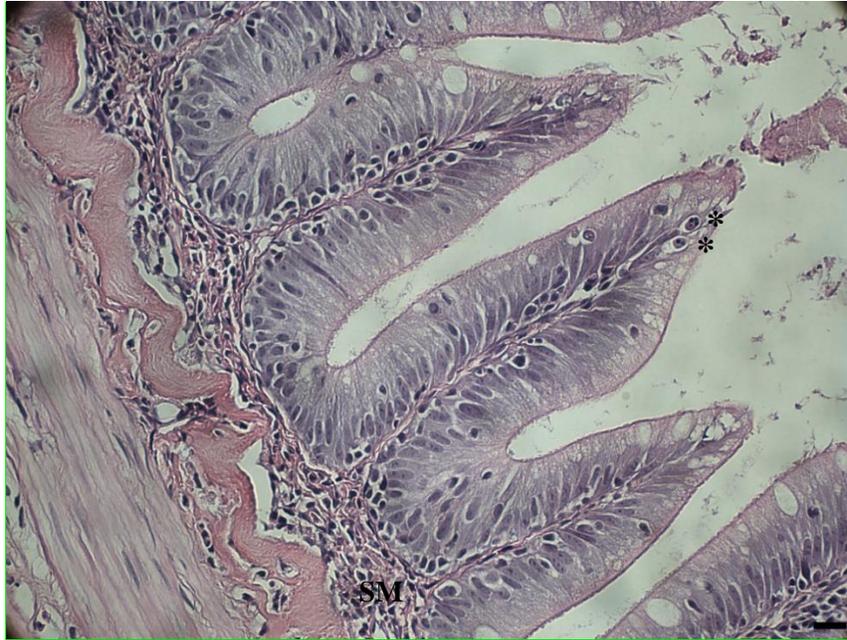


Figure 4.4 – Villus in distal intestine of Strain 1 Arctic charr fed Diet C (25% SBM) exhibiting extremely shortened villi, near complete loss of enterocyte vacuolization, infiltration of polymorphonuclear leukocytes (*), organizational loss of enterocyte nuclei, and thickened lamina propria and submucosa (SM) (bar length=35µm).

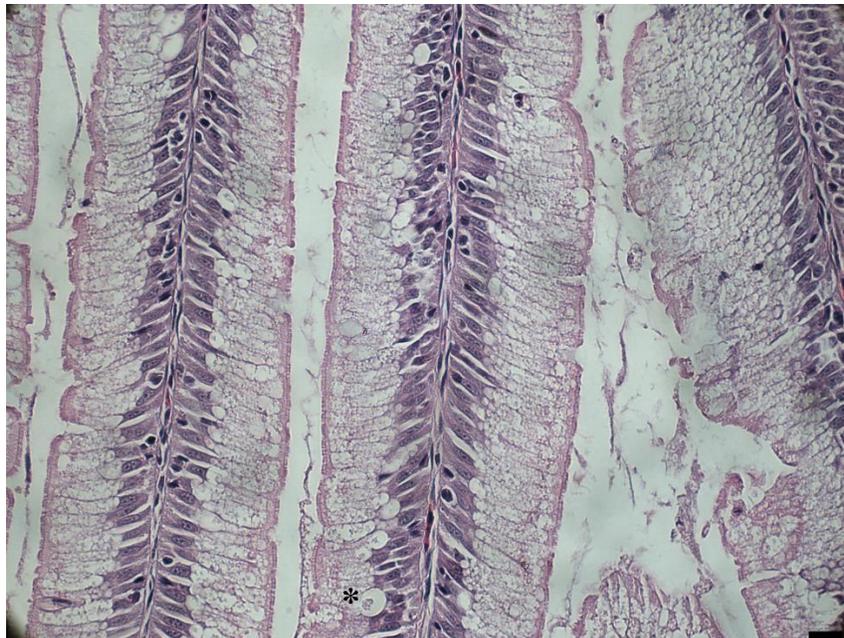


Figure 4.5 – Villus in distal intestine of Strain 1 Arctic charr fed Diet D (12.5% HPSFM) showing increase in supranuclear vacuole size (*), and maintained lamina propria thickness, nuclei organization and microvilli (bar length=35µm).

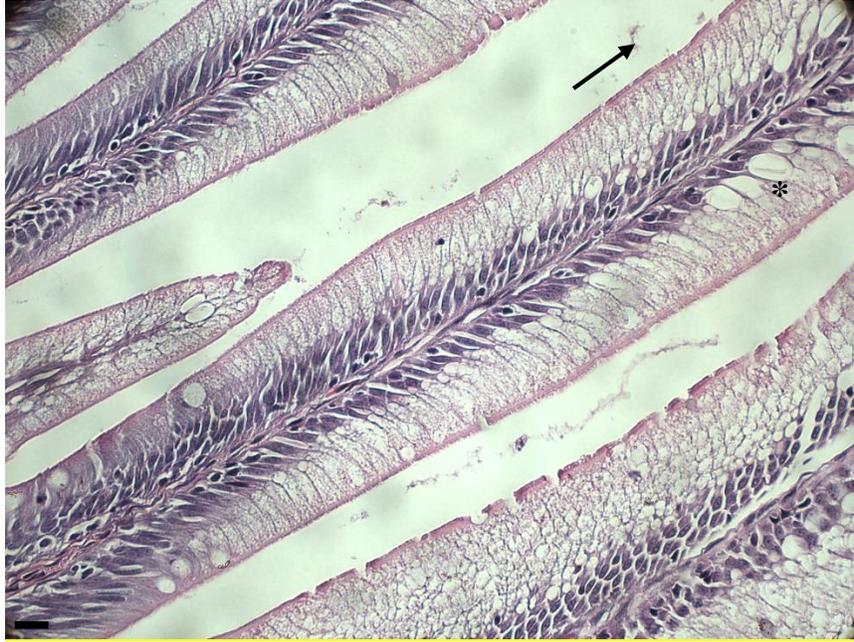


Figure 4.6 – Villus in distal intestine of Strain 1 Arctic charr fed Diet E (25% HPSFM) showing increase in supranuclear vacuole size (*), towards the apical end of the villi (arrow), minor lamina propria thickening, maintained nuclei organization and slightly reduced microvilli (bar length=35 μ m).

Table 4.7 – Fold-change mRNA expression of pregnane X receptor (PXR) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) mRNA in the distal intestine of Strain 1 Arctic charr exposed to the experimental diets.

	PXR	NF- κ B
<i>Strain 1</i>		
Diet A, Control	1	1
Diet B, 10% SBM	2.8	1.2
Diet C, 20% SBM	1.1	2.2
Diet D, 10% SFM	1.5	1.3
Diet E, 20% SFM	0.9	1.6
<i>Significance^a</i>		
Linear	N.S. ^c	N.S.
Quadratic	N.S.	N.S.
S.E.M. ^b	0.7	0.3
<i>Effects of</i>		
Level	N.S.	P<0.05
Ingredient	N.S.	N.S.
Level*Ingredient	N.S.	N.S.

^aSignificance = significance of the orthogonal linear and quadratic contrasts of dependent variables across the Ingredient*Level interaction.

^bS.E.M. = Standard error of the mean.

^c N.S. = Not statistically significant or a trend ($P \geq 0.1$).

Table 4.8 – Chemical body composition of the whole carcass of Strain 1 Arctic charr (initial average weight = 330.1±8.9g/fish) fed the experimental diets for 84 days, T_{avg}=10°C.

	H₂O (%)	CP^a (%)	Lipid (%)	Ash (%)	GE^b KJ/g
Diet A, Control	69.1	15.6	11.4	3.3	8.4
Diet B, 12.5% SBM	67.6	15.8	12.6	3.4	8.9
Diet C, 25% SBM	68.5	16.6	11.6	3.0	8.6
Diet D, 12.5% HPSFM	67.8	16.5	12.3	3.3	8.9
Diet E, 25% HPSFM	68.0	15.8	12.3	3.4	8.7
<i>Significance^c</i>					
Linear	N.S. ^e	N.S.	N.S.	N.S.	N.S.
Quadratic	N.S.	N.S.	N.S.	N.S.	N.S.
S.E.M. ^d	0.9	0.3	0.7	0.2	0.3
<i>Effects of</i>					
Ingredient	N.S.	N.S.	N.S.	N.S.	N.S.
Level	N.S.	N.S.	N.S.	N.S.	N.S.
Level*Ingredient	N.S.	N.S.	N.S.	N.S.	N.S.

^a CP = Crude protein.

^b GE =Gross energy.

^cSignificance = significance of the orthogonal linear and quadratic contrasts of dependent variables across Ingredient*Level interaction.

^d S.E.M. = Standard error of the mean.

^e N.S. = Not statistically significant or a trend (P≥ 0.1).

4.5 – Discussion

4.5.1 – Growth Performance

Grow-out stage Strain 1 Arctic charr (initial weight = 330g) displayed relatively linear growth across the experimental time period compared to juvenile stage fish in the first experiment within this study. This transition in growth trajectory is commonly observed in many fish species, and is suggestive of a potential for different growth stanzas in charr (Dumas et al., 2007; Chowdhury et al, 2013). Divergence in average tank weight amongst diets occurred between day 28 and 56 of the study and resulted in tanks fed both SBM diets and 25% HPSFM showing reduced gain compared to a typical formulated trout diet (A) and 12.5% HPSFM. This was not attributed to differences in feed intake or dissimilarities in dietary nutrient (amino acid) content, as there was no noted effect of either ingredient utilized or inclusion level on elective intake, nor were there differences in amino acid or macronutrient composition between experimental diets. A notable linear depressive effect of SBM on weight gain with inclusion level was observed in the current study, and is in agreement with trends detected by Krogdahl et al. (2003) examining the effect of graded levels of SBM in 200g+ Atlantic salmon.

Growth rate (TGC) of Strain 1 charr noted in the practical diet was approximately 0.25, similar to that observed in Canadian hatcheries and grow out operations (Andre Dumas, CZRI). TGC also showed a linear reduction with SBM inclusion, an extremely well-documented depressive effect noted in both salmon and trout fed diets with relatively high levels of SBM (Refstie et al., 1999; Refstie et al., 2000; Krogdahl et al., 2003; Mundhem et al., 2004). Reduction in growth rate was not observed in fish fed the high protein sunflower meal diets, likely a result of a reduced ANF content in the HPSFM diets compared to the SBM diets. Studies comparing growth performance in salmonids fed either SBM or more highly-processed plant ingredients (protein

concentrates, protein isolates) indicate a greater tolerance, thus higher plausible inclusion level of value-added protein products (Kaushik et al., 1994; Médale et al., 1998; Mambrini et al., 1999). Increased tolerance is attributed to improved nutrient concentration of the plant ingredient, and enhanced nutrient digestibility and retention, thus overall improved feed efficiency by the fish. Supporting this, in the present study, tanks fed diets with HPSFM maintained a similar FE to the control diet, while those fed SBM showed a trend towards FE reduction.

4.5.2 – Nutrient utilization and deposition

The nitrogen retention observed for a practical diet in the present study was within the same range indicated by previous findings with rainbow trout (Mambrini et al., 1999; Refstie et al., 2000). High-level inclusion of common commercial plant protein ingredients such as soybean meal has often been seen to result in a reduced capacity for nutrient digestibility, thus altering overall nitrogen deposition and retention in carnivorous fish species. Both Atlantic salmon and rainbow trout have shown reduced nitrogen retention (% ingested nitrogen) with addition of 30% dietary SBM compared to a fishmeal-based diet, although the depression in trout was insignificant (Refstie et al., 2000). In contrast, additional studies have also shown that the inclusion of high levels of plant-sourced protein concentrates and isolates often do not result in markedly depressed N retention (Kim et al., 1998; Mambrini et al., 1999; Storebakken et al., 2000) thus these ingredients may be added at higher levels in nutrient-dense diets. Compared to a practical diet, NRE (% ingested N) in Strain 1 charr was slightly, although not significantly elevated with SBM and HPSFM addition, likely a result of a marginally reduced dietary N content with no increase in elective feed intake amongst tanks with plant protein inclusion. Interestingly, nitrogen retention and deposition appeared to be higher in diet D (12.5% HPSFM) than in both the practical and 25%

HPSFM, resulting in a significant 2nd order polynomial contrast when compared to SBM as well as with inclusion level. The increase in NRE was paired with a slightly elevated feed efficiency and final carcass protein, however no notable increase in growth rate nor any difference in dietary essential amino acid or macronutrient composition was observed. In the present study, 12.5% HPSFM inclusion in diet D resulted in a 4% reduction of corn gluten meal and wheat middlings, a 3% reduction in fishmeal, and a 1% reduction in poultry by-product meal compared to the control diet. Apparent crude protein digestibility of HPSFM has been reported as higher than 95% in the rainbow trout (Cheng et al., unpublished data). The replacement of other ingredients with lower apparent crude protein digestibility by HPSFM likely increased dietary crude protein digestibility, allowing for both enhanced NRE and growth rates in fish fed 12.5% HPSFM.

4.5.3 – Organosomatic indices and intestinal histology

The addition of select plant protein ingredients to diets inherently increases the carbohydrate fraction, either digestible or indigestible depending on plant source, and ingredient and diet processing steps. Greater concentrations of digestible carbohydrates in salmonid diets has been noted to stimulate the production of hepatic lipogenic enzymes, and increase liver and total body lipid deposition, possibly through reduced oxidation of dietary lipid for energy (Brauge et al., 1994; Gélinau et al., 2002). In the present study, diets were formulated such that there was very little variation in total carbohydrate content between diets. As such, neither the addition of HPSFM or SBM resulted in a significantly linear or quadratic pattern in HSI. Interestingly, and unlike other studies with salmonid species, HSI was on average reduced with SBM inclusion when compared to a control diet.

Comparing the SBM and HPSFM, there was a significant linear difference in distal intestine somatic index (ISI) with increasing inclusion level between ingredients where addition of SBM up to 25% resulted in a reduction in ISI while HPSFM had no effect. Similarly, in studies examining the effect of SBM on intestinal parameters in Atlantic salmon, the addition of 25% dietary SBM resulted in significantly reduced ISI values compared to a control diet which the authors attributed to a reduction in simple fold height (Bakke-McKellep et al., 2007). Supporting these conclusions, Atlantic salmon exposed to 33% dietary SBM for three weeks have reduced intestine simple and complex fold height (Baeverfjord and Krogdahl, 1996). In the current study, >50% decrease in fold height with 25% SBM inclusion was observed through measurements of distal intestine simple folds. This is a classic pathology of ANF-induced non-infectious sub-acute gastrointestinal enteritis in salmonids (Van den Inge et al., 1991; Baeverfjord and Krogdahl, 1996; Bakke, 2011), and has been strongly associated with the presence of lectins and their ability to induce enterocyte sloughing (Buttle et al., 2001). Enhanced processing steps in HPSFM likely resulted in reduction of the concentration of dietary ANF including lectins, thus feeding diets with this ingredient did not result in shortening of simple fold height.

SBM-induced enteritis in salmonids also presents with other key pathologies including reduced vacuolization of enterocytes, immune cell infiltration and swelling of lamina propria and submucosal tissues, and increased number of mucosal cells (Van den Inge et al., 1991; Baeverfjord and Krogdahl, 1996; Bakke, 2011). However, select pathologies have been observed to differ between salmonid species. Comparing the inflammatory response in rainbow trout and Atlantic salmon exposed to 30% dietary protein supplied by SBM, Atlantic salmon presented with a markedly higher infiltration of mixed leukocyte population than did trout (Refstie et al., 2000). In the present study, reduced numbers of supranuclear vacuoles, as well as potentially increased

number of goblet cells were observed, however inflammation of the lamina propria was not noted in charr fed the 25% SBM diet. This may be indicative of a reduced species-specific immune sensitivity towards the allergenic constituents of soybean meal in charr compared to salmon, or could possibly be a result of adaptation to SBM diets during the 12-week experimental period.

4.5.4 – Inflammatory mRNA expression

RT-qPCR analysis indicated that distal intestine mRNA fold-change of PXR and NF- κ B did not differ either linearly or quadratically between the investigated plant protein ingredients with increasing dietary inclusion. However there was a significant effect of level, whereby on average 25% inclusion of plant protein resulted in a greater fold-difference than both 12.5% and a practical diet. This elevation in fold-change was noted more so in the SBM diets than in HPSFM diets.

The development of SBM-induced enteritis is also associated with the enhanced localized expression of genes and gene products involved in immune cell function and development, xenobiotic sensing, and cell damage sensing and apoptosis. Inclusive in this broad suite of mRNA is pregnane X receptor (PXR) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B). PXR is a nuclear transcription factor, and functions as a xenobiotic-sensing molecule which once activated transcribes genes responsible for xenobiotic targeting and removal. NF κ B is a fast-acting cellular transcription factor present in all differentiated cell types, and is involved in cellular responses to stress, cytokines, and bacterial/viral antigens (Hoffman and Baltimore, 2006). It can be directly activated by cytokines such as IL1 β or tumour necrosis factor alpha (TNF α), and, depending on the host cell, will transcribe genes related to inflammation, apoptosis, cell cycle arrest, differentiation or proliferation (Hoffman and Baltimore, 2006). In a study investigating

270g Atlantic salmon fed pea protein concentrate supplemented with soyasaponin representing 20-30% SBM, there was a 2-fold upregulation in mRNA expression of the P100 subunit of NF- κ B (Kortner et al., 2012). Although a precursor, the upregulation of the P100 subunit in the study by Kortner et al. (2012) closely matches that of the 2.2-fold change in mRNA expression in the current study, suggesting increased concentration of saponins in SBM-based diets and a significant effect of these soyasaponins on NF- κ B mRNA expression in Arctic charr.

4.6 – Conclusions

Graded levels of soybean meal and high protein sunflower meal from 0 – 25% fed to a Canadian strain of Arctic charr resulted in significant differences in linear patterns of weight gain, growth rate, distal intestine somatic indices, and in measurements of distal intestine simple folds, whereby tanks fed SBM displayed the most noteworthy depressive effects. Significant quadratic contrasts for nitrogen retention and deposition rate for HPSFM-fed tanks indicated that 12.5% inclusion level promoted NRE and marginally increased feed efficiency and final carcass nitrogen, which may imply a potential for the sparing of nitrogen by digestible carbohydrates in charr when highly-processed, reduced ANF plant protein ingredients are included at moderate levels.

Histological analysis indicated a strong effect on SBM on height and width of distal intestine simple folds and enterocyte vacuolization, and these effects corresponded directly with reduced weight gain and growth rate in SBM fed tanks, indicating a strong effect of SBM anti-nutrients on intestinal physiology in charr. However, the noted physiological changes to the distal intestine did not include enhanced inflammation of the lamina propria or submucosal tissues, a classic pathophysiology of SBM-induced enteritis in salmonids. This, combined with relatively similar responses in pro-inflammatory mRNA expression between the two examined ingredients, suggests

a reduced immune sensitivity of Arctic charr towards anti-nutritive constituents of plant proteins. Dietary HPSFM addition did not seem to elicit any of the negative effects associated with enteritis in salmonids.

5 – GENERAL DISCUSSION

The increased utilization of plant proteins in aquafeed is currently an important issue in the culture of salmonids, given present environmental and economic pressures on producers and feed formulators to reduce utilization of marine sources. Historically, the first plant protein ingredient included in salmonid feed is soybean meal. Since then, several different plant protein ingredients have been applied to aquafeeds, inclusion levels highly dependent on plant species, fish species and life stage nutrient requirements, and species-specific sensitivity to select plant ingredient constituents. However, much research has implicated enhanced plant source inclusion in feed formulations as the cause of deleterious effects on growth performance and nutrient digestibility and utilization in key cultured salmonid species, including Atlantic salmon and rainbow trout (Baeverfjord and Kroghdahl, 1996; Refstie et al., 1999; Bennetau-Pelissero et al., 2001; Refstie et al., 2001; Glencross et al., 2003; Kroghdahl et al., 2003; Aslaksen et al., 2007). Antinutritional factors inherent in many of the common plant protein sources, including protease inhibitors, saponins, lectins, antigenic proteins, and indigestible carbohydrates have been associated with the aforementioned effects on fish performance (Kroghdahl et al., 1994; Olli et al., 1994; Refstie et al., 1999; Glencross et al., 2003; Sinha et al., 2011; Taliercio et al., 2014), as well as for the onset and continuation of sub-acute gastro-intestinal enteritis, an inflammatory condition presented in the distal intestine of salmonids (Van den Inge et al., 1991; Baeverfjord and Kroghdahl, 1996; Bakke, 2011). However, there are significant differences in overall performance and physiological/immunological reaction between salmonid species (Bureau et al., 1998; Refstie et al., 2000; Burr et al., 2011), life stages, and genetic strains (Pierce et al., 2008; Overturf et al., 2012; Venold et al., 2012; Overturf et al., 2013) when exposed to specific plant proteins and/or antinutritional factors. Much of the key research surrounding replacement of fishmeal by plant

protein ingredients investigates their effects in Atlantic salmon and rainbow trout, with very little information on the Arctic charr, an emerging alternative species in Canadian aquaculture. Therefore, the objective of these studies was to compare the effects of commercial soybean and sunflower meals, as well as a high protein sunflower meal in two Canadian strains of Arctic charr at the juvenile and grower stages.

The findings from the current study indicate that there are significant differences in the utilization of energy-yielding nutrients for growth between Canadian strains of juvenile Arctic charr. Although contrast analysis revealed no differences either linearly or quadratically between Strain 1 and Strain 2 with graded inclusion of either SBM or SFM, Strain 2 experienced 10 -15% higher feed efficiency (gain:feed) than Strain 1 at all diets examined. This was attributed to a lower elective feed intake in this strain paired with a comparatively similar growth trajectory and growth rate (measured as the thermal-unit growth coefficient, TGC). Nutrient retention analysis revealed that Strain 2 also experienced approximately 5% greater nitrogen retention efficiency (% ingested N), and 2% less total carcass lipid than Strain 1 when fed both the practical (Diet A) and SBM diets. This may indicate a shift in the preferential utilization of lipids, as opposed to protein as a ready source of metabolic energy in Strain 2 fish, sparing ingested protein for growth. Protein sparing by lipids is commonly noted in salmonids, and may differ dramatically between species. Examining Atlantic salmon (1kg) fed diets of lowering DP/DE ratios, Einen and Roem (1997) observed a reduction in body lipid content, corresponding to relatively similar nitrogen retention efficiencies across diets. This was theorized to be a result of the enhanced role of dietary lipid for maintenance energy, sparing dietary proteins for growth (body deposition). Comparing the nitrogen retention capacity in Atlantic salmon and rainbow trout, a salmonid not known for heightened protein-sparing capability, retention (% ingested) is shown to be approximately 17%

higher in salmon (Refstie et al., 2000). Furthermore, the addition of 30% dietary SBM significantly reduced the NRE within salmon, however not in trout, likely due to enhanced sensitivity of salmon to soybean meal anti-nutrients. As expected, the NRE discrepancy between genetic strains of Arctic charr in the present study was much less severe than between differing salmonid species. Similarly, the effect of 20% SBM did not depress NRE as markedly in Strain 2 compared to Atlantic salmon, a likely result of 10% less SBM inclusion utilized in the present study than in the study by Refstie et al. (2000). However the difference in NRE reduction between species may also indicate the possibility of a relatively diminished sensitivity towards SBM anti-nutrients in the Arctic charr.

Comparing performance in Strain 1 and Strain 2 fish, the potential effects of ploidy level cannot be ignored. Strain 2 charr represents a 4th generation induced triploid hybrid strain, while Strain 1 is a 6th generation pure strain diploid. Previous studies comparing triploid to diploid fish have shown varying and inconclusive results. In a study by Tibbetts et al., (2013) growth rate, nitrogen retention, and overall feed efficiency did not differ between diploid and triploid Atlantic salmon. Comparing genetically-derived (4N + 2N parents) and induced (pressure shock) triploid rainbow trout to diploids, body weight and fork length were enhanced with genetic, however reduced with induced triploidy (Weber et al., 2014). In contrast to these results, Strain 2 fish did not show markedly reduced weight gain compared to Strain 1, however there was noted visually a very slight increase in the incidence of skeletal deformities. Furthermore, size variation amongst Strain 2 individuals was much higher than Strain 1, a likely result of a relatively shorter domestication history, and less selection pressure for uniform growth in Strain 2 stocks.

Findings in juvenile as well as grower stage charr also indicate a marked effect of type of plant protein ingredient and degree of processing on growth parameters. Soybean meal is

classically recognized to be a significant source of plant anti-nutrients, including soyasaponins, trypsin inhibitors, lectins, antigenic proteins, and isoflavones, which limits its utilization in salmonid aquafeed. Studies investigating the effects of individual, combined, and plant-protein borne anti-nutrients in other salmonids have identified them as causative agents of reduced nutrient digestibility, feed efficiency and overall growth performance, as well as their combinative roles in the development of gastro-intestinal enteritis (Krogdahl et al., 1994; Olli et al., 1994; Refstie et al., 1999; Glencross et al., 2003; Sinha et al., 2011; Taliercio et al., 2014). Consistent with past research, graded levels of SBM in the present study negatively affected feed efficiency, and tended to reduce growth rate in both strains of juvenile stage (9g – 60g) Arctic charr, and resulted in significant depressions in body weight gain and growth rate at the grower stage (330g – 700g), while sunflower meal (SFM), or high-protein sunflower meal (HPSFM) had less of an effect. Grower-stage fish also displayed increasing severity of several of the physiological changes in the distal intestine associated with the enteritis pathology, including simple fold shortening and visually reduced enterocyte vacuolization with increasing SBM, however not with HPSFM inclusion. Past research has implicated the glycoprotein agglutination properties of lectins in enterocyte sloughing and resulting shortening of intestinal folds (Buttle et al., 2001), thus it is probable that the SBM utilized in this study retained lectin agglutination properties throughout ingredient and feed processing.

In relation to the inflammatory response seen in Atlantic salmon, it appears as though Arctic charr show a similar degree of structural changes, apart from the noted lack of immune cell infiltration into lamina propria and submucosal tissues. However, similar 2-fold up-regulation of mRNA for the pro-inflammatory transcription factor NF- κ B in the current study and a precursor to NF- κ B in a study by Kortner et al. (2012) in Atlantic salmon may indicate either a temperature-

induced delayed immune response, a total lack of significant response, or a time-course acclimation to SBM in charr over the 84-day experimental period. As such, further investigation into the time course of inflammatory gene expression, and its relation to the physiological enteritis response in Arctic charr exposed to high levels of dietary soybean meal is warranted.

As a method to avoid ANF-associated reduction in salmonid growth performance, additionally-processed plant protein ingredients may be implemented. Studies comparing growth performance in salmonids fed either SBM or more highly-processed plant ingredients indicate a greater tolerance, thus higher plausible inclusion level of value-added protein products (Kaushik et al., 1994; Médale et al., 1998; Mambrini et al., 1999). In agreement with previous findings, the present study revealed that tanks of grower stage fish fed either 12.5% or 25% HPSFM did not portray any of the typical pathologies associated with enteritis, nor did HPSFM have a negative effect on any growth or nutrient parameters or feed acceptance. Although ANF were not measured in this study, it is presumed by these results that additional processing of HPSFM resulted in comparatively lower ANF levels than in SBM diets.

Based on the evidence provided in this thesis, it is apparent that different strains of Canadian Arctic charr present with clearly different patterns in macronutrient utilization, and this protein-sparing effect of lipids should be further investigated. However, Arctic charr also maintain a similar reaction towards increased inclusion of commercially-processed plant proteins both within the species, and when compared to other cultured salmonids. Thus, it is suggested that current limitations of dietary SBM inclusion set for rainbow trout (~20%) also be utilized in Arctic charr, especially at the grow-out stage in order to avoid significant effect on growth performance and productivity. Additionally, given significant differences between Arctic charr strains at the juvenile stage in regards to feed efficiency, nitrogen retention, and apparent tolerance to dietary

plant proteins inclusion, the implementation of a breeding program targeted to hybridize strains may be beneficial towards future Canadian production of this species.

6 – REFERENCES

- Alvarez, M.J., Díez, A., López-Bote, C., Gallego, M., Bautista, J.M. (2000). Short-term modulation of lipogenesis by macronutrients on rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *British Journal of Nutrition*. 84: 619-628.
- Amin, M.N., Barnes, R.K., Adams, L.R. (2014). Effect of temperature and varying level of carbohydrate and lipid on growth, feed efficiency and nutrient digestibility of brook trout, *Salvelinus fontinalis* (Mitchill, 1814). *Animal Feed Science and Technology*. 193: 111 – 123.
- Arnason, T., Gunnarsson S., Inslund, A. K., Thorarensen, H., Smáradóttir, H., Steinarsson, A., Gústavsson, A., Johansson M., Björnsson, B. T. (2014). Long-term rearing of Arctic charr *Salvelinus alpinus* under different salinity regimes at constant temperature. *Journal of Fish Biology*. 85: 1145 – 1162.
- 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
- Arnesen A.M., Halvorsen, M., Nilssen K.J. (1992). Development of hypoosmoregulatory capacity in Arctic char (*Salvelinus alpinus*) reared under either continuous light or natural photoperiod. *Canadian Journal of Fisheries and Aquatic Sciences*. 49: 229 – 237.

- 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.
- Ayles, G.B., Baker, R.F. (1983). Genetic differences in growth and survival between strains and hybrids of rainbow trout (*Salmo gairdneri*) stocked in aquaculture lakes in the Canadian prairies. *Aquaculture*. 33:269-280.
- Azevedo, P.A., Leeson, S., Cho, C.Y., Bureau, D.P. (2004). Growth, nitrogen and energy utilization of juveniles from four salmonid species: diet, species and size effects. *Aquaculture*. 234: 393-414.
- Azevedo, P.A., Bureau, D.P., Leeson, S., Cho, C.Y. (2002). Growth and efficiency of feed usage by Atlantic salmon (*Salmo salar*) fed diets with different dietary protein:energy ratios at two feeding levels. *Fisheries Science*. 68: 878-888.
- Baeverfjord, G., Krogdahl, A. (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. *Journal of Fish Diseases*. 19: 375 – 387.

- Bakke, A.M. (2011). Pathophysiological and Immunological Characteristics of Soybean Meal-Induced Enteropathy in Salmon: Contribution of Recent Molecular Investigations. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., Hernández-Hernández, L. (Eds), Avances en Nutrición Acuícola XI - Memorias del Décimo Primer Simposio Internacional de Nutrición Acuícola, 23-25 de Noviembre, San Nicolás de los Garza, N. L., México. Universidad Autónoma de Nuevo León, Monterrey, México, pp. 345-372
- Bakke-McKellep, A.M., Koppang E.O., Gunnes, G., Sanden, M., Hemre G.I., Landsverk, T., Krogdahl, A. (2007). Histological, digestive, metabolic, hormonal and some immune factor responses in Atlantic salmon, *Salmo salar* L., fed genetically modified soybeans. *Journal of Fish Diseases*. 30: 65 – 79.
- Bakke-McKellep, A.M., Press, C.M., Baeverfjord, G., Krogdahl, A., Landsverk, T. (2000). Changes in immune and enzyme histochemical phenotypes of cells in the intestinal mucosa of Atlantic salmon, *Salmo salar* L., with soybean meal-induced enteritis. *Journal of Fish Diseases*. 23: 115 – 127.
- Becker, K., Makkar, H.P.S. (1999). Effects of dietary tannic acid and quebracho tannin on growth performance and metabolic rates of common carp (*Cyprinus carpio* L.). *Aquaculture*. 175: 327-335.

- Bendiksen, E.A., Berga, O.K., Jobling, M., Arnesen, A.M., Masøval, K. (2003). Digestibility, growth and nutrient utilisation of Atlantic salmon parr (*Salmo salar* L.) in relation to temperature, feed fat content and oil source. *Aquaculture*. 224: 283 – 299.
- Benfey, T.J. (2001). Use of sterile triploid Atlantic salmon (*Salmo salar* L.) for aquaculture in New Brunswick, Canada. *ICES Journal of Marine Sciences*. 58: 525 – 529.
- 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*. *General and Comparative Endocrinology*. 121: 173-187.
- Birk, Y. (2003). Plant Protease inhibitors: Significance in nutrition, plant protection, cancer prevention, and genetic engineering. Springer-Verlag Berlin Heidelberg, Germany. 175pp.
- Blaxter, K.L. (1989). *Energy Metabolism in Animals and Man*. Cambridge, UK: Cambridge University Press.
- Bodin, N., Mambrini, M., Wauters, J.P., Abboudi, T., Ooghe, W., Le Boulgerge, E., Larondelle, Y., Rollin, X. (2008). Threonine requirements in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) at the fry stage are similar. *Aquaculture*. 274:353-365.

- Boeuf, G., Payan, P. (2001). How should salinity influence fish growth? *Comparative Biochemistry and Physiology Part C*. 130: 411 – 423.
- Bower, N.I., Li, X., Taylor, R., Johnston, I.A. (2008). Switching to fast growth: the insulin-like growth factor (IGF) system in skeletal muscle of Atlantic salmon. *The Journal of Experimental Biology*. 211: 3859 – 3870.
- Brännäs, E., Linnér, J. (2000). Growth effects in Arctic charr reared in cold water: feed frequency, access to bottom feeding and stocking density. *Aquaculture International*. 8: 381-389.
- Brauge, C., Medale, F., Corraze, G. (1994). Effect of dietary carbohydrate levels on growth, body composition and glycaemia in rainbow trout, *Oncorhynchus mykiss*, reared in seawater. *Aquaculture*. 123: 109-120.
- Bureau, D.P., Cho, C.Y. (1999). Measuring Digestibility in Fish. UG/OMNR Fish Nutrition Research Laboratory Technical Document, University of Guelph, Ontario, Canada. 9pp.
- Bureau, D.P., Harris, A.M., Cho, C.Y. (1999). Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 180: 345-358.

- 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.
- Burr, G.S., Barrows, F.T., Gaylord, G., Wolters, W.R. (2011). Apparent digestibility of macronutrients and phosphorus in plant-derived ingredients for Atlantic salmon, *Salmo salar*, and Arctic charr, *Salvelinus alpinus*. *Aquaculture Nutrition*. 17: 570 – 577.
- 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. *Veterinary Immunology and Immunopathology*. 72: 277 – 288.
- Buttle, L.G., Burrells A.C., Good J.E., Williams P.D., Southgate. P.J., Burrells C. (2001). The binding of soybean agglutinin (SBA) to the intestinal epithelium of Atlantic salmon, *Salmo salar* and rainbow trout *Oncorhynchus mykiss*, fed high levels of soybean meal. *Veterinary Immunology and Immunopathology* 80: 237-244.
- Buyukcapar, H.M., Atalay, A.I., Kamalak, A. (2011). Growth performance of Nile tilapia (*Oreochromis niloticus*) fed with diets containing different levels of hydrolysable and condensed tannin. *J.Agr. Sci. Tech*. 13: 1045-1051.

Canola Council of Canada. Current Canola Oil, Meal, and Seed Prices.

<<http://www.canolacouncil.org/markets-stats/statistics/current-canola-oil,-meal,-and-seed-prices>>. Accessed April 5, 2015.

Castillo, J., Codina, M., Martínez, M.L., Navarro, I., Gutiérrez, J. (2004). Metabolic and mitogenic effects of IGF-I and insulin on muscle cells of rainbow trout. *American Journal of Physiology – Regulatory and Integrative Comparative Physiology*. 283:R647–R652.

Cheng, Z., Hardy, R.W. (2002). Apparent digestibility coefficients of nutrients and nutritional value of poultry by-product meals for rainbow trout *Oncorhynchus mykiss* measures in vivo using settlement. *Journal of the World Aquaculture Society*. 33(4): 458-465.

Cheng, Z., Hardy, R.W. (2002b). Effect of microbial phytase on apparent nutrient digestibility of barley, canola meal, wheat and wheat middlings, measures in vivo using rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*. 8: 271-277.

Chikwati, E.M., Sahlmann, C., Holm, H., Penn, M.H., Krogdahl, Å., Bakke, A.M. (2013). Alterations in digestive enzyme activities during the development of diet-induced enteritis in Atlantic salmon, *Salmo salar* L. *Aquaculture*. 402-403: 28 – 37.

- Chikwati, E.M., Gu, J., Penn, M.H. (2013b). Intestinal epithelial cell proliferation and migration in Atlantic salmon, *Salmo salar* L.: effects of temperature and inflammation. Cell and Tissue Research. 353: 123 – 137.
- Chikwati, E.M., Venold, F.F., Penn, M.H., Rohloff, J., Refstie, S., Guttvik, A., Hillestad, M., Krogdahl, A. (2012). Interaction of soyasaponins with plant ingredients in diets for Atlantic salmon, *Salmo salar* L. British Journal of Nutrition. 107: 1570 – 1590.
- Cho, C.Y., Kaushik, S.J., Woodward, B. (1992). Dietary arginine requirement of young rainbow trout (*Oncorhynchus mykiss*). Comparative Biochemistry and Physiology Part A. 102: 221-216.
- Chowdhury, M.A.K., Siddiqui, S., Hua, K., Bureau, D.P. (2013). Bioenergetics-based factorial model to determine feed requirement and waste output of tilapia produced under commercial settings. Aquaculture. 410-411: 138-147.
- Cleveland, B.M , Manor, M.L. (2015) Effects of phytoestrogens on growth-related and lipogenic genes in rainbow trout (*Oncorhynchus mykiss*). Comparative Biochemistry and Physiology, Part C. 170: 28-37.
- Cleveland, B.M. (2014). In vitro and in vivo effects of phytoestrogens on protein turnover in rainbow trout (*Oncorhynchus mykiss*) white muscle. Comparative Biochemistry and Physiology, Part C. 165:9-16.

- Cleveland, B.M., Weber, G.M. (2013). Effects of triploidy on growth and protein degradation in skeletal muscle during recovery from feed deprivation in juvenile rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part A*. 166: 128 – 137.
- Cole, J.T., Fahey, G.C., Merchen, N.R., Patil, A.R., Murray, S.M., Hussein, H.S., Brent, J.R. (1999). Soybean hulls as a dietary fibre source for dogs. *Journal of Animal Science*. 77(4): 917-924.
- Cotter, D., O'Donovan, V., O'Maoileidigh, N. Rogan, G., Roche, N., Wilkins, N.P. (2000). An evaluation of the use of triploid Atlantic salmon (*Salmo salar* L.) in minimising the impact of escaped farmed salmon on wild populations. *Aquaculture Research*. 186, 1-2: 61 – 75.
- Delabbio, J.L, Glebe, B.D., Sreedharan, A. (1990). Variation in growth and survival between two anadromous strains of Canadian Arctic charr (*Salvelinus alpinus*) during long-term saltwater rearing. *Aquaculture*. 85: 1259 – 1270.
- Dempsen, J.B. (1993). Salinity tolerance of freshwater-acclimated, small-sized Arctic charr, *Salvelinus alpinus* from northern Labrador. *Journal of Fish Biology*. 43: 451 – 462.
- Drew, M.D., Borgeson, T.L., Thiessen, D.L. (2007). A review of processing of feed ingredients to enhance diet digestibility in finfish. *Animal Feed Science and Technology*. 138:118-136.

- Dumas, A., France, J., Bureau, D.P. (2007). Evidence of three growth stanzas in rainbow trout (*Oncorhynchus mykiss*) across life stages and adaptation of the thermal-unit growth coefficient. *Aquaculture*. 267(1):139-146.
- Düsterhöft, E.M., Voragen, A.G.J. (1991). Non-starch polysaccharides from sunflower (*Helianthus annuus*) meal and palm kernel (*Elaeis guineensis*) meal – Preparation of cell wall material and extraction of polysaccharide fractions. *J. Sci. Food Agric*. 55: 411-422.
- Duston, J., Astatkie, T., Murray, S.B. (2007). Effect of salinity at constant 10 °C on grow-out of anadromous Arctic charr from Labrador. *Aquaculture*. 273: 679 – 686.
- Encarnação, P., de Lange, C.F.M., Rodehutschord, M., Hoeler, D., Bureau, W., Bureau, D.P. (2004). Diet digestible energy content affects lysine utilization, but not dietary lysine requirements of rainbow trout (*Onchorhynchus mykiss*) for maximum growth. *Aquaculture*. 235: 569-586.
- Fagbenro, O.A., Adeparusi, E.O, Jimoh, W.A. Effect of processing on the nutrients and anti-nutrient composition of sunflower (*Helianthus annuus*) seed meals. *International Jour. Chem*. 20(3): 165-170.
- FAO (Food and Agriculture Organization of the United Nations) (2015). *Aquaculture Feed and Fertilizer Resources Information System. Rainbow Trout – Feed formulation*. <http://www.fao.org/fileadmin/user_upload/affris/docs/troutT2.pdf>. Accessed April 5, 2015.

FAO FIGIS (Fisheries and Agriculture Organization of the United Nations, Fisheries Global Information System). Global Aquaculture Production 1950-2013.

<http://www.fao.org/figis/servlet/SQServlet?file=/work/FIGIS/prod/webapps/figis/temp/hqp_6659506817571978070.xml&outtype=html>. Accessed April 5, 2015.

Fenwick, D.E., Oakenfull, D. (1981). Saponin content of soya beans and some commercial soya bean products. *J. Sci. Food Agric.* 32: 273-278.

Fowler, L.G. (1980). Substitution of soybean and cottonseed products for fish meal in diets fed to chinook and coho salmon. *The Progressive Fish-Culturist*, 42(2): 87-91.

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.

Frantzen, M., Arnesen, A.M., Damsgard, B., Tveiten, H., Johnsen, H.K. (2004). Effects of photoperiod on sex steroids and gonad maturation in Arctic charr. *Aquaculture*. 240: 561 – 574.

Fuentes, J., Eddy, F.B. (1997). Drinking in Atlantic salmon psmolts and smolts in response to growth hormone and salinity. *Comparative Biochemistry and Physiology Part A*. 117: 87 – 91.

- Gélineau, A., Corraze, G., Boujard, T., Larrouquet, L., Kaushik, S. (2001). Relation between dietary lipid level and voluntary feed intake, growth, nutrient gain, lipid deposition and hepatic lipogenesis in rainbow trout. *Reproduction Nutrition Development*. 41: 487-503.
- Gill, N., Higgs, D.A., Skura¹, B.J., Rowshandeli, M., Dosanjh, B.S, Mann, J., Gannam, A.L. (2006). Nutritive value of partially dehulled and extruded sunflower meal for post-smolt Atlantic salmon (*Salmo salar* L.) in sea water. *Aquaculture Research*. 37: 1348 – 1359.
- Gill, N. (2002). Effect of varying concentrations of partially dehulled and extruded sunflower-meal on growth performance and sensory attributes of post-juvenile Atlantic salmon (*Salmo salar*). University of British Columbia. 202pp.
- Gjedrem, T., Gunnes, K. (1978). Comparison of growth rate in Atlantic salmon, pink salmon, Arctic char, sea trout and rainbow trout under Norwegian farming conditions. *Aquaculture*. 13: 135-141.
- Glencross, B., Evans, D., Dods, K., McCafferty, P., Hawkins, W., Maas, R., Sipsas, S. (2005). Evaluation of digestible value of lupin and soybean protein concentrates and isolates when fed to rainbow trout, *Ocorhynchus mykiss*, using wither stripping or settlement faecal collection methods. *Aquaculture*. 245: 211-220.

- Glencross, B.D., Boujard, T., Kaushik, S.J. (2003). Influence of oligosaccharides on the digestibility of lupin meals when fed to rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*. 219: 703-713.
- Goda, Y., H. Akiyama, E. Suyama, S. Takahashi, J. Kinjo, T. Nohara, and M. Toyoda. (2002). Comparison of soyasaponin and isoflavone contents between genetically modified (GM) and non-GM soybeans. *Journal of the Food Hygienics Society of Japan* 43:339-347.
- Grisdale-Hellend, B., Shearer, K.D., Helland, S.J. (2007). Energy and nutrient utilization of Atlantic cod, Atlantic salmon and rainbow trout fed diets with differing energy contents. *Aquaculture Nutrition*. 13:321 – 334.
- Gulseth O.A., Nilssen, K.J. (2000). The brief period of spring migration, short marine residence, and high return rate of a northern Svalbard population of Arctic char. *Transactions of the American Fisheries Society*. 129: 782 – 796.
- Gunnarsson, S., Johansson, M., Gustavsson, A., Arnason, T., Arnason, J., Smaradottir, H., Bjornsson, B.T., Thorarensen H., Imsland A. K. (2014). Effects of short-day treatment on long-term growth performance and maturation of farmed Arctic charr *Salvelinus alpinus* reared in brackish water. *Journal of Fish Biology*. 85: 1211 – 1226.
- Gurure RM, Moccia RD, Atkinson JL (1995) Optimal protein requirements of young Arctic charr (*Salvelinus alpinus*) fed practical diets. *Aquaculture Nutrition*. 1:227-234.

- Hadidi, S., Glenney, G.W., Welch, T.J., Silverstein, J.T., Weins, G.D. (2008). Spleen size predicts resistance of rainbow trout to *Flavobacterium psychrophilum* challenge. The Journal of Immunology. 180: 4156-4165.
- Halver, J.E., Hardy, R.W. (2002). Fish Nutrition. Third Edition. Academic Press, San Diego, California, USA. 500pp.
- Hammersmith Marketing LTD. Weekly Feed Grain and Protein Report – April 5, 2005. <<http://hammersmithltd.blogspot.ca/>>. Accessed April 5, 2005.
- Han, H., Baik, B.K. (2006). Oligosaccharide content and composition of legumes and their reduction by soaking, cooking, ultrasound, and high hydrostatic pressure. Cereal Chemistry. 83(4): 428-433.
- Hardy, R.W. (2015). Use of soybean meals in the diets of salmon and trout. Soybean Meal INFOCentre. <<http://www.soymeal.org/FactSheets/SalmonidTechReview.pdf>>.
- Hardy, R. (2008). Utilization of plant proteins in fish diets: effects of global demands of grains and oilseeds. Suárez, L.E.C., Marie, D.R., Salazar, M.T., López, M.G.N., Cavazos, D.A.V., Ma, J.P.L., Viana, T. (Eds.). Avances en Nutrición Acuícola IX. IX Simposio Internacional de Nutrición Acuícola. 24-27 Noviembre. Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, México.

Hart, S.D., Barrows, F.T., Dabrowski, D., Dasgupta, S., Garling, D.L., Hardy, R.W., Malison, J.A., Skonberg, D.I., Weeks, C., Brown, P.B. Soybean antinutritional factors and their relative importance in limiting the use of soybean meal in salmonid diets. The U.S. Soybean Export Council.

<http://www.soyaqua.org/sites/default/files/reports/siasalmonidtechbull__0.pdf>.

Hartviksen, M., Bakke, A.M., Vecino, J.G., Ringø, E., Krogdahl, A. (2014). Evaluation of the effect of commercially available plant and animal protein sources in diets for Atlantic salmon (*Salmo salar* L.): digestive and metabolic investigations. *Fish Physiology and Biochemistry*. 40: 1621 – 1637.

Heikkinen, J., Vielma, J., Kemiläinen, O., Tirola, M., Eskelinen, P., Kiuru, T., Navia-Paldanius, D., Wright, 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.

Hertrampf, J.W., and Piedad-Pascual, F. (2000). *Handbook for Ingredients for Aquaculture Feeds*. Kluwer Academic Publishers, Dordrecht, The Netherlands. 573pp.

Hillestad M., Johnsen, F. (1994). High-energy/low-protein diets for Atlantic salmon: effects on growth, nutrient retention and slaughter quality. *Aquaculture*. 124: 109-116.

- Hilton, J.W., Atkinson, J.L., Slinger, S.L. (1983). Effect of increased dietary fibre on the growth of rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Science*. 40: 81-85.
- Hoffman, A., Baltimore, D. (2006). Circuitry of Nuclear Factor κ B Signalling. *Immunological Reviews*. 210: 171 – 186.
- Hu, J., Lee, S., Hendrich, S., Murphy, P.A. (2002). Quantification of the group B soyasaponins by high-performance liquid chromatography. *Journal of Agriculture and Food Chemistry*. 50(9): 2587-2594.
- Ireland, P.A., Dziedzic, S.Z., Kearsly, 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.
- Iwashita Y., Yamamoto T., Furuita H., Sugita T., Suzuki N. (2008). Influence of certain soybean antinutritional factors supplemented to a casein-based semipurified diet on intestinal and liver morphology in fingerling rainbow trout *Oncorhynchus mykiss*. *Fisheries Science* 74: 1075–1082.
- Jensen, A.J. (1994). Growth and age distribution of a river-dwelling and lake-dwelling population of anadromous Arctic char at the same latitude in Norway. *Transactions of the American Fisheries Society*. 123: 370 – 376.

- Jensen, J.L.A., Rikardsen, A.H. (2012). Archival tags reveal that Arctic charr *Salvelinus alpinus* and brown trout *Salmo trutta* can use estuarine and marine waters during winter. *Journal of Fish Biology*. 81: 735 – 749.
- Jobling, M., Tveiten, H., Hatlen B. (1998) Cultivation of Arctic charr: an update *Aquaculture International*. 6: 181–196.
- Jobling, M., Jørgensen, E.H., Arnesen A.M., Ringø, E. (1993). Feeding, growth, and environmental requirements of Arctic char: A review of aquaculture potential. *Aquaculture International*. 1: 20 – 46.
- Johnsen, H.K., Eliassen, R.A., Saether, B.S., Larsen, J.S. (2000). Effects of photoperiod manipulation on development of seawater tolerance in Arctic charr. *Aquaculture*. 189(1-2): 177 – 188.
- Johnston G. (2002). Arctic charr Aquaculture. Fishing New Books, Oxford, UK. 272pp.
- Jonsson, B., Jonsson, N. (2001). Polymorphism and speciation in Arctic charr. *Journal of Fish Biology*. 58: 605 – 638.
- Kaankuka, F.G., Balogun, T.F., Tegbe, T.S.B. (1996). Effects of duration of cooking of full-fat soya beans on proximate analysis, levels of antinutritional factors, and digestibility by weanling pigs. *Animal Feed Science and Technology*. 62: 229 – 237.

- Kamalam, B.S., Medale, F., Kaushik, S., Polakof, S., Skiba-Cassy, S., Panserat, S. (2012). Regulation of metabolism by dietary carbohydrates in two lines of rainbow trout divergently selected for muscle fat content. *The Journal of Experimental Biology*. 215: 2567-2578.
- Karr-Lilienthan, L.K., Grieshop, C.M., Spears, J.K., Fahey, C.G. 2005. Amino acid, carbohydrate, and fat composition of soybean meals prepared at 55 commercial U.S. soybean processing plants. *Journal of Agriculture and Food Chemistry*. 53:2146-2150.
- Kaushik, S.J., Cravedi, J.P., Lalles, J.P., Sumpter, J., Fauconneau, B., Laroche, M. (1995). Partial or total replacement of fishmeal 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.
- Kim, J.D., Kaushik, S.J., Breque, J. (1998). Nitrogen and phosphorus utilization in rainbow trout (*Oncorhynchus mykiss*) fed diets with or without fish meal. *Aquat. Living Resour.* 11(4): 261-264.
- Kim, J.D., Kaushik, S.J. (1992). Contribution of digestible energy from carbohydrates and estimation of protein/energy requirements for growth of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 106: 161-169.

- Kim, K.I. (1993). Requirement for phenylalanine and replacement value of tyrosine for phenylalanine in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 113:243-250.
- 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.). *British Journal of Nutrition* 100: 120-129.
- Knudsen D, Ron O, Baardsen G, Smedsgaard J, Koppe W & Frøkiaer H (2006) Soyasaponins resist extrusion cooking and are not degraded during gut passage in Atlantic salmon (*Salmo salar* L.). *J Agric Food Chem* 54: 6428–6435.
- Kocher, A., Choct, D., Porter, M.D., Broz, J. (2000). The effects of enzyme addition to broiler diets containing high concentrations of canola or sunflower meal. *Poultry Science*. 79: 1767-1774.
- 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 nutrigenomics interactions underlying enteropathy in Atlantic salmon (*Salmo salar*). *BMC Veterinary Research*. 8: 101.
- Krogdahl, A., Bakke-McKellep, A.M., 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.). *Aquaculture Nutrition*. 9: 361 – 371.

- Krogdahl A., Lea, T.B., Olli J.J. (1994). Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilities in rainbow trout (*Oncorhynchus mykiss*).
Comparative Biochemistry and Physiology, Part A. 107,1: 215 – 219.
- Leid, E., Decken, A.V.D., Espe, M., Berge, E. (1998). Nutrition and Protein Metabolism in Fish
Nordic Council of Ministers, Copenhagen, 95pp.
- Lilleeng, E., Froystad, M.K., Ostby, G.C., Valen, E.C., Krogdahl, A. (2007). Effects of diets containing soybean meal on trypsin mRNA expression and activity in Atlantic salmon (*Salmo salar* L). Comparative Biochemistry and Physiology, Part A. 147: 25 – 36.
- Lim, C., Webster, C.D., Lee, C. (Eds.) (2008). Alternative Protein Sources in Aquaculture Diets.
The Hawthorn Press, Taoylor and Francis Group, New York, NY. 571pp.
- Maenz, D. D., Irish G. G., Classen, H.L., (1999). Carbohydrate-binding and agglutinating lectins in raw and processed soybean meals. Animal Feed Science and Technology 76: 335-343.
- Mambrini, M., Roem, A.J., Cravedi, J.P., Lallès, J.P., Kaushik, S.J. (1999). Effects of replacing fishmeal 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*. Journal of Animal Science. 77:2990 – 2999.

- Marjara, I.S., Chikwati, E.M., Valen, E.C., Krogdahl, A., Bakke, A.M. (2012). Transcriptional regulation of IL-17A and other inflammatory markers during the development of soybean meal-induced enteropathy in the distal intestine of Atlantic salmon (*Salmo salar* L.). *Cytokine*. 60: 186 – 196.
- Mazur, C.N., Higgs, D.A., Plisetskaya, E., March, B.E. (1992). Utilization of dietary starch and glucose tolerance in juvenile chinook salmon (*Oncorhynchus mykiss*) of different strains in seawater. *Fish Physiology and Biochemistry*. 10(4): 303-313.
- McCormick, S. (1996). Effects of growth hormone and Insulin-like Growth Factor I on salinity tolerance and gill Na⁺, K⁺-ATPase in Atlantic salmon (*Salmo salar*): interaction with cortisol. *General and Comparative Endocrinology*. 101: 3 – 11.
- Médale, F., Boujard, T., Vallee, F., Blanc, D., Mambrini, M., Roem, J., Kaushik, S.J. (1998). Voluntary feed intake, nitrogen and phosphorus losses in rainbow trout (*Oncorhynchus mykiss*) fed increasing levels of soy protein concentrate. *Aquatic Living Resources*. 11(4): 239-246.
- Melzig, M.F., Bader, G., Loose, R. (2001). Investigations of the mechanism of membrane activity of selected triterpenoid saponins. *Planta Medica*. 67(1): 43-48.

- Merrifield, D.L., Dimitroglou, A., Bradley, G., Baker, R.T.M., Davies, S.J. (2009). Soybean meal alters autochthonous microbial populations, microvilli morphology and compromises intestinal enterocyte integrity of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*. 32: 755-769.
- Metusalach, Brown, J.A., Shahidi, F. (1999). Effects of stocking density on composition and performance of reared Arctic charr (*Salvelinus alpinus*). *Journal of Aquatic Food Product Technology*. 8(2): 39-57.
- Mugford, S.T., Osborne, A. (2013). Saponin Synthesis and Function. In: Bach, T.J., Rohmer, M. (Eds.) *Isoprenoid Synthesis in Plants and Microorganisms: New Concepts and Experimental Approaches*. Springer, NY. Pp 405 – 424.
- 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*. 236: 315-331.
- Muzquiz M. (1993). The saponin content and composition of sweet and bitter lupin seed. *Journal of the Science of Food and Agriculture* 63: 47-52.
- Naeve, S.L., Orf, J.H., Miller-Garvin, J. (2013). Quality of the United States soybean crop 2012. <<http://ussec.org/wp-content/uploads/2012/09/2012-U.S.-Food-Soybean-Quality-Report-January-2-2012.pdf>>.

Näslund, I., Henricson, J. (1996). Growth of five landlocked Arctic charr stocks under hatchery conditions. *Aquaculture International*. 4: 105 – 116.

National Sunflower Association, 2015. Sunflower Statistics.

<<http://www.sunflowernsa.com/stats/historical-prices-values/>>. Accessed April 5, 2015.

Nel, A.A., Loubster, H.L., Hammes, P.A. (2000). The effect of environment and cultivar on sunflower seed. II. Composition and processing quality. *South African Journal of Plant and Soil*. 17(3): 138-142.

Ng, W.K., Sigholt, T., Bell, J.G. (2004). The influence of environmental temperature on the apparent nutrient and fatty acid digestibility in Atlantic salmon (*Salmo salar* L.) fed finishing diets containing different blends of fish oil, rapeseed oil and palm oil. *Aquaculture Research*. 35: 1228-1237.

Ng, W.K., Campbell, P.J., Dick, J.R., Bell, J.G. (2003). Interactive effects of dietary palm oil concentration and water temperature on lipid digestibility in rainbow trout, *Oncorhynchus mykiss*. *Lipids*. 38(10): 1031 – 1038.

Nilsson, J. (1993). Arctic charr strain crosses: effects on growth and sexual maturity. *Journal of Fish Biology*. 43: 163 – 171.

Nordrum, S., Bakke-McKellep, A.M., Krogdahl, A., Buddington R.K. (2000). Effects of soybean meal and salinity on intestinal transport of nutrients in Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part B*. 125: 317 – 335.

NRC (National Research Council). (2011). *Nutrient Requirements of Fish and Shrimp*. National Academy of Sciences, USA.

Ojima, D., Pettersen, R.J., Wolkers, J., Johnsen, H.K., Jorgensen, E.H. (2009). Growth hormone and cortisol treatment stimulate seawater tolerance in both anadromous and landlocked Arctic charr. *Comparative Biochemistry and Physiology A – Molecular & Integrative Physiology*. 153(4): 378 – 385.

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

Olli, J.J., Hjelmeland, K., Krogdahl, A. (1994). Soybean trypsin inhibitors in diets for Atlantic salmon (*Salmo salar*, L): effects on nutrient digestibilities and trypsin in pyloric caeca homogenate and intestinal content. *Comparative Biochemistry and Physiology, Part A*. 109,4: 923 – 928.

- Olsen, R.E, Ringø, E. (1998). The influence of temperature on the apparent nutrient and fatty acid digestibility of Arctic charr, *Salvelinus alpinus* L. *Aquaculture Research*. 29: 695 – 701.
- Olsen, Y.A., Reitan, L.J., Røed, K. H. (1993). Gill Na⁺,K⁺-ATPase activity, plasma cortisol level, and non-specific immune response in Atlantic salmon (*Salmo salar*) during parr-smolt transformation. *Journal of Fish Biology*. 43(4): 559 – 573.
- Oppedal, F., Taranger, G.L., Hansen, T. (2003). Growth performance and sexual maturation in diploid and triploid Atlantic salmon (*Salmo salar* L.) in seawater tanks exposed to continuous light or simulated natural photoperiod. *Aquaculture*. 215: 145 – 162.
- Overturf, K., Casten, M.T., LaPatra, S.L., Rexroad III, C., Hardy, R.W. (2003). Comparison of growth performance, immunological response, and genetic diversity of five strains of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 217:93-106.
- Parsons, C.M., Hashimoto, K., Wedekind K.J., Han, Y., Baker, D.H. (1992). Effect of overprocessing on availability of amino acids and energy in soybean meal. *Poultry Science*. 71:133-140
- Pratt, D., Birac, P.M. (1979). Source of antioxidant activity of soybeans and soy products. *J. Food Sci*. 44:541-544,

- Qin, G.X., Verstegen, M.W.A., van der Poel, A.F.B. (1998). Effect of temperature and time during steam treatment on the protein quality of full-fat soybeans from different origins. *J. Sci. Food. Agric.* 77:393-398.
- Qin, G., ter Elst, E.R., Bosch, M.W., van der Poel, A.F.B. (1996). Thermal processing of whole soya beans: Studies on the inactivation of antinutritional factors and effects on ileal digestibility in piglets. *Animal Feed Science and Technology.* 57: 313-324.
- Rahma, E.H., Rao, M.S.N. (1981). Removal of polyphenols from sunflower meal by various solvents: effects on functional properties. *Journal of Food Science.* 46: 1521-1526.
- 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., 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., 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. *Animal Feed Science and Technology.* 79: 331-345.

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., Helland, S.J., Storebakken, T. (1997). Adaptation to soybean meal in diets for rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*. 153: 262 – 272

Rodehutschord, M., Becker, A., Pack, M., Pfeffer, E. (1997), Response of rainbow trout (*Oncorhynchus mykiss*) to supplements of individual essential amino acids in a semipurified diet, including an estimate of the maintenance requirement of essential amino acids. *Journal of Nutrition*. 127:1166-1175.

Rodehutschord, M., Jacobs, S., Pack, M., Pfeffer, E. (1995). Response of rainbow trout (*Oncorhynchus mykiss*) growing from 50 to 170g to supplements of either l-arginine or l-threonine in a semipurified diet. *Journal of Nutrition*. 125:964-969.

Romarheim, O.H., Skrede, A., Gao, Y., Krogdahl A., 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.

Rosa, P.M., Antoniassi, R., Freitas, S.C., Bizzo, H.R., Zanotto, D.L., Oliveira, M.F., Castiglioni, V.B.R. (2009). Chemical composition of Brazilian sunflower varieties. *Helia*. 32(50): 145-156.

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. *Veterinary Immunology and Immunopathology*. 41: 323 – 339.

Saez, P., Borquez, A., Dantagnana, P., Hernández, A. (2015). Effects of dehulling, steam-cooking and microwave-irradiation on digestive value of white lupin (*Lupinus albus*) seed meal for rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Archives of Animal Nutrition*. 69(2): 143-157.

Sahlmann, C., Sultherland, B.J.G., Kortner, T., Koop, B.F., Krogdahl, A., Bakke, A.M. (2013). Early response of gene expression in the distal intestine of Atlantic salmon (*Salmo salar*) during the development of soybean meal induced enteritis. *Fish & Shellfish Immunology*. 34: 599-609.

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.

Shepherd, B.S., Drennon, K., Johnson, J., Nichols, J.W., Playle, R.C., Singer, T.D., Vijayan, M.M. (2005). Salinity acclimation affects the somatotropic axis in rainbow trout. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*. 288: R1385 – R1395.

Sealey, W.M., Barrows, F.T., Smith, C.E., Overturf, K., LaPatra, S.E. (2009). Soybean meal level and probiotics in first feeding fry diets alter the ability of rainbow trout *Oncorhynchus mykiss* to utilize high levels of soybean meal during grow-out. *Aquaculture*. 293: 195 – 203.

Siegler, D.S. (1998). *Plant Secondary Metabolism*. Kluwer Academic Publisher, Massachusetts, USA. 759pp.

Siikavuopio S.I., Foss A., Saether B-S., Gunnarsson S., Imsland A.K. (2013). Comparison of the growth performance of offspring from cultures versus wild populations of arctic charr, *Salvelinus alpinus* (L.), kept at three different temperatures. *Aquaculture Research* 44: 995-1001.

Silverstein, J.T., Bosworth, B.G., Waldbeiser, G.C., Wolters, W.R. (2001). Feed intake in channel catfish: is there a genetic component? *Aquaculture Research*. 32(Suppl.1): 199-205.

- Silverstein, J.T., Wolters, W.R., Holland, M. (1999). Evidence of differences in growth and food intake regulation in different genetic strains of channel catfish. *Journal of Fish Biology*. 54: 607-615.
- Simmons L., Moccia R.D., Bureau D.P., Herbert, S.J.G. (1999). Dietary methionine requirements of juvenile Arctic charr *Salvelinus alpinus* (L.). *Aquaculture Nutrition* 5: 93-100.
- Singer, T.D., Raptis, S. Sathiyaa, R., Nichols, J.W., Playle, R.C., Vijayan, M.M. (2007). Tissue-specific modulation of glucocorticoid receptor expression in response to salinity acclimation in rainbow trout. *Comparative Biochemistry and Physiology Part B*. 146: 271 – 278.
- Sinha, A.K., Kumar, V., Makkar, H.P.S., Boeck, G.D., Becker, K. (2011). Non-starch polysaccharides and their role in fish nutrition – A review. *Food Chemistry*. 127: 1409-1425.
- Skúlason, S., Snorrason, S.S., Ota, D., Noakes, D.L.G. (1993). Genetically-based differences in foraging behaviour among sympatric morphs of arctic charr (Pisces: Salmonidae). *Animal Behaviour*. 45: 1179 – 1192.

- Smith, R.R., Kincaid, H.L., Regenstein, J.M., Rumsey, G.L. (1988). Growth, carcass composition and taste of rainbow trout of difference strains fed diets containing primarily plant or animal protein. *Aquaculture*. 70:309-321.
- Solberg, M.F., Skaala, Ø., Nilsen, F., Glover, K.A. (2013). Does domestication cause changes in growth reaction norms? A study of farmed, wild, and hybrid Atlantic salmon families exposed to environmental stress. *PLOS ONE*. 8(1): e54469.
- Solsulski, F., Fleming, S.E. (1977). Chemical, functional, and nutritional properties of sunflower protein products. *J. Am. Oil Chemists' Soc.* 54: 100A – 104A.
- Sørensen, M., Penn, M., El-Mowafi, A., Storebakken, T., Chunfang, C., Øverland, M., Krogdahl, A. (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.
- Statistics Canada (2014). Table 003-0001 - Aquaculture, production and value, annual, CANSIM (database). <<http://www5.statcan.gc.ca/cansim/a26>>. Accessed April 5, 2015.
- Stefansson, S.O., Haugland, M., Björnsson, B.T., McCormick S.D., Holm, M., Ebbesson, L.O.E., Holst, J.C., Nilsen, T.O. (2012). Growth, osmoregulation and endocrine changes in wild Atlantic salmon smolts and post-smolts during marine migration. *Aquaculture*. 362 – 363: 127 – 136.

- Stickney, R.R., hardy, R.W., Koch, K., Harrold, R., Seawright, D., Masee, K.C. (2007). The effects of substituting selected oilseed protein concentrates for fish meal in rainbow trout *Oncorhynchus mykiss* diets. *Journal of the World Aquaculture Society*. 27(1): 57-63.
- 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. *Aquaculture Nutrition*. 6:103-108.
- Storebakken, T., Kvein, I.S., Shearer, K.D., Grisdale-Helland, B., Helland, S.J., Berge, G.M. (1998). The apparent digestibility of diets containing fish meal, soybean meal or bacterial meal fed to Atlantic salmon (*Salmo salar*): evaluation of different faecal collection methods. *Aquaculture*. 169: 195-210.
- 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. (1985). Binders in fish feeds I. Effect of alginate and guar gum on growth, digestibility, feed intake and passage through the gastrointestinal tract of rainbow trout. *Aquaculture*. 47: 11-26.

- Sutterlin A.M., and Stevens E.D. (1992). Thermal behaviour of rainbow trout and Arctic char in cages moored in stratified water. *Aquaculture* 102: 65-75.
- Synder, H.E., Kwon, T.W. (1987). *Soybean Utilization*. Van Nostrand Reinhold Company Inc., New York, NY. 348pp.
- Tabachek, J.L. (1986) Influence of dietary protein and lipid levels on growth, body composition and utilization efficiencies of Arctic charr, *Salvelinus alpinus* L.. *Journal of Fish Biology*. 29:139-151.
- Tacon, A.J.G., Webster, J.L., Martinez, C.A. (1984). Use of solvent extracted sunflower seed meal in complete diets for fingerling rainbow trout (*Salmo gairdneri* Richardson). *Aquaculture*. 43: 381 – 389.
- Taliercio, E., Loveless, T.M., Turanob, M.J., Kim, S. (2014). Identification of epitopes of the β subunit of soybean β -conglycinin that are antigenic in pigs, dogs, rabbits and fish. *Journal of the Science of Food and Agriculture*. 94: 2289 – 2294.
- Thodeson, J., Grisdale-Helland, B., Helland, S., Gjerde, B. (1999). Feed intake, growth and feed utilization of offspring from wild and selected Atlantic salmon (*Salmo salar*). *Aquaculture*. 180: 237-246.

- Thorsen, J., Lilleeng, E., Valen, E.C., Krogdahl, A. (2008). Proteinase-activated receptor-2: two potential inflammatory mediators of the gastrointestinal tract in Atlantic salmon. *Journal of Inflammation*. 5:18.
- Tibbetts, S.M., Wall, C.L., Barbosa-Solomieu, V., Bryenton, M.D., Plouffe, D.A., Buchanan, J.T., Lall, S.P. (2013). Effects of combined ‘all-fish’ growth hormone transgenics and triploidy on growth and nutrient utilization of Atlantic salmon (*Salmo salar* L.) fed a practical grower diet of known composition. *Aquaculture*. 406-407: 141 – 152.
- USDA (United States Department of Agriculture) (2015). Oilseeds: World Market and Trade. <<http://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf>>. Accessed April 5, 2015.
- Van den Ingh, T.S.G.A.M., Krogdahl, A., Olli, J.J., Hendriks, H.G.C.J.M., Koninkx, J.G.J.F. (1991). Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. *Aquaculture*. 94: 297 – 305.
- Vandenberg, J.W., de la Noüe, J. (2001). Apparent digestibility comparison in rainbow trout (*Oncorhynchus mykiss*) assessed using three methods of faeces collection and three digestibility markers. *Aquaculture Nutrition*. 7: 237-245.
- Vergnolle, N., Luger, T.A., Hollenberg, M.D. (2005). Proteinase-activated receptors: Transducers of proteinase-mediated signaling in inflammation and immune response. *Endocrinology Review*. 26:1-43.

- Vergnolle, N. (1999). Proteinase-activated receptor-2-activating peptides induce leukocyte rolling, adhesion, and extravasation in vivo. *Journal of Immunology*. 163: 5064-5069.
- Wassmur, B., Gräns, J., Kling, P., Celandier, M.C. (2010). Interactions of pharmaceuticals and other xenobiotics on hepatic pregnane X receptor and cytochrome P450 3A signaling pathway in rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology*. 100:91-100.
- Weber, G.M., Hostuttler, M.A., Cleveland, B.M., Leeds, T.D. (2014). Performance comparison of inter-cross-triploid, induced triploid, and diploid rainbow trout. *Aquaculture*. 433: 85-93.
- Weeks, C., Garling, D., Barrowsm F.T., Faisal, M. (2010). The effect of feeding varying levels of soybean meal in high-nutrient-density diets on growth performance and body composition of juvenile Atlantic salmon. *North American Journal of Aquaculture*. 72: 279-289.
- Weins, G.D., Marancik, D.P., Zwollo, P., Kaattari, S.L. (2015). Reduction of rainbow trout spleen size by splenectomy does not alter resistance against bacterial cold water disease. *Developmental and Comparative Immunology*. 49: 31-37.
- Yin, Y., Fatufe, A.A., Blachier, F. (2011). Soya Bean Meal and Its Extensive Use in Livestock Feeding and Nutrition. In: El-Shemy, H.A. (Ed.) *Soybean and Nutrition*. InTech. <<http://www.intechopen.com/books/soybean-and-nutrition>>.

Yo, F., Classen, H.L., Garlich, J.D., Swaisgood, H.E., Clare, D.A. (2003). Investigating the possibility of monitoring lectin levels in commercial soybean meals intended for poultry feed using steam- heated soybean meal as a model. *Poultry Science*. 82(4): 648-656.

Zdunczyk, Z., Minakowski, D., Frejnagel, S., Flis, M. (1999). Comparative study of the chemical composition and nutritional value of pumpkin seed cake, soybean meal and casein. *Nahrung*. 43(6): S392-S395.

Zhang, Y., Parsons, C.M. (1994). Effects of overprocessing on the nutritional quality of sunflower meal. *Poultry Science*. 73:436-442.