EVALUATION OF CAMELINA (*CAMELINA SATIVA*) BYPRODUCTS FED TO ATLANTIC SALMON (*SALMO SALAR*) IN PRACTICAL DIETS

By

Chang Lin Ye

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
August 2014

List of tables

List of Figures

Abstract

Camelina byproducts tested were camelina oil (CO), high oil residue camelina meal (HOCM) and solvent extracted camelina meal (SECM). Growth trials using Atlantic salmon parr (initial weight: 8.4±0.2g) and smolts (initial weight: 242±46g in salt water) were conducted for 16 weeks to evaluate the effects of CO and SECM on the growth performance, carcass composition and hindgut histology. Total replacement of fish oil by CO was resulted in excellent growth by fish, and SECM could be effectively included at 8% in the diet of smolts and 10% in the diet of parr. Hindgut enteritis was found in fish fed diets with greater than 15% of SECM. Smolts (initial weight: 61.8±9.1g) in freshwater were fed graded levels of HOCM diets for 16 weeks. Fish growth was depressed by the lowest level of HOCM, 8%. The early stage of hindgut enteritis was detected in fish fed HOCM diets.

List of Abbreviations Used

ALA	α-linolenic acid
ANOVA	Analysis of variance
CF	Condition factor
CO	Camelina oil
CPC	Camelina protein concentrate
CS	Compound symmetry
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FC	Feed consumption
FCR	Feed conversion ratio
FM	Fish meal
GC	Goblet cell
HIS	Hepatosomatic index
HOCM	High oil residue camelina meal
LNA	Linolenic acid
LP	Lamina propria
MF	Mucosal fold
MUN	Memorial University of Newfoundland
NSP	Non-starch polysaccharides
NRC	National Research Council
PRR	Protein retention ratio

PUFA	Polyunsaturated fatty acid
SC	Stratum compactum
SD	Standard deviation
SECM	Solvent extracted camelina meal
SEFM	Solvent extracted fish meal
SGR	Specific growth rate
SM	Sub-mucosal
SNV	Supranuclear vacuoles
TMS	Tricaine methane sulfonate
VI	Viscera index
WG	Weight gain

Acknowledgements

I would like to express the deepest appreciation to my supervisor Dr. Derek Anderson for his guidance and support during the last three years. I would like to acknowledge the advice and guidance given by my committee members Dr. Jim Duston and Dr. Santosh Lall. Many thanks to Paul MacIsaac and Scott Jeffrey for their technical support during the growth trials. I would like to thank Michael McConkey for the help in feed mill, thank Margie Hartling for supervising me on the nutrient analyses, and thanks to Cara Kirkpatrick, Janice MacIsaac and Marilyn Roberts for answering lots of questions and the help on ordering of experimental consumables and shipping of experimental samples. I would like to appreciate the students in the aquaculture program for their help during sample collections. Many thanks to the camelina group at the Ocean Sciences Centre, Memorial University of Newfoundland for providing fish tissue samples. Thanks to the financial support from GenomeAtlantic. Last but not least, my grateful thanks to my parents, Chang Hui Ye and Bing Wang, who gave me great support and encouragement.

Chapter 1 : Introduction

1.1 Atlantic Salmon Production

Aquaculture is growing and plays an important role in providing protein food to the world. World aquaculture production has increased from 41.9 to 63.6 million tonnes between 2004 and 2011(FAO, 2010; 2012). Atlantic salmon is a species with high quality protein and fat. The production of Atlantic salmon reached 2.0 million tonnes in 2012. Norway and Chile are the two largest Atlantic salmon producers in the world, contributing 1.2 and 0.4 million tonnes respectively (Global Aquaculture Production, FishStat, data.fao.org/dataset-data-filter, accessed on June 17, 2014). By the 1990s, the production of Atlantic salmon on the west and east coasts of Canada grew rapidly, which contributed to a 40% increase in Canadian finfish aquaculture (Olin et al., 2010). British Columbia and New Brunswick shared 61% and 33% of Canadian salmon farming respectively, Nova Scotia and Newfoundland and Labrador contributed 6% in total (Olin et al, 2010). During 2002 to 2012, the production of Atlantic salmon in Canada remained stable around 0.1 million tonnes (Global Aquaculture Production, FishStat, data.fao.org/dataset-data-filter, accessed on June 17, 2014).

1.2 Fish Meal and Fish Oil

Fish meal and fish oil are important protein and fat sources in diets for Atlantic salmon. Salmon consumed 36.6% of global fish oil usage in compounded aquafeeds, and consumed 13.7% of global fish meal production in compounded aquafeeds in 2008 (FAO, 2012). The expansion of farmed Atlantic salmon has increased the demand for fish meal and fish oil dramatically in recent years. Other aquaculture and terrestrial species production shared a part of fish meal and fish oil consumed. An estimated 31.7 million tonnes of fish and crustaceans, accounting for 46.1% of world aquaculture production, were fed as manufactured aquafeeds in 2008 (FAO, 2012). These aquafeeds utilized various levels of fish meal and fish oil. In 2008, 20.8 million tonnes of captured fish was used for fish meal and fish oil (FAO, 2010). These two ingredients are mainly processed from small pelagic species, particular peruvian anchovy (*Engraulis ringens*) (FAO, 2010). The capture fisheries production was unchanged in the world, around 90 million tonnes during the years from 2004 to 2011 (FAO, 2010, 2012). In general, a decreasing production trend of fish meal

(7.48 to 5.74 million tonnes) and fish oil (1.5 to 1.07 million tonnes) occurred between 1994 and 2009 due to the reduction in captured anchovy (Tacon et al., 2011; FAO, 2012). Fish meal and fish oil productions were 6.10 and 0.98 million tonnes respectively between 2010 and 2012 (FAO, 2014).Thus, this accounted for the increase of fish meal and fish oil prices in the last 20 years.

The price trends of fish meal and fish oil in Germany and Netherlands are similar to the world trend. Fish meal price has jumped from 300 USD/tonne in 1986 to about 1500 USD/tonne in January 2014, the highest price was 1919 USD/tonne in 2013. By contrast, the soybean meal price was about 500 US$/tonne in 2014 (FAO, 2014). Meanwhile, an increase in price of fish oil was seen in Netherlands, from 300 USD/tonne in 1986 to 1600 USD/tonne January 2014, the highest price was 2400 USD/tonne in 2013. Soybean oil was about 1000 USD/tonnes in 2014 (FAO, 2014). Fish meal usage did not grow with the expansion of aquaculture production, because of the limited supply of capture fish and increasing use of protein substitutes (FAO, 2012). On the contrary, it decreased from 2005 to 2008, and was predicted to be further decline to 3.63 million tonnes by 2015 (FAO, 2012). These changes were reflected in the inclusion levels of fish meal in compound aquafeeds. Fish meal inclusion level in feed will decrease by 10-22%, while fish oil levels will drop by 0.5-7% for carnivorous fish and crustacean species in the next decade (FAO, 2012). For salmon, the fish meal inclusion level will be reduced from 25% in 2008 to 12% in 2020 (FAO, 2012). The average level of fish meal in the salmon feed has been reduced to 18.1% in 2013 (EWOS Reporting Centre). Fish oil use is predicted to be reduced to 6% in salmonid diets by 2020 (Tacon and Metian, 2008). It was 9.8% in the salmon feed in 2013 (EWOS Reporting Centre). Therefore, there will be more space in diet formulation for alternative protein and lipid sources in the salmon feed.

1.3 Alternative Ingredients

Currently, the available non-fish meal protein sources include soybean protein concentrate, pea protein concentrate, maize gluten (EWOS Reporting Centre, 2013), soybean meal, wheat gluten meal, corn gluten meal, canola meal, cottonseed meal, sunflower seed meal, peanut meal, mustard oil cake, lupine kernel meal, and broad bean meal, meat by-product meals, poultry by-product meal, hydrolysed feather meal, and blood meal (FAO, 2012).

Canola oil, soybean oil, palm oil, and poultry oil can be used as non-fish oil lipid sources (FAO, 2012). The levels of these ingredients for salmon diets were recommended by Tacon et al. (2011) to be: poultry by-product meal (10-30%), hydrolysed feather meal (5-12%), blood meal (1-8%), meat meal (10-30%), poultry oil (1-15%), soybean meal (3-12%), wheat gluten meal (2-10%), sunflower seed meal (5-9%), corn gluten meal (10-40%), canola meal (3-10%), lupine kernel meal (5-15%), faba bean meal (5%), field pea meal (3%), canola oil (5-15%), soybean oil (5-10%). Burr et al. (2013) recommended 10% canola protein concentrate in salmon diet. The de-phytate canola protein concentrate could replace 75% fish meal protein in rainbow trout diet (Thiessen et al., 2004). Soy protein concentrate has been used in commercial salmon feed at 15.7% (EWOS reporting centre,2013).

Camelina sativa belongs to the *Cruciferae* (*Brassicaceae*) family. It is called false flax and gold of pleasure (Downey, 1971). The extracted oil has been re-investigated in recent years because of its high level of omega-3 fatty acid linolenic acid (38%) (Crowley and Frohlich, 1998). The camelina meal contains about 33.9% crude protein, which is similar to canola meal (32.9-38%) and full fat soybean meal (35.2%) (NRC, 2011), making it a potential protein source in aquafeeds. However, camelina has not been approved as an animal feed ingredient in Canada. Very little is known regarding the effects of feeding camelina to Atlantic salmon. The current research investigated graded levels of camelina byproducts in practical diets fed to Atlantic salmon at different life stages. The byproducts of camelina seed tested included high oil residue meal, solvent extracted meal, and camelina oil. In addition, two different genetic samples of camelina, Calena and 007 line, contain higher oil content, oil yield, protein yield and polyunsaturated fatty acids (Jiang et al., 2014), thus these two sources of *Camelina sativa* were used. Calena was used in Chapter 3 and 4, while the 007 line was used in Chapter 5. Both Calena cultivar and 007 line were grown at Lyndhurst Farms Ltd, Canning, Nova Scotia, Canada.

Chapter 2 : Literature Review

2.1 Camelina

2.1.1 History of the Crop

Camelina is an ancient oil crop. However, it was not successful as a major crop in the Middle Ages in eastern and northern Europe (Robinson, 1987). During the Middle Ages, camelina was cultured in Denmark, France, Belgium, Holland, the Balkan region and Russia (reviewed by Zubr, 1997). Most camelina grown in Europe and Asia since 1934 is a variety of *Camelina sativa* Crantz (Plessers et al., 1962). The development of camelina was replaced by canola during the 1950s due to the difficulty and high cost of oil hydrogenation (Crowley and Frochlich, 1998). There were still small areas of camelina production in Europe in 1971, aiming to replace winter canola in the cold months (Downey, 1971). In recent decades, interest in camelina has revived due to its high content of omega-3 fatty acids (Putnam, et al., 1993; Francis and Campbell, 2003).

Camelina sativa seed was not only desirable for oil production, but the residue cake after oil pressing is a potential feed ingredient. Camelina oil (CO) can be extracted at an industrial scale by crushing and pressing (Zubr, 1997). The residue after oil extraction was referred to as oil cake with about 10% residual oil (Zubr, 1997). This high oil residue meal (HOCM) was cited in the Nutrient Requirement of Shrimp and Fish (NRC, 2011). The use of solvent extracted camelina meal (SECM) was investigated by Korsrud and Bell in 1967. Ground camelina seed was mixed with petroleum ether at 35-58°C for 24 hours (Korsrud and Bell, 1967). The production of camelina protein concentrate (CPC) can be feasible using an appropriate procedure like that used to make canola protein concentrate, which contains higher protein and less antinutritional factors, such as glucosinolates and phytate (Tzeng, et al., 1990). Camelina byproducts include CO, HOCM, SECM, and CPC. Camelina has not been approved as feed ingredients in Canada. The results from the current study will be a portion of application materials submitted to Canadian Food Inspection Agency as part of the approval process.

2.1.2 Fatty Acids in Camelina Oil

The oil content and fatty acid composition in camelina seed is affected by the varieties of *Camelina sativa*, the environmental conditions in different countries, and the seeding

season (Table 2.1). Genetics accounted for most to the variation in fatty acid composition (Francis and Campbell, 2003; Gugel and Falk, 2006). The oil content ranged from 31.4-43% among all studies (Table 2.1). The ranges of oleic and linoleic from American studies were greater than those from Canada, Australia and Europe, but the range of linolenic acid (omega-3 fatty acid) was lower (Table 2.1). Camelina oil (calena cultivar) processed in Nova Scotia contained 35.6% α-linolenic acid and 18.4% linoleic acid (Hixson et al., 2013). The α-linolenic acid and linoleic acid in camelina oil are higher than that in canola oil, 11% linolenic acid and 21% linoleic acid (Canola Council of Canada, accessed on June 17, 2014). Erucic acid is toxic to animals' growth and heart health (Sauer and Kramer, 1983; Corner, 1983). Low level erucic acid (0.2% vs. 3.7% as diet basis) reduced the negative impact on feed consumption, egg weight and egg production of hens (Vogtmann et al., 1974). High level of erucic acid (50% in rapeseed oil) had negative effects on coho salmon fed diets containing 6% and 12% rapeseed oil (Hendricks, 2002).

2.1.3 Protein in Camelina Meal

High oil residue camelina (HOCM) contains 33.9% crude protein and 12% crude lipid (NRC, 2011). The protein content in solvent extracted camelina meal (SECM) at Fort Vermilion, Alberta was 46.9% and Morden, Manitoba was 45.1% (Korsrud et al., 1978). It was similar to the 42.5% on a fat free dry matter basis in Europe reported by Zubr (2003 a). The highest level of crude protein reported was 49.6% in fat extracted meal (Miller et al., 1962). For essential amino acids, SECM had similar levels of arginine, histidine, phenylalanine, threonine and valine to fish meals, but was lower for isoleucine, leucine, lysine, and methionine (Table 2.2). The amino acid profile was similar among HOCM, SECM and solvent extracted canola meal (Table 2.2).

Table 2.1 The oil content (% in seed) and primary fatty acid composition (% total fatty acids) in *Camelina sativa* seed.

		Locations						
		Australia[1]	Canada[2]	Canada[3]	USA[4]	USA[5]	USA[6]	Europe[7]
Seeding season		Winter	-	Fall	Spring/Fall	Spring	Spring	Summer
number of cultivar		32	10	19	1	10	8	3
Oil content		32-39	36.7	38-43	32-39	31.4	35.5	-
Palmitic acid	C 16:0	-	4.5-5.5	4.6-5.2	6	6.1-8.4	5.7-6.6	5.3-5.6
Stearic acid	C 18:0	-	2.0-2.5	2.2-2.5	2.8	1.4-3.0	2.6-3.5	2.3-2.7
Behenic acid	C 22:0	-	1.5-2.0	-	0.4	-	-	0.2-0.3
Palmitoleic acid	C 16:1	-	-	trace	0.2	-	-	0.1
Oleic acid	C 18:1	12.4-18.7	13.0-16.0	12.8-14.7	17.5	14.1-17.1	14.8-19.4	14.7-15.0
Linoleic acid	C 18:2	15.1-22.5	15.5-17.0	16.3-17.2	18.7	20.0-24.0	19.0-22.3	15.1-15.4
Linolenic acid	C 18:3	31.2-36.9	34.5-38	36.2-39.4	27.9	30.9-34.5	27.1-31.1	36.6-37.1
Eicosenoic acid	C 20:1	13.5-16.7	16.5-18	14.0-15.5	16.4	12.3-12.6	13.6-14.7	15.1-15.8
Eicosadienoic acid	C20:2	-	2.0-2.5	1.8-2.4	2	-	-	1.7-2.0
Erucic acid	C 22:1	2.5-4.2	3.5-4.5	2.5-3.1	3.5	0-3.6	3.2-4.0	2.8-2.9

- not reported.

References:

1. Francis and Campbell, 2003
2. Plessers et al., 1962
3. Gugel and Falk, 2006
4. Robinson, 1987
5. trial 1. Budin et al., 1995
6. trial 2. Budin et al., 1995
7. Zubr and Matthaus, 2002

Table 2.2 Composition of the amino acid in camelina byproducts and two types of fish meal.

	HOCM[a]	Solvent extracted camelina meal		Solvent extracted canola meal	Fish meal	
	NRC 2011	Zubr[b]	Miller[c]	Bell and Keith[d]	Menhaden[e]	Anchovy[e]
Crude protein (%)	33.9	42.5	49.6	41.9	67.9	70.4
Amino acids						
Alanine	-	4.61	3.89	4.4	6.07	6.93
Arginine	7.73	8.15	7.65	6.21	7.73	6.48
Aspartic acid	-	8.71	8.02	7.74	12.56	11.93
Cysteine	1.95	2.12	2.48	2.87	0.93	0.17
Glutamine	-	16.4	16.74	19.07	11.11	11.78
Glycine	-	5.44	4.66	5.21	8.11	6.97
Histidine	2.21	2.6	2.22	3.68	2.16	2.64
Isoleucine	3.54	3.96	3.68	4.25	5.35	5.61
Leucine	6.28	6.63	6.19	7.26	8.29	8.92
Lysine	4.54	4.95	4.19	5.95	9.04	8.74
Methionine	1.80	1.72	1.79	2.05	3.47	3.03
Phenylalanine	4.13	4.19	3.92	4.09	4.33	4.76
Proline	-	5.09	5.09	6	6.57	5.21
Serine	-	5.04	3.58	4.66	4.71	3.79
Threonine	3.83	4.25	3.65	4.54	4.73	3.95
Tryptophan	1.24	1.15	-	1.27	0.64	0.37
Tyrosine	-	3.04	2.64	3.03	3.6	3.49
Valine	4.75	5.42	5.01	5.47	6.28	5.84

a. HOCM: High oil residue camelina meal
b. Zubr, 2003b.
c. Miller et al., 1962.
d. Bell and Keith, 1991. Mean of seven commercial canola meals.
e. Anderson et al., 1995.

2.1.4 Glucosinolates

Glucosinolates exist widely in the plants of the *Brassicaceae* family (Francis et al., 2001). More than 130 glucosinolates have been identified, which are divided into three classes, aliphatic glucosinolates, aromatic glucosinolates and indole glucosinolates (Redovnikovic

et al., 2008). Kjaer et al (1956) isolated glucocamelinin (Figure 2.1), as a nonvolatile glucosinolate from *Camelina sativa*.

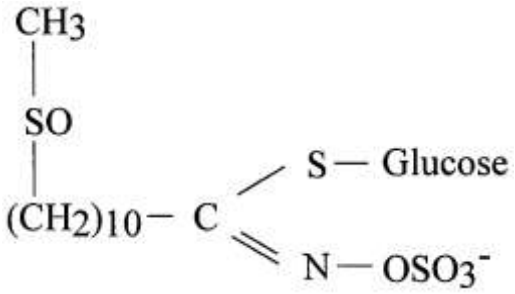


Figure 2.1 Chemical structure of glucocamelinin (Kjaer et al., 1956; Schuster and Friedt, 1998).

Camelina meal contained neither thiooxazolidone nor oxazolidinethione, but a trace level of isothiocyanate was detected at 0.036 mg/g (Plessers et al., 1962) and 1.3 mg/g (Daxenbichler et al., 1964). The acceptable daily intake of allyl isothiocyanate, one type of isothiocyanate, is 0.04-0.08 mg/kg bw/day for children and 0.16 mg/kg bw/day for adult consumers (European Food Safety Authority, 2010). The range of total glucosinolate content was 14.2 to 36.2 μmol/g in dry camelina seed (Schuster and Friedt, 1998), 14.5 to 23.4μmol/g in camelina oilseed cake (Mathaus and Zubr, 2000), while conventional toasted canola meal samples in western Canada showed average 6.16 μmol/g glucosinolates, and non-toasted canola meal samples contain average 10.5 μmol/g (Newkirk, et al., 2003). Glucosinolate was 11μmol/g in the expeller-pressed canola meal reported in some studies (Shafaeipour, et al., 2007; Landero et al., 2012). Glucocamelinin constitutes 65% in the total glucosinolates content in camelina (Schuster and Friedt, 1998). Another study indicated that glucocamelinin (10-methyl-sulfinyl-decyl-glucosinolate) dominated with 62-72% of the total glucosinolates, while the other two major glucosinolates with different side chains were 9-methyl-sulfinyl-nonyl-glucosinolate and 11-methyl-sulfinyl-undecyl-glucosinolate (Mathaus and Zubr, 2000). The toxicity of these three types of glucosinolates has not been published. The negative effects to animals is not due to glucosinolates themselves, but from their degraded products (Schuster and Friedt, 1998). Glucosinolates

could be hydrolysed by myrosinase into isothiocyanates, nitriles, thiocyanates or oxazolidithione (Figure 2.2).

The enzyme myrosinase is released as a defence response by plants. This type of enzyme can be produced by intestinal microflora (Rask et al., 2000; Vaughn and Berhow, 2005; reviewed by Tripathi and Mishra, 2007). Steam stripping is an effective way to destroy myrosinase (Korsrud and Bell, 1967). Thiocyanate competes with iodine in the thyroid system and induced goitrogenesis in rats. This could be mitigated by supplying additional iodine in the diet (Lakshmy et al., 1995). However, 5-vinyl-oxazolidine-2-thione inhibits thyroxine (T4) synthesis directly, and this is independent of the availability of iodine (Mithen et al., 2000). Glucosinolate and isothiocyanate hydrolysis products were the precursors of detoxication enzymes, these enzymes could protect humans from carcinogens (Talalay and Fahey, 2001; Shapiro et al., 2001). Whether this positive effect is expressed in fish remains unknown.

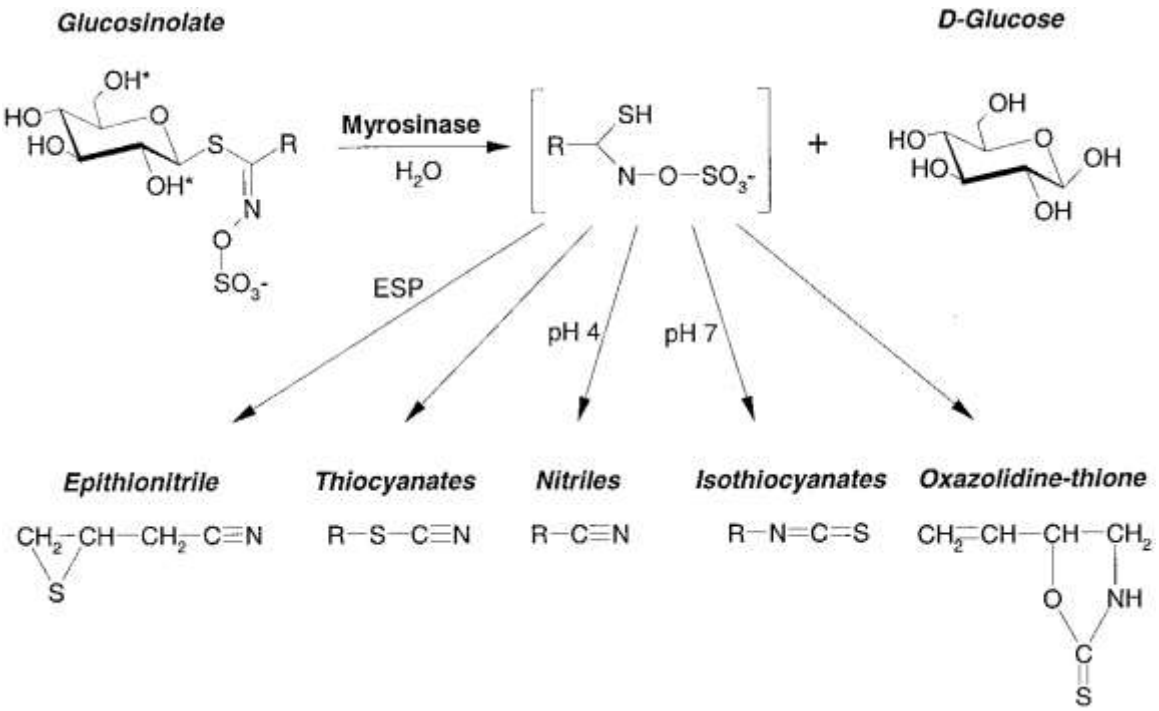


Figure 2.2 The general structure of glucosinolates and their degradation products hydrolyzed by myrosinase (Rask et al., 2000).

Palatability was improved when the glucosinolate level was reduced to 6 μmol/g in calf and dairy cow diets, 3 μmol/g in pig grower diets or 4.5 μmol/g in finisher diets (Mawson et al., 1993). The bitterness was due to the glucosinolate breakdown products, isothiocyanates (Mithen et al., 2000). Growth and feed intake reduction in rats fed SECM above 10% was caused by volatile isothiocyanates and non-volatile compounds. These volatile compounds could be reduced by two hours steam stripping at 110 °C (Korsrud and Bell, 1967). The total glucosinolates in oil extracted rapeseed meal was reduced from 41 to 5 μmol/g via a thermal treatment (Burel et al., 2000). Growth (65.2 g vs. 90.6g) and feed efficiency (0.68 vs. 1) were reduced severely in rainbow trout fed a diet containing glucosinolates up to 19.3 μmol/g. By comparison, slight reduction of final weight was seen at 1.4 μmol/g diet compared to control (82.5 g vs. 90.6 g respectively) (Burel et al., 2000). The suppression of thyroid function was observed (Burel et al., 2000; Burel et al., 2001). Since other antinutritional factors in rapeseed may depress growth at the same time, glucosinolates at 1.4 μmol/g in the diet was regarded as a safe upper limit for rainbow trout (Burel et al., 2000; NRC, 2011). The major components of glucosinolate degradation in rapeseed meals varied dependent on the oil extraction and the dgree of thermal treatment. The oil extracted rapeseed meal with thermal treatment contained progoitrine (2.2 μmol/g) and gluconapine (1.2 μmol/g) in major amounts. Another type of rapeseed meal had high levels of progoitrine (17.2 μmol/g), gluconapine (6.9 μmol/g), glucobrassicanapine (2.2 μmol/g), sinalbine (4.5 μmol/g) and glucobrassicine (7.6 μmol/g) (Burel et al., 2000). Camelina seeds, by comparison to rapeseed, contain different types of glucosinolates (Mathaus and Zubr, 2000). Other antinutritional factors (described in next section) in camelina should not be ignored. Therefore, a safe upper limit of 1.4 μmol/g glucosinolates in the diet may not be appropriate for Atlantic salmon fed camelina byproducts.

2.1.5 Other Antinutritional Factors in Camelina Seed/Meal

Mean values in Camelina seed samples were 2.2 mg/g tannins, 19 mg/g phytic acid and 2 mg/g sinapine (Matthaus, 1997). Both sinapine (1.7-4.2 mg/g vs. 7 mg/g) and condensed tannins (1.0-2.4 mg/g vs. 3.84 mg/g) measured in camelina oilseed cake were lower than rapeseed meal, but phytic acid in camelina oilseed cack was higher than rapeseed (21.9-30.1% vs. 17.4 mg/g) (Matthaus and Zubr, 2000). There was 11.8-26.6 TUI (trypsin units inhibited)/mg of trypsin inhibitor activity in seed, although it was much lower than raw

soybean with 110.7 TUI/mg (Budin et al., 1995). Camelina seed contained 6.7% mucilage (Zubr, 2010), which should be higher in HOCM and SECM.

Tannins include hydrolysable tannins and condensed tannins (Stobiecki and Makkar, 2004) that can reduce digestibility by forming less digestible complexes with proteins, starch and digestive enzymes (Reed, 1995). In an in *vitro* study, the protease, lipase and amylase activity in Indian major carps (body weight: 10-17 g) were inhibited by the tannin (12.5-200 μg) extracted from *Pistia* (Mandal and Ghosh, 2010). The inhibited growth of common carp (*Cyprinus carpio* L.) was partially associated to the 0.57-1.14% tannins in the diet (Hossain and Jauncey, 1989). It was recommended to use dehulled seeds with lower tannins (NRC, 2011).

Sinapine has a bitter taste, and subsequently reduced the palatability of feed (McCurdy and March, 1992). The individual effect of sinapine on salmonids was difficult to address due to accompanying factors, such as glucosinolates and phytate (McCurdy and March, 1992).

Phytic acid (phytate) can disrupt mineral utilization (Francis et al., 2001). The digestibility of zinc and magnesium decreased as phytic acid increased from 1 g/kg to 20.7g/kg in the Atlantic salmon diet (Denstadli et al., 2006). Atlantic salmon parr can tolerate phytic acid at 4.7-10.0 g/kg in the diet without compromising either growth or feed intake (Denstadli et al., 2006). In the same experiment, phytic acid diets did not alter the morphology in the distal intestine (Denstadli et al., 2006). The growth and appetite of juvenile chinook salmon (*Oncorhynchus tshawytscha*) were reduced by high ratio of calcium to phytic acid (48-51 to 1.62 g/kg) (Richardson et al., 1985). Phytase is an enzyme that can release phosphorus from phytate and enhance the availability of other minerals and trace elements (Cao et al., 2007).

Mucilage is a type of non-starch polysaccharides (NSP) with high water solubility, and forms a gel (Mazza and Biliaderis, 1989). Thus, mucilage may have a similar manner to guar gum that slows down the food passage rate in the gastro-intestinal tract (Storebakken, 1985). The apparent digestibility of protein and fat and feed intake in rainbow trout was reduced by guar gum, and moisture content increased in the faeces (Storebakken, 1985). The soluble NSP was suspected to absorb water and increase the viscosity of digesta in the

intestine, subsequently blocking the contact with digestive enzymes (Francis et al., 2001). Mucilage in the flax seed coat could be removed from flax seed meal by water or polysaccharide-degrading enzymes (Wanasundara and Shahidi, 1997). Its viscosity can be reduced in NaCl solutions (Mazza and Biliaderis, 1989). However, no literature was found specifically discussing the mucilage from camelina.

2.1.6 Special Components

Camelina seed contained higher levels of Vitamin E at 17.4 mg/100g seed compared to canola (9.27 mg/100g), flax (12.74 mg/100g), soybean (10.99 mg/100g), and sunflower (13.29 mg/100g) (Budin et al, 1995). Vitamin E had positive effects on the stability of camelina oil (Eidhin et al., 2003). This vitamin E will be in the CO, and in the oil residue in HOCM and SECM.

2.1.7 Experiments of Camelina Byproducts as Feed Ingredients

Use of camelina as a feed ingredient has been reported in mammals, poultry and fish. Camelina seed up to 15% in the diet did not affect the growth and carcass composition of rabbits (Peiretti et al., 2007a). Camelina seed inclusion ranging from 10 to 15% increased the digestibility of organic matter, dry matter, nitrogen-free extract, ether extract and gross energy in rabbits (Peiretti et al., 2007b). In rats, camelina oil with a high level of α-linolenic acid lowered the high levels of triglyceride and cholesterol in liver and plasma induced by high fat diet, and relieve the symptoms of dyslipoproteinemia (Deng et al., 2011).

DHA levels in hen eggs increased as camelina meal percentage increased in feed, but the camelina meal level recommended was not higher than 10% due to the increased lipid oxidation in the eggs (Cherian et al., 2009). Ten percent camelina meal in broiler diets provided similar growth compared to the control group, with n-3 fatty acid composition enhanced in meat, liver and adipose tissues (Aziza et al., 2010). Five percent camelina meal inclusion in diet did not reduce the growth of turkeys. Levels above 5% should be used with caution (Frame et al., 2007). Other vegetable oils in turkey diets could be substituted with camelina oil (Frame et al., 2007).

Similar specific growth rates were observed in rainbow trout fed 15% full fat camelina seed cake diet compared to fish fed the control diet (Anderson et al., 2008). Diet containing 16%

high fat residue camelina meal (HOCM) did not affect weight gain, feed consumption, feed conversion ratio, hepatosomatic index (HSI) and carcass composition of rainbow trout (Pan et al., 2011). Higher level of HOCM (20%) did not inhibit the growth of rainbow trout (Bullerweel and Anderson, 2012). Inclusion levels of camelina meal greater than 20% in the diet have not been reported. For canola/rapeseed meal, a level up to 30% can either inhibit or not inhibit growth of rainbow trout, but 50% inclusion level was not acceptable (Burel et al., 2000; 2001; Shafaeipour et al., 2007; Collins et al., 2012). The conflicting results might be due to different fish size and different content of antinutritional factors in the diet.

Atlantic cod (*Gadus morhua*) fed up to 80% (Hixson et al., 2013) or 100% (Morais, et al., 2012) replacement of fish oil with CO diets exhibited similar growth performance to the control diets. Both studies found significant increases in 18:2n-6 (LNA) and 18:3n-3 (ALA) levels, and a decrease in 20:5n-3 (EPA) and the ratio of n-3/n-6 polyunsaturated fatty acid (PUFA) in liver and muscle (Morais et al., 2012; Hixson et al., 2013). This trend was also found in cod intestine (Morais et al., 2012). Complete substitution of fish oil with CO in diets was acceptable for rainbow trout, but the fatty acid composition in muscle, skin, and viscera fat shifted in a similar manner to Atlantic cod (Hixson et al., 2014a). Although the fatty acid profile changed in Atlantic salmon, the sensory quality (texture, odour, and appearance) of salmon fillets was similar between fish fed fish oil and 100% CO diets (Hixson et al., 2014b). Sunflower oil, rapeseed/canola oil, and oil blend of rapeseed, palm and camelina oil at 5:3:2 did not disrupt fish growth, but tended to decrease EPA, DHA and the ratio of n-3/n-6 PUFA, and increase LNA and ALA in the fatty acid profiles in various fish tissues (Bell et al., 2003; Bransden et al., 2003; Drew et al., 2007; Bell et al., 2010). The reduced EPA and DHA in fish tissues were due to the lack of EPA and DHA in vegetable oils. Salmonid fish can convert 18:3n-3 to DHA (Tocher et al., 1997; Bell et al. 2001). Hixson et al. (2014a) found that ALA in camelina oil contributed 27% of the DHA in rainbow trout. but was not efficient enough to compensate for the depletion of DHA in tissues when replacing fish oil with vegetable oil. Atlantic salmon can convert 25% of ALA to long chain polyunsaturated fatty acids (Hixson et al., 2014c).

The weight gain of Atlantic salmon post-smolts (*Salmo salar*) was unaffected by 100% CO as the only lipid source, but was compromised by feeding diets containing 10% solvent extracted camelina meal (SECM) or a combination of SECM and CO (Hixson et al., 2014b). The levels of LNA and ALA increased, and the ratio of n-3/n-6 PUFA decreased in both white and dark muscle tissues, when salmon were fed diets containing 100% CO. The study in Hixson et al. (2014b) and the study in Chapter 4 were based on the same growth experiment, but the diets containing graded levels of SECM were not published by Hixson et al. (2014b). The study in Chapter 4 evaluated all the test diets, and included carcarss analysis and evaluation of hindgut histology as novel parts. The optimal levels of camelina byproducts fed to Atlantic salmon at different life stages remains unknown. No literature indicated the effects of camelina byproducts on the carcass composition and hindgut histology of Atlantic salmon.

2.2 Life Stages of Atlantic Salmon: Pre-smolt, Smolting and Post-smolt

Smoltification occurs in freshwater when Atlantic salmon parr are ready to migrate to seawater, which is accompanied with the changes in morphology, physiology and behavior (Stefansson et al., 2008). This process can be divided into three stages: pre-smolt, smolting and post-smolt. Pre-smolts (parr) exhibit visible parr-marks and live in freshwater. The behavior of parr is territorial, bottom-dwelling, and swim against the water current to keep position. Smolts have dark fin margins, loose silvery scales and disappearance of parr marks, and adapt to the seawater. Post-smolts complete smoltification and can tolerate full-strength seawater. The behavior of smolts is pelagic and schooling (Handeland and Stefansson, 2002; Stefansson et al., 2008).

In freshwater, fish tend to lose ions (predominantly Na^+ and Cl^-) passively, because they are faced with passive osmotic influx of water. Fish exhibit active ions uptake in the gills, low drinking rate and high production of urine (reviewed by Varsamos et al., 2005). The development of a hypoosmoregulation mechanism in Atlantic salmon occurs and the Na^+, K^+ -ATPase in the chloride cells of the gill increases, when salmon are smolting (McCormick and Saunders, 1987; Duston, 1994). To overcome the dehydration and ions invasion in the seawater, fish increase the drinking rate, actively absorb ions along the

digestive tract accompanying with an osmotic intake of water, actively excrete ions via the gills, and produce a limited amount of urine (reviewed by Varsamos et al., 2005).

The plasma thyroid hormones in smolts increased compared to parr (McCormick et al., 2007). The lipid and glycogen content in the carcass decreased, while the protein content did not change during wild Atlantic salmon migrating from the river to the marine environment (Stefansson et al., 2003). It indicated that smolts have low energy reserves during migration, while post-smolts maintains a positive protein balance during the early marine phase, and the protein contributed to the somatic growth of post-smolts (Stefansson et al., 2003). Atlantic salmon post-smolts in seawater tended to retain protein content in fish body and increased dry matter and lipid content, while parr showed a growing trend of protein, lipid and dry matter in fish body (Shearer, et al., 1994a). A temporary peak of biosynthesis of highly unsaturated fatty acids occurred at the point of seawater transfer (Tocher et al., 2003). Parr exhibited increasing fluid transport rate and Na^+, K^+ -ATPase activity in the anterior intestine, but decreasing paracellular permeability in the posterior intestine during parr-smolt transformation. The direction of water flow shifted from the paracellular route to a transcellular pathway, which was driven by Na^+, K^+ -ATPase activity (Sundell et al., 2003). Smolts (>16 cm fork length) in freshwater were more sensitive to stress than parr (<12 cm) (Carey and McCormick, 1998).

When smolts remained in freshwater, desmoltification occurred and was accelerated by increased temperature (Duston et al., 1991). These fish did not revert to the morphology of parr, but failed to adapt to seawater (Stefansson et al., 2008). In Chapter 5, Atlantic salmon smolts (initial mean weight: 61.8g) were reared in freshwater, thus the desmoltification should occur during the experiment.

2.3 Intestine in Atlantic Salmon in Response to Plant Feed Ingredients

2.3.1 Morphology and Histology of Salmon Intestine

Terrestrial plant ingredients are common lipid or protein sources in farmed salmon feed. However, histopathological damage was observed in the gastro-intestinal tract due to plant ingredients, particular soybean meal. Soybean-induced enteritis in the distal intestine was reported (Van den Ingh et al., 1991; Van den Ingh et al., 1996; Baeverfjord and Krogdahl,

1996; Bakke-McKellep et al., 2007; Knudsen et al., 2007; Uran, 2008a; Overland et al., 2009). Thus, the morphology and histology of distal intestine need to be examined when salmon are fed diets containing camelina byproducts.

Atlantic salmon intestine consists of five different regions: pyloric caeca, first segment of the mid-intestine with pyloric caeca, first segment of the mid intestine posterior to pyloric caeca, second segment of the mid-intestine and posterior intestinal segment (Figure 2.3, Lokka et al., 2013). The second segment of the mid intestine also was defined as distal intestine (Baeverfjord and Krogdahl, 1996; Krogdahl et al., 2003; Bakke-McKellep et al., 2007). Sometimes, it is difficult to distinguish the dividing line between mid-intestine and distal intestine. Externally, the midgut is much longer and thinner than hindgut. Internally, the surface of midgut presents irregular or net-like folding. Conversely, there are abundant circular or loop-like folding in hindgut (Lokka et al 2013).

The gut wall of both the midgut and hindgut comprise of four layers radially. The *tunica mucosa*, which includes mucosal epithelium and lamina propria, is a vascularized connective tissue with nerves and leukocytes. The submucosa is a connective tissue layer. Then the *tunica muscularis*, below the submucosa, contains striated and smooth muscle in circular or longitudinal layers. Lastly the *tunica serosa*, refers to the outside layer of gut

Figure 2.3 Diagram of the gastrointestinal tract in Atlantic salmon.
(ca) Cardiac stomach; (1) pyloric caeca; (2) first segment of the mid-intestine with pyloric caeca; (3) first segment of the mid intestine posterior to pyloric caeca (short for mid gut); (4) second segment of the mid-intestine (between two arrows, also called distal intestine or hindgut); (5) posterior segment (Modified based on Lokka et al., 2013).

wall (Wilson and Castro, 2010). The *tunica mucosa* also refers to the mucosal fold (Baeverfjord and Krogdahl, 1996; Krogdahl et al., 2003; Bakke-McKellep et al., 2007;

Wilson and Castro, 2010) or villi (Merrifield et al., 2011). The mucosal fold in hindgut includes two types of folds, the simple mucosal fold and complex mucosal fold (Van den Ingh, et al., 1991). Complex mucosal folds contain branched central connective tissue stroma. The simple mucosal fold is shorter than the complex fold, with only one central stroma, which is located along the intestinal wall or on the complex fold (Baeverfjord and Krogdahl, 1996). Many microvilli make up the brush borders on the epithelium of the mucosal fold (Merrifield et al., 2009).

The intestinal surface area is increased by multiple folding of the mucosa. Brush border microvilli contribute to the increased surface area (Wilson and Castro, 2010). Epithelial cells, either vacuolated or non-vacuolated, are on the surface of individual mucosal folds and the connection between mucosal folds. In vacuolated cells, the nuclei exist in the base of the cell. Various sizes of translucent vacuoles fill the space of the supranuclear cytoplasm (SNV, Figure 2.4). These columnar vacuoles are also called enterocytes, where nutrient uptake and ion regulation occur via the transepithelial transport processes. The processes are essentially driven by Na^+/K^+ ATPase (Wilson and Castro, 2010). Macromolecular proteins can be taken up by enterocytes (Wilson and Castro, 2010). In non-vacuolated cells at the base of the simple fold, mitotic figures are common (Baeverfjord and Krogdahl, 1996). Goblet cells (GC) are challis-like or goblet shape, and contains acidic mucosubstance sialomucin and lesser amounts of sulfomucins (Wilson and Castro, 2010). These mucins were secreted by goblet cells as an innate defense response (Marchetti et al., 2006). Goblet cells (Figure 2.4) are scattered within the vacuolated epithelial cells on the mucosal fold. A prominent collagenous stratum compactum is in the center of lamina propria (LP) (Figure 2.4) with plenty of eosinophilic granular cells distributed on both sides (Baeverfjord and Krogdahl, 1996). The locations of sub-mucosal (SM), stratum compactum (SC), and serosa are labeled (Figure 2.4).

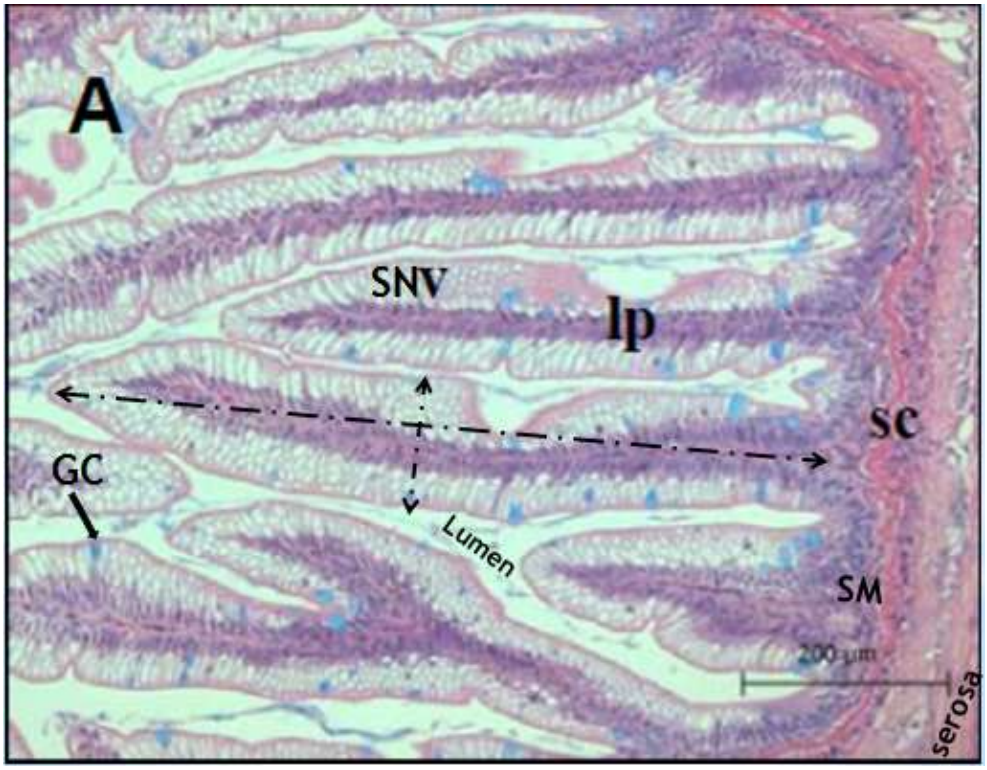


Figure 2.4 Normal morphology of distal intestine in Atlantic salmon.
Supranuclear vacuoles (SNV) and lamina propria (LP) in mucosal folds; connective tissue (or called submucosa, SM) between the base of folds and stratum compactum (SC). Double arrows represent length (long arrow) and width (short arrow) of mucosal folds respectively (modified from Knudsen et al., 2007b).

2.3.2 Soybean Induced Enteritis in Salmonids

The changes of soybean induced enteritis included a decreased height of mucosal folds. The supranuclear vacuoles were reduced or disappeared in the intestinal epithelium. The lamina propria in the centre of mucosal fold was widened, and leucocytes infiltrated into the lamina propria. The condition was defined as non-infectious subacute enteritis (Baeverfjord and Krodahl, 1996). More details of morphological changes in rainbow trout were detected using electron microscopy. They included irregular shaped enterocytes, lower density, shorter and irregular microvilli in the hindgut (Merrifield, et al., 2009) and increased numbers of goblet cells (Van den Ingh, et al., 1991). Uran, et al. (2008a) demonstrated that the disappearance of superanuclear vacuoles was initiated by the endocytosis block. The causative agents were suspected to be antinutritional factors in the soybean meal, such as protease inhibitors and soybean lectin (Van den Ingh, et al., 1991),

but were not clearly identified. Because the activities of proteinase inhibitors and lectin were reduced by 90% after heating, these two antinutritional factors were absent in a later study on heated soybean meal diets and Velasse diet (proteinase inhibitors and lectin free). Two other enteritis inducing compounds were alcohol soluble components, oligosaccharides and saponines (Van den Ingh, et al., 1996). The alcohol extract from soybean meal reduced the digestibility of fat in salmon. The effects of alcohol soluble saponines were found in the alcohol extract (Olli and Krogdahl, 1995). Enteritis-like signs in hindgut were observed in both soybean meal diet and the alcohol extract of soybean meal diet, and elevated levels of lysozyme and IgM as inflammatory responses in hindgut were reported in salmon fed soybean molasses (Krogdahl et al., 2000). One hypothesis was that the saponins increased intestinal epithelial permeability in the hindgut (Knudsen et al., 2008). The membrane of the intestine was disturbed, allowing foreign antigens to trigger inflammation in the gut (Knudsen et al., 2008; Merrifield et al., 2009). The soybean meal increased the permeability of distal intestinal epithelium, and reduced the capacity of nutrient absorption (Nordrum et al., 2000). Another hypothesis is that the combination of changes in gut microbiota and hindgut epithelial cell protective responses induced enteritis, due to increasing numbers and types of adherent bacteria in the gut of fish fed soybean meal (Bakke-McKellep et al., 2007). Soybean protein concentrate altered the bacteria composition in hindgut of Atlantic salmon, but mannan-oligosaccharide could ameliorate the intestinal-microbiome (Green et al., 2013).

2.4 Summary of the Current Knowledge and Thesis Objectives

Camelina oil and camelina meal contain sufficient quantities of linolenic (omega-3 fatty acid) and crude protein to make them attractive as feed ingredients. Camelina oil can replace all the fish oil without reducing the growth of Atlantic cod, rainbow trout, and Atlantic salmon post-smolt. However, there is a challenge in incorporating high levels of camelina meal in finfish diets due to some antinutritional factors and lower protein compared to fish meal. The antinutritional factors in camelina meal include glucosinolates, phytic acid, sinapine, tannins and trypsin inhibitor.

High fat residue camelina meal at 16% in the diet is acceptable for rainbow trout (Pan et al., 2011), but there is no literature concerning higher inclusion level of HOCM. The

combination of 100% CO and 10% SECM in the diet inhibits the growth of Atlantic salmon smolts (Hixson et al., 2014b). The reasons are unclear, and knowledge about the double substitution of low level CO and SECM is absent. Because the nutrient requirements differ at different weight range (NRC, 2011), and the physiology and living conditions of salmon alter after smoltification (Stefansson et al., 2008), studies at different salmon life stages should be conducted to determine appropriate inclusion levels of camelina byproducts.

The histological changes in salmon distal intestine, under the condition of soybean induced enteritis, has been described in detail, but the causative agent is not clear. The changes in distal intestine in salmon fed camelina have not been described in the scientific literature. The observations from soybean studies may be useful to study the intestine histology assessment of Atlantic salmon fed camelina products.

The objectives of this thesis were to investigate the optimal levels of various camelina byproducts as novel ingredients fed to Atlantic salmon at different life stages. The evaluation parameters included growth performance, fish carcass composition, and hindgut histology. In Chapter 3, Atlantic salmon parr were reared in freshwater, and fed test diets containing four graded levels of SECM (5, 10, 15 and 20%) and two levels of CO (50% and 100% replacing fish oil). In Chapter 4, Atlantic salmon post-smolts were reared in seawater, and the experimental diets included Control containing local fish oil and fish meal, 100% CO+solvent extracted fish meal (SEFM), 100% CO+10% solvent extracted camelina meal (SECM)+SEFM, 100% CO+fish meal (FM), 100% CO+10% SECM+FM, 8% SECM, 16% SECM, 24% SECM. In Chapter 5, Atlantic salmon smolts were fed three graded levels (8, 16, and 24%) of high oil residue camelina meal (HOCM) in freshwater.

Chapter 3 : The Effects of Camelina Oil and Solvent Extracted Camelina Meal on the Growth, Carcass Composition and Hindgut Histology of Atlantic Salmon Parr (*Salmo salar*) in Freshwater

3.1 Abstract

Generally, the camelina byproducts had no negative impacts on growth performance of Atlantic salmon parr in a 16 week trial. Fish (initial weight: 8.4g) were fed control, 5, 10, 15 or 20% solvent extracted camelina meal (SECM), or 50% and 100% camelina oil (CO) substituted for fish oil in diets. Fish were reared in 11.7 °C freshwater. The average final mean body weight was 46.7g. Weight gain from week 13 to week 16 and SGR among fish fed 15% SECM was inhibited, but no inhibition was detected in fish fed 20% SECM diet. Final condition factor, feed conversion ratio and hepatosomatic index were similar among all groups ($p \geq 0.05$). Feed consumption and specific growth rates were similar between control and all other groups ($p \geq 0.05$), these were higher in fish fed CO compared to SECM ($p < 0.05$). The safe upper range of glucosinolates was 1.8-3.3 μmol/g in the diet. Carcass protein, fat, ash and moisture were unaffected at week 16. Protein retention ratio was similar in all treatments. The length, width and area of hindgut villi were unaffected by treatments. Increased size of lamina propria was observed in 15 and 20% SECM groups. The promising diets were 50% CO, 100% CO, 5% and 10% SECM in this study, but 10% SECM should be used with caution.

3.2 Introduction

Intensive research on alternative plant meals and oils for fish feed has been conducted in recent years. Camelina (*Camelina sativa*) oil (CO) is rich in α-linolenic (18:3n-3, 35.6%) and linoleic (18:2n-6, 18.4%) (Hixson et al., 2013), and it can replace all fish oil in the diet without inhibiting fish growth (Morais, et al., 2012; Hixson et al., 2014a; Hixson et al., 2014b). Feeding CO to Atlantic salmon parr has not been studied, so 50% and 100% CO were tested in the present study. Freshwater fish and salmonid species can convert α-linolenic to docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) (reviewed by Tocher, 2003). Therefore, the shortage of DHA and EPA in camelina oil can be compensated somewhat by the high level of α-linolenic in CO via bio-synthesis.

Soybean meal has become a major plant protein to substitute for some degree of fish meal in salmonid feed (Carter and Hauler, 2000). However, the soybean induced intestinal enteritis in the distal intestine of Atlantic salmon remains a problem, and the causative agent is unknown (Van den Ingh et al., 1991; Baeverfjord and Krogdahal, 1996; Krogdahl et al., 2000; Refstie et al., 2000; Uran et al., 2008a). Canola meal, also belongs to the brassicaceae family, and can be included in salmoind diets up to 20% (Canola Council of Canada, accessed on June 17, 2014). In previous studies, high fat residue camelina meal

did not suppress the growth of rainbow trout (*Oncorhynchus mykiss*) at 20% in the diets (Bullerwell and Anderson, 2012). The growth of rainbow trout was not inhibited by 10% solvent extracted camelina meal (SECM) at 12 week, but was reduced at week 16 (Bullerwell, personal communication 2012). The carcass composition of rainbow trout fed 16% high fat residue camelina meal was unchanged compared to control (Pan et al., 2011). The SECM with higher protein (39%) is expected to be included at higher level in the diet, but the content of antinutritional factors tend to increase in this process. Based on previous studies, the levels of SECM tested in the current study were 5, 10, 15 and 20% in the diet.

Camelina meals and oil are potential alternative protein and lipid sources, respectively, but are not yet approved as feedstuffs by the Canadian Food Inspection Agency. The optimal levels of each type of camelina byproduct for Atlantic salmon needs to be investigated. To define the levels of these new ingridients to maintain gut health, it was necessary to evaluate the intestinal histology in the current investigation.

3.3 Objective

Based on previous studies, a range of camelina meal from 0 to 20%, and 50% and 100% CO substitute for fish oil were worth testing on Atlantic salmon. This study quantified growth performance, carcass composition, gut histology in Atlantic salmon parr fed practical diets containing four graded levels of SECM (5, 10, 15 and 20%) and two levels of CO (50% and 100% replacement of fish oil).

3.4 Hypothesis

The growth performance would be similar among fish fed control, 5%SECM, 10%SECM, 50%CO and 100%CO diets. Fish fed 15 and 20% SECM would show slower growth.

The carcass composition of all treatments would be similar to fish fed the control diet.

Fish fed 20% SECM diet would show histological changes in the hindgut compared to fish fed control diet.

3.5 Materials and Methods

3.5.1 Preparation of Test Ingredients

Camelina (Calena cultivar) was grown by Lyndhurst Farms Ltd. In Canning, Nova Scotia, Canada. Camelina oil was pressed from seeds using a KEK 0500 press at Atlantic Oilseed Processing Ltd., in Summerside, Prince Edward Island, Canada. Solvent extracted camelina meal was made in the Department of Plant and Animal Sciences nutrition lab in a fume hood at room temperature (20 ºC) away from any heat source. Camelina meal (400 g) was mixed with petroleum ether (Fisher Scientific product# E139-4) at a ratio of 1g meal / 3ml ether. Petroleum ether is composed of 50-60% pentane and isomers of hexane (Johnson, personal communication). The mixture was held in the beaker for one hour, and was stirred every 15 minutes. At the end of 1 hour, the ether was decanted and the wet camelina meal was placed on an absorbent pad in the fume hood for two hours to air dry. The composition of SECM was in Appendix A.

3.5.2 Feed Formulation and Preparation

Each of the seven diets was balanced to be isonitrogenous (44% crude protein) and isocaloric (4400 Kcal/kg) to meet National Research Council (NRC, 2011) requirements for Atlantic salmon. All ingredients are typical of those used in commercial salmon feed production in Atlantic salmon. A control treatment based on fish meal and fish oil, four diets including solvent extracted camelina meal (SECM) at 5%, 10%, 15% and 20%, and two diets where 50% or 100% of the fish oil is replaced by camelina oil (CO) were prepared. The fish oil level in SECM diets was the same as the control diet, excluding CO diets. The extra lipid required was added as camelina oil. Whey (2%) and gelatinized starch (4%) were added to help bind the feed particles together. To achieve the protein balance of feed formulations by including SECM, ingredients with high level of proteins were added, including wheat gluten meal (80%), Empyreal 75® (75%), poultry byproduct meal (66%), D/L methionine (Table 3.1). The essential amino acids were calculated according to the ingredient amino acid composition values listed in Nutrient Requirements of Fish and Shrimp (NRC, 2011) (Table 3.2). All diets met the requirements for essential amino acids. The major nutrients, minerals and glucosinolates were analysed (Table 3.3). The analysed protein levels were similar at 43±0.3%. The crude lipid levels increased (21.8-25.1%) as

SECM level increased in the diet, compared to the control diet (19.9%). The CO diets had similar lipid levels to the control diet (20.6% vs. 20.1% vs. 19.9%). The levels of calcium, phosphorus, sodium, potassium and magnesium were similar in all diets. In 50% CO diet, manganese (119.5 g/kg), copper (68 g/kg) and zinc (121.5 ppm) were lowest among all diets. Glucosinolates were not detected in the control and CO diets, and were positively correlated with the level of SECM in the experimental diets.

All diets were mixed according to the formulations (Table 3.1), and steam pelleted by a California pellet mill (San Francisco, USA), dried at 54 °C, and cooled to room temperature at the Chute Animal Nutrition Centre, Faculty of Agriculture, Dalhousie University.

Table 3.1 The formulations of seven practical diets for Atlantic salmon parr (% as fed basis).

Ingredient of diet	Control	SECM				CO	
		5%	10%	15%	20%	50%	100%
Wheat Gluten Meal	5	5	5	5	5	5	5
Poultry Byproduct Meal	5	5	5	5	5	5	5
Empyreal 75®[1]	15	15	15	15	15	15	15
D/L Methionine	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Vitamin Mineral Premix[2]	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Special Premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline Chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.05
Whey	2	2	2	2	2	2	2
Pregelatinized starch	4	4	4	4	4	4	4
Fish Meal	32.1	30.3	28.4	26.5	24.6	32.1	32.1
Fish Oil	16.1	16.1	16.1	16.1	16.1	8.0	0
Ground wheat	19.7	15	10.3	5.7	1.0	19.7	19.7
SECM	0	5	10	15	20	0	0
Camelina Oil	0	1.5	3.1	4.6	6.1	8.0	16.1
Total	100	100	100	100	100	100	100

SECM: solvent extracted camelina meal; CO: camelina oil.

1. Empyreal 75® is a high-energy corn protein concentrate manufactured by Cargill Corn Milling, Nebraska, USA.

2. Vitamin and mineral premix contains: zine 77.5 mg, manganese 125 mg, iron 84 mg, copper 2.5 mg, iodine 7.5 mg, vitamin A 5000 IU, vitamin D 4000 IU, vitamin K 2 mg, vitamin B12: 4 µg, thiamin 8 mg, riboflavin 18 mg, pantothenic acid 40mg, niacin 100mg, folic acid 4 mg, biotin 0.6 mg, pyridoxine 15 mg, inositol 100 mg, ethoxyquin 42 mg, wheat shorts 1372 mg (per kg of diet).

3. Special Premix contains per kg: selenium 0.22 mg, vitamin E 250 mg, vitamin C 200 mg, astaxanthin 60 mg, and wheat shorts 1988mg.

Table 3.2 The calculated nutrients in practical diets for Atlantic salmon (% as fed basis).

	Control	SECM				CO		Requirements[α]
		5%	10%	15%	20%	50%	100%	
CP (%)	44	44	44	44	44	44	44	44
DE(Kcal/kg)	4400	4400	4400	4400	4400	4400	4400	4400
Amino acids:								
Arginine	2.11	2.16	2.21	2.27	2.32	2.11	2.11	1.6-2.2
Histidine	1.04	1.04	1.05	1.05	1.06	1.04	1.04	0.7
Isoleucine	1.7	1.69	1.68	1.67	1.66	1.7	1.7	1
Leucine	4.09	4.09	4.08	4.07	4.07	4.09	4.09	1.6
Lysine	2.26	2.23	2.2	2.17	2.14	2.26	2.26	2.0-2.2
Methionine	1.18	1.17	1.16	1.15	1.14	1.18	1.18	0.7
Methionine+ Cysteine	1.65	1.65	1.66	1.67	1.67	1.65	1.65	1.1
Phenylalanine	1.93	1.93	1.94	1.94	1.94	1.93	1.93	1.7
Phenylalanine +Tyrosine	3.32	3.32	3.31	3.31	3.3	3.32	3.32	1.8
Threonine	1.48	1.49	1.5	1.51	1.52	1.48	1.48	1.1
Tryptophan	0.37	0.36	0.36	0.35	0.35	0.37	0.37	0.1-0.3
Valine	2.01	2.01	2.01	2.01	2	2.01	2.01	0.8-1.6

SECM: solvent extracted camelina meal; CO: camelina oil
CP: crude protein; DE: digestible energy
[α]Nutrients requirements for Atlantic salmon, National Research Council (NRC, 2011).

Table 3.3 Analysis of nutrients in practical diets for Atlantic salmon parr (% as fed basis).

	Control	SECM				CO	
		5%	10%	15%	20%	50%	100%
Crude protein (%)	43.6	42.9	43.4	43.1	42.8	43.5	43.2
Crude lipid (%)	19.9	21.8	22.5	25.1	25.1	20.6	20.1
Dry matter (%)	92.8	93.2	91.6	89.4	91.4	91.5	92.1
Calcium (%)	1.9	1.8	1.7	1.6	1.5	1.9	1.9
Total phosphorus (%)	1.2	1.2	1.2	1.1	1.0	1.2	1.2
Sodium (%)	0.4	0.3	0.3	0.3	0.3	0.4	0.4
Potassium (%)	0.4	0.5	0.5	0.5	0.6	0.4	0.4
Magnesium (%)	0.1	0.1	0.1	0.1	0.2	0.1	0.1
Manganese (mg/kg)	128.2	131.8	133.3	135.7	137.4	119.5	126.3
Copper (mg/kg)	7.3	9.2	9.5	8.3	12.0	6.8	11.0
Zinc (mg/kg)	133.7	132.4	138.1	138.9	131.5	121.5	132.1
Glucosinolates (μmol/g)[α]	0.0	1.8	3.3	5.5	7.4	0	0

SECM: solvent extracted camelina meal; CO: camelina oil
Feed was analysed at Nova Scotia Agriculture quality evaluation division laboratory services, 176 College Road, Harlow Institute, Truro, NS, Canada.
[α] Glucosinolates were analysed by HPLC at Agriculture and Agri-Food Canada, Saskatoon.

All feeds were stored at -20 °C until needed. The original pellet size was 2.5 mm diameter. A portion of each feed was crumbled to a suitable size of 0.75~1.0 mm particles (Jobling, 1993) and sifted on a screen. The smaller particles were fed to the small fish during the first four weeks. The size of feed increased to 1.4~2.0 mm from week 5 to week 8, then intact 2.5 mm pellets were used for the remaining period of the experiment.

3.5.3 Fish Rearing Conditions

Fish were sourced from the 2012 year class of St. John River stock at Big Falls Fish Grower Ltd., in Wolfville, Nova Scotia. There were 1050 Atlantic salmon parr (initial weight: 8.4±0.2 g) divided equally and randomly among 21 tanks, 50 fish per tank. The lower part of the tank is a cone shape with a height of 20cm, and the upper part of the tank is cylinder shape with a height of 45 cm and a diameter of 72cm. The volume of each tank is 210L. Each tank was supplied 3 L/min flow through freshwater. The average temperature and oxygen saturation were 11.7 °C and 120% respectively. Seven different diets were randomly assigned to tanks with three replicates per treatment. Fish were fed with the control diet for one week acclimation in the system. Fish were then hand fed test diets three times per day to satiation for 16 weeks, week 1 started from this time point (December 26, 2012). Faeces in all the tanks were purged after morning and afternoon feeding. This experiment was conducted in according with animal care protocol (File: 2012-016) approved by animal care committee in Faculty of Agriculture, Dalhousie University using the guidelines of the Canadian Council on Animal Care (2005).

3.5.4 Sampling

Food was withheld for one day prior to the sampling day. Average mean body weight per tank was determined every four weeks. Feed consumption was calculated every four weeks. At the beginning of the experiment, twenty-one fish, 1 fish per tank were euthanized using overdose of buffered tricaine methane sulfonate (TMS) for carcass analysis and six fish of the sampled fish were randomly selected for gut histology evaluation. Eleven muscle samples were randomly taken from the tanks, 1 fish per tank, and sent to Memorial University of Newfoundland (MUN) for other studies. At 8 weeks, 6 fish per tank were euthanized and dissected for gut histology analysis at Faculty of Agriculture, Dalhousie University and gut genomic study at the Ocean Sciences Centre of MUN. The same fish

were retained for carcass analysis. Gut samples for histology analysis included mid gut and hindgut. These samples were stored in 10% neutral buffered formalin (source: Fisher Scientific) in 20 ml vials. At 16 weeks, the sampling procedures were repeated as described for the 8-week sampling. In addition, muscle samples from three fish per tank were collected for another study at Ocean Science Centre of MUN. All sampled fish were weighed individually and the fork length measured. The liver from each sampled fish (6 fish per tank) was weighed at 16 weeks for hepatosomatic index, because nutrient deficiency can cause hepatocytes swollen (Ellis et al., 1978)

3.5.5 Calculations

The specific growth rate (SGR), feed conversion ratio (FCR), and protein retention ratio (PRR), Hepatosomatic index (HSI) and glucosinolates intake were calculated.

SGR = $\frac{\ln Mf - \ln Mi}{t} * 100$; M_f and M_i are the mean final and initial individual masses, t refers to time

FCR = $\frac{\text{Total feed fed}}{\text{body weight gain}} * 100$

PRR= $\frac{\text{(final body weight*final body protein content as wet weight basis)-(initial body weight*initial protein content as wet weight basis)}}{\text{total feed intake*Protein content in diet}}$

Condition factor (CF) = $\frac{\text{body weight}}{\text{fork length\^3}} * 100$

Hepatosomatic index (HSI) = $\frac{\text{liver weight}}{\text{body weight}} * 100$

Glucosinolates intake = $\frac{\text{Feed consumption} * \text{glucosinolate in the diet（μmol/g）}}{\text{time} * \text{(Initial body weight+final body weight)/2}} * 100$ (Burel et al., 2000)

3.5.6 Carcass Analysis

Fish carcass referred to the whole fish with the viscera and kidney removed. Twenty one fish sampled from week 1 were homogenized in a manual meat grinder. At 8 weeks carcass samples, six fish from each tank were homogenized together. However, at 16 weeks samples, three fish from the same tank were pooled as one carcass sample so that there were two mixed carcass samples for each tank. One hundred grams of wet sample was needed for carcass analysis of dry matter, crude protein, lipid and ash. All ground samples were weighed and stored at -20 °C for at least 24 hours. Samples were put into a ModulyoD

freeze dryer (Source: Thermo Fisher Scientific) for 24 hours and weighed again, and water content of carcass samples was calculated.

The freeze dried samples were ground using a small coffee grinder (Brand: Intertek) in preparation for analysis of crude protein, crude fat and ash. Dry matter in prepared samples was measured by AOAC International (2011) procedure (method no. 934.01, 2011). Crude protein was analysed using a Leco FP-528 nitrogen gas analysis, nitrogen*6.25. (AOAC, 2011; method no. 992.15). Crude fat was measured by using ANKOM XT15 solvent extraction system and ANKOMRD dryer (AOAC, 2011; method no. 920.39). Ash was measured as the residue that remained after 550 °C heating in Isotemp Muffle Furnace for at least 2 hours (AOAC, 2011; method no. 942.05). Each sample was analysed in two replicates.

3.5.7 Hindgut Histology Evaluation

The second segment of the mid-intestine (Figure 2.3, Chapter 2) was defined as distal intestine or hindgut. All samples were stored in 10% neutral buffered formalin. Histology samples were prepared by the Animal Health Laboratory, Agriculture and Food Operations Branch, Nova Scotia Dept. of Agriculture. A small piece of each sample was placed in a cassette and immersed in 10% buffered formalin. The cassettes were washed by cleaning solutions at 40 °C in a vacuum infiltration processor (Tissue-TEK VIP). Prepared cassettes were fixed in paraffin in Tissue-TEK TEC. Each sample was cut into three 5 microns cross sections using a microtome (LEICA RM2255). The cross sections were fixed in paraffin solution again and placed on slides. After 40 minutes drying at 62±1°C, the slides were stained with haematoxylin and eosin (H&E) in an auto-staining machine. Prepared slides were scanned by Nikon Coolscan 4000ED in the image lab, Haley Institute. Ten intact shaped villi were selected clockwise in each slide, and they were evenly distributed around the cross section. The 10 villi included one complex villus and nine simple villi. Tiny villi (<0.1mm) were not quantified. The length, width and area of a villus were measured respectively in the photographs by SigmaScan Pro 5 software. Mucosal fold, supranuclear vacuoles, lamina propria, goblet cells, and sub-epithelial mucosa were assessed using the semi-quantitative scoring system (Uran et al., 2008b). The slides were evaluated blindly,

and each parameter was scored from 1-5, and half point was given when it was difficult to distinguish the difference (Table 3.4). The higher value reflected more severe enteritis.

In this study, the mid gut was the section from the end of pyloric caeca to where the diameter increased. The posterior section was the hind gut, excluding the end segment close to the anus. There were two types of villi: complex and simple villus (Baeverfjord and Krogdahl, 1996). In a simple villus, there was only one central connective tissue. In contrast, a long central stroma connected with several simple villi was a complex villus. The outline looked like a branch. A villus consists of supranuclear vacuoles in epithelium and connective tissue in the centre(lamina propria). The basal layer with the same color as lamina propria that crosses all villi was defined as submucosa. Stratum compactum was next to the submucosa. Muscular layers including circular and longitudinal layers were between stratum compactum and serosa. Serosa was the outside layer. The goblet cells were present in the epithelium of the villi (Figure 3.1).

Table 3.4 The criteria of each morphology parameter measured in the hindgut of Atlantic salmon (adapted from Uran et al., 2008b).

Supranuclear Vacuoles (SNV)	**Goblet Cells (GC)**
1 Basal SNV size	1 Scattered cells
2 Some size reduction	2 Increased number and sparsely distributed
3 Diffused size reduction	3 Diffused number widely spread
4 Onset of extinction	4 Densely grouped cells
5 No SNV	5 Highly abundant and tightly-packed cells
Lamina Propria (LP)	**Sub-epithelial Mucosa (SM)**
1 Normal size LP	1 Normal SM
2 Increased size of LP	2 Increased size SM
3 Medium size LP	3 Medium size SM
4 Large LP	4 Large SM
5 Largest LP	5 Largest SM
Mucosal fold (MF)	
1 Basal length	
2 Some shrinkage and bloating	
3 Diffused shrinkage and onset of tissue disruption	
4. Diffused tissue disruption	
5 Total tissue disruption	

A value of 0.5 was given between each score if necessary
Images were taken from the current trial, and attached in Appendix C-G

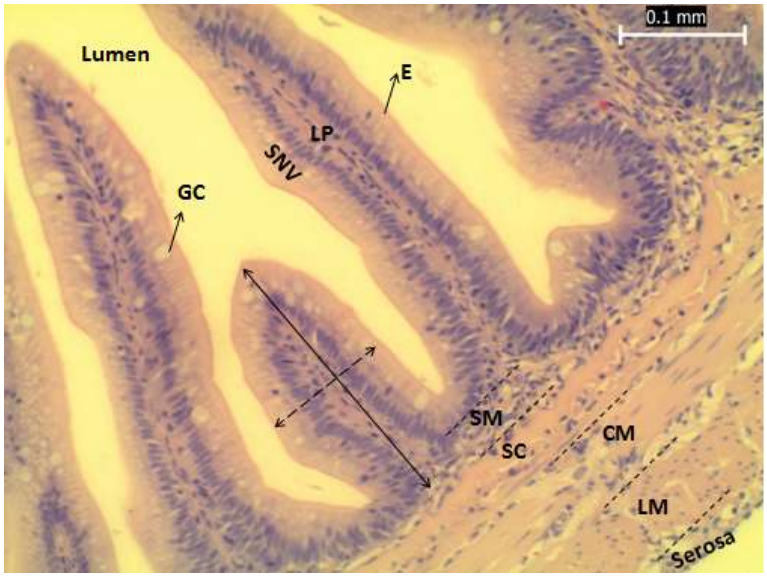


Figure 3.1 Morphology of the hindgut in Atlantic salmon parr fed the control diet (X200). Supranuclear vacuoles (SNV) in epithelium (E) and lamina propria (LP) in villi; connective tissue or called submucosa (SM) between the base of villi and stratum compactum (SC). The intestinal wall consists of SC, inner circular (CM) and an outer longitudinal (LM) layers, serosa is the outside layer of the wall. Double arrows (long arrow) and (dash arrow) showed the height and width of a villus respectively. Each layer in the wall was cut by dash lines (image was taken from the current study).

3.5.8 Statistical Analysis

The experiment was a completely randomized experiment with diet as the main effect. Analysis of variance was conducted using the Mixed model of SAS (Littell et al, 2006). If the residuals of data (all parameters measured except the scoring data) did not meet normality, an appropriate transformation was applied. When time was not a factor, one-way ANOVA with Tukey-Kramer test was applied. When time was a factor, the repeated measures statement (SAS 9.3) was included. The experimental unit was tank and the type of covariance structure was compound symmetry (CS). When the interaction of time and treatment was significant ($p<0.05$), then the data was sliced into each time point or period. If ANOVA was significant ($P<0.05$), Tukey-Kramer test (Hayter, 1984) as a conservative method was used to differentiate the means ($P<0.05$). Parameters measured included weight, weight gain, fork length, condition factor, specific growth rate (SGR), protein retention ratio (PRR), feed consumption, feed conversion ratio (FCR), hepatosomatic index (HSI), and carcass composition (% fat, ash, protein and dry matter).

Ho: there is no difference among dietary treatment (Ho: $\mu=0$)

Ha: there is a difference among dietary treatment (Ho: $\mu\neq0$)

The model for a single time point:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where: Y = the response of interest

μ = the overall mean

T_i = the effect of treatment (i= 1-7)

ε_{ij} = the effect of error

The model for over all time points:

$$Y_{ijk} = \mu + T_i + \beta_j + (T\beta)_{ij} + \varepsilon_{ijk}$$

Where: Y = the response of interest

μ = the overall mean

T_i = the effect of treatment (i = 1-7)

β_j = the effect of period (j = 1-4)

$(T\beta)_{ij}$ = the interactive effect of treatment and period (i = 1-7 and j = 1-4)

ε_{ijk} = the effect of error

The length, width and area of villi were analysed as one-way ANOVA, Tukey-Kramer test (Minitab 16.2.2). For scoring evaluation, Kruskal-Wallis test was conducted (Minitab 16.2.2). If it was significant ($P<0.05$), the Mann-Whitney U-test was applied to pairwise comparisons. Results were significantly different when p-values were less than 0.05. Parameters included mucosal fold (MF), goblet cells (GC), sub-mucosal (SM), lamina propria (LP) and supranuclear vacuoles (SNV).

3.6 Results

3.6.1 Growth Performance

All fish had normal feeding behavior and appetite, and their mean body weight increased over five-fold in 16 weeks. Only four fish died during the experiment. The significant interaction effects (week×trt) were seen in the parameters of body weight, weight gain and feed consumption (FC) ($p<0.05$) (Table 3.5). ANOVA indicated that body weight, weight gain and FC were significantly different between treatments at weeks 8, 12 and 16 ($p<0.05$).

However, the Tukey-Kramer test did not reveal the significant differences among means in weight gain at weeks 8 and 12, and FC at week 12.

In the first period (week 0-4), fish consumed similar amounts of feed among all treatments and had similar weight gain ($p \geq 0.05$) (Table 3.6). Between weeks 5-8, there were no significant differences between the control (7.5 g/fish) and any other treatments in feed consumption. However, fish had higher intake of 50% CO (8.5g/fish) than 15% and 20% SECM ($p<0.05$), and consumed more of 100% CO (9.1g/fish) than 10, 15 and 20% SECM ($p<0.05$). For weight gain, ANOVA showed $p<0.05$, but Tukey-Kramer test, as a conservative procedure, did not reveal significant differences in the weight gain among all dietary treatments. In the third period (weeks 9 to 12), all fish exhibited similar feed consumption and weight gain (Tukey-Kramer test did not reveal significant differences). In the final period (week 13-16), fish consumed similar amounts of test diets to the control diet (16.6 g/fish). The consumption of 50% CO (17.2 g/fish) was higher than 20% SECM (14.0 g/fish) ($p<0.05$), and the consumption of 100% CO (19.3 g/fish) was higher than the four SECM diets ($p<0.05$). The weight gain in fish fed 15% SECM diet (14.9 g/fish) was lower than that of fish fed control diet (18.3 g/fish) ($p<0.05$), and the weight gains in fish fed the other test diets were similar to that of fish fed control diet. From week 1 to week 16 (Table 3.7), total feed consumption of 50% CO (40.4 g/fish) was higher than 10%, 15% and 20%SECM ($p<0.05$). Fish consumed more 100% CO diet (43.6 g/fish) than any of the SECM diet ($p<0.05$).

All fish had similar initial mean weight (mean±SD: 8.4±0.2 g/fish). Final mean body weight in the other six treatments were similar to the control (49.2 g) ($p \geq 0.05$) (Table 3.7). Fish fed 50% and 100% CO (52.8 g and 52.1 g respectively) were heavier than 10%SECM (42.6g), 15%SECM (42.6 g) and 20% SECM at week 16 (42.5 g) ($p<0.05$). Fish fed either 50% CO (44.4 g/fish), 100% CO (43.9 g/fish) or 5% SECM (36.9 g/fish) diet exhibited a similar weight gain to fish fed the control diet (40.8 g/fish) ($p \geq 0.05$). By comparison, a lower weight gain was exhibited by fish fed 10%, 15% and 20% SECM diets (mean: 34.2 g/fish) compared to the control diet ($p<0.05$). Condition factor, feed conversion ratio (FCR), and (hepatosomatic index) HSI were independent of dietary treatments ($p \geq 0.05$). Specific growth rate (SGR) in the CO groups was higher than SECM groups ($p<0.05$). Fish fed 15%

Table 3.5 Significance of treatment, week and treatment×week on weight, weight gain and feed consumption of Atlantic salmon parr, and the effects of treatment at each sliced week point or period in Proc Mixed Model, SAS 9.3 ($p<0.05$ was significant).

Parameters	trt	week	week×trt	Sliced week				
				initial	4 or (1-4)	8 or (5-8)	12 or (9-12)	16 or (13-16)
Body weight	<.0001	<.0001	0.0031	0.9913	0.2111	<.0001	<.0001	<.0001
Weight gain	<.0001	<.0001	0.0104	-	0.6995	0.0026	0.0021	<.0001
Feed consumption	<.0001	<.0001	0.038	-	0.5698	<.0001	0.0001	<.0001

Table 3.6 The feed consumption (FC, gram fish^{-1}) and weight gain (WG, gram fish^{-1}) of Atlantic salmon parr fed different diets at four periods during 16 weeks.

	Weeks							
	0-4		5-8		9-12		13-16	
	FC	WG	FC	WG $^{\alpha}$	FC $^{\alpha}$	WG $^{\alpha}$	FC	WG
Control	3.7±0.2	3.4±0.3	7.5±0.2 abc	7.4±0.5	10.5±1.1	11.6±1.2	16.6±1.2 abc	18.3±1.7 ab
50%CO	3.4±0.4	3.6±0.6	8.5±0.4 ab	8.5±0.5	11.1±0.6	12.7±1.0	17.2±1.4 ab	19.6±1.6 a
100%CO	3.9±0.1	3.6±0.2	9.1±0.5 a	8.3±0.4	11.3±0.6	11.8±1.1	19.3±1.6 a	20.3±1.2 a
5%SECM	3.7±0.6	3.1±0.2	7.2±0.5 abc	6.7±0.3	10.0±1.2	10.9±0.9	15.7±0.1 bc	16.2±0.8 bc
10%SECM	3.5±0.2	2.7±0.4	6.6±0.3 bc	6.1±0.6	9.0±0.5	9.9±0.8	14.4±0.2 bc	15.4±1.0 bc
15%SECM	3.3±0.4	2.6±0.8	6.5±1.0 c	6.1±1.4	9.2±0.9	10.5±1.4	14.6±1.1 bc	14.9±0.4 c
20%SECM	3.5±0	2.8±0.1	6.8±0.4 bc	6.1±0.8	9.0±0.7	10.0±0.6	14.0±0.6 c	15.4±1.4 bc

CO: camelina oil; SECM: solvent extracted camelina meal

a-c: mean±SD, values with different letters in each column are significantly different ($p<0.05$)

The letter grouping of each parameter at each sliced period depended on the results of Turkey-Kramer test (Table 3.5)

$^{\alpha}$ $p<0.05$ (Table 3.5), but Tukey-Kramer test did not reveal any significant differences among treatments

Table 3.7 The growth performance, hepatosomatic index and protein retention ratio (mean±SD, per fish) of Atlantic salmon parr fed different diets for 16 weeks.

Treatments	Initial body weight (g)	Final body weight(g)	Final condition factor	FC(g)	SGR (% day^{-1})	FCR	HSI (%)	PRR (%)
Control	8.4±0.1	49.2±3.1 ab	1.06±0.04	38.4±2.0 abc	1.58±0.05 ab	0.94±0.04	1.24±0.15	38.4±2.1
50%CO	8.4±0.2	52.8±1.8 a	1.03±0.05	40.4±1.6 ab	1.65±0.03 a	0.91±0.02	1.06±0.09	40.5±1.9
100%CO	8.2±0.3	52.1±2.5 a	1.08±0.05	43.6±2.7 a	1.65±0.07 a	0.99±0.02	1.18±0.13	36.7±0.4
5%SECM	8.4±0.1	45.3±0.1 ab	1.03±0.02	36.8±2.2 bc	1.51±0.01 bc	0.99±0.06	1.11±0.02	36.8±2.6
10%SECM	8.5±0.4	42.6±5.5 b	1.03±0.05	33.6±1.1 c	1.44±0.05 bc	0.99±0.05	1.15±0.19	35.9±2.0
15%SECM	8.5±0.3	42.6±3.1 b	1.00±0.07	33.6±3.4 c	1.44±0.04 c	0.98±0.02	1.11±0.07	36.0±0.9
20%SECM	8.3±0.3	42.5±1.8 b	1.02±0.00	33.3±1.4 c	1.46±0.07 bc	0.97±0.03	1.17±0.03	36.8±0.6
p-value	0.9913	<0.0001	0.4633	0.0002	0.0002	0.1011	0.6065	0.0517

Initial measurement: n=50 per tank, Final measurement: n= 32-44 per tank
CO: camelina oil; SECM: solvent extracted camelina meal
FC: feed consumption (per fish over 16 weeks), FCR: feed conversion ratio, SGR: specific growth ratio, HSI: hepatosomatic index, PRR: protein retention ratio
a-c: mean±SD, values with different letters in each column are significant different ($p<0.05$)

SECM diet grew slower (1.44 % day^{-1}) than fish fed the control (1.58% day^{-1}) ($p<0.05$). The protein retention ratio after 16 weeks was similar in all treatments.

3.6.2 Carcass Composition

Crude protein content in fish carcass was not affected by the interaction of week and treatment (Table 3.8). The initial carcass protein was 15.9% on wet weight basis (Table 3.9). It was independent of dietary treatment or time. However, the mean by treatment in fish fed 50% CO diet was higher than the fish fed 10-20% SECM diets. The interaction of week and treatment was significant in carcass lipid (Table 3.8). There were no significant differences between test diets and the control diet at both week 8 and week 16. The crude lipid content in fish carcass increased from 6.5% at week 0 to 10.3% at week 16 (Table 3.9). Ash content in fish carcass was not affected by the interaction, time and treatment (Table 3.8). Moisture content in fish carcass was affected by time and treatment alone, but was independent of the interaction of time and treatment (Table 3.8). It was 72.5% at week 0, and decreased from 71.7% at week 8 to 69.7% at week 16 (Table 3.9). Mean by treatment was lower in fish fed 100% CO diet compared to the fish fed the control diet.

Table 3.8 P-values for treatment (trt), week and treatment×week on carcass composition (crude protein, crude lipid, ash and moisture) of fish fed various experimental diets at week 8 and week 16.

Parameters	trt	week	week×trt
Crude protein	0.0061	0.2836	0.6898
Crude lipid	0.0153	<0.001	0.003
Ash	0.0829	0.1756	0.0610
Moisture	0.0374	<0.001	0.3231

In Proc Mixed Model, SAS 9.3 ($p<0.05$ was significant).

Table 3.9 Carcass composition of Atlantic salmon parr fed various experimental diets for 16 weeks (mean±SD, on wet weight basis).

	Control	Camelina oil		Solvent extracted camelina meal				Mean by week
		50%	100%	5%	10%	15%	20%	
Crude protein (%)								
Initial	15.9±0.1							
Wk 8	15.3±0.2	16.1±0.7	15.8±0.3	15.4±0.2	15.3±0.2	15.4±0.3	15.3±0.3	15.5±0.5
Wk 16	15.8±0.4	16.0±0.4	15.8±0.3	15.7±0.2	15.4±0.3	15.4±0.1	15.4±0.2	15.6±0.3
Mean by trt	15.5±0.4 ab	16.0±0.6 a	15.8±0.3 ab	15.6±0.2 ab	15.3±0.3 b	15.4±0.2 b	15.4±0.3 b	
Crude lipid (%)								
Initial	6.5±0.0							
Wk 8	8.0±0.1ef	8.7±0.7 cdef	9.4±0.1bcde	8.3±0.5 def	7.9±0.2 f	8.0±0.5 ef	8.8±0.4 cdef	8.4±0.6
Wk 16	9.7±0.4 abcd	9.9±0.2 abc	10.0±0.3 abc	9.8±0.9 abcd	10.6±0.5 ab	11.1±0.6 a	11.0±0.4 a	10.3±0.7
Mean by trt	8.9±0.9	9.3±0.8	9.7±0.4	9.0±0.1.0	9.3±1.5	9.5±1.7	9.9±1.1	
Ash (%)								
Iinitial	2.9±0.1							
Wk 8	1.8±0.3	2.1±0.3	2.1±0.3	2.4±0.1	2.4±0.1	2.3±0.3	2.2±0.2	2.2±0.3
Wk 16	2.3±0.1	2.3±0.2	2.2±0.4	2.3±0.2	2.3±0.2	2.2±0.3	2.3±0.3	2.3±0.2
Mean by trt	2.0±0.3	2.2±0.3	2.2±0.4	2.3±0.2	2.3±0.2	2.3±0.3	2.3±0.2	
Moisture (%)								
Initial	72.5±0.0							
Wk 8	72.6±0.6	70.9±1.2	70.4±1.1	71.8±1.2	72.6±0.3	72.3±0.6	71.7±0.3	71.7±1.1a
Wk 16	70.2±0.4	69.7±0.3	69.2±1.0	69.9±1.0	69.5±1.4	69.5±0.3	69.6±0.4	69.7±0.7 b
Mean by trt	71.4±1.4 a	70.3±1.0 ab	69.8±1.1 b	70.9±1.4 ab	71.1±1.9 ab	70.9±1.6 ab	70.6±1.2 ab	

Wk: week; trt: treatment

a-f: mean±SD, Values with different letters refer to significant differences

Initial values were not included in the statistical analysis

3.6.3 Hindgut Histology

The length and area of simple villi in the hindgut increased from week 1 to week 16, but the width did not change (Table 3.10). The length, width and area of simple villi were unaffected by the treatment ($p \geq 0.05$). The length of simple villi in the hindgut grew from 0.36 to 0.59 mm in the fish fed the control diet, and the area increased from 0.05 to 0.08 mm^2, but the width (0.13 mm) did not change after 16 weeks. The size of complex villi (length, width and area) in the hindgut increased from week 1 to week 16. There were no significant differences among treatments ($p \geq 0.05$).

Table 3.10 The length, width, and area of simple villi and complex villi (mean±SD) in Atlantic salmon parr hindgut at Week 16 (n=18 per treatment).

	Simple villi			Complex villi		
	Length (mm)	Width (mm)	Area (mm^2)	Length (mm)	Width (mm)	Area (mm^2)
Initial	0.36±0.07	0.13±0.04	0.05±0.02	1.66±0.67	0.30±0.06	0.57±0.27
Week 16						
Control	0.59±0.14	0.13±0.02	0.08±0.03	2.95±1.36	0.63±0.30	2.30±2.11
50%CO	0.53±0.11	0.14±0.02	0.08±0.02	2.64±1.04	0.52±0.17	1.28±0.58
100%CO	0.60±0.12	0.14±0.02	0.09±0.02	2.07±1.08	0.55±0.25	1.14±0.76
5%SECM	0.57±0.14	0.14±0.03	0.09±0.03	3.31±1.53	0.57±0.28	2.02±1.40
10%SECM	0.59±0.18	0.15±0.02	0.09±0.03	2.99±1.23	0.86±0.35	2.34±1.59
15%SECM	0.55±0.15	0.13±0.02	0.08±0.02	2.09±0.67	0.62±0.25	1.28±0.61
20%SECM	0.63±0.15	0.15±0.03	0.10±0.04	2.76±1.46	0.56±0.29	1.57±1.04
P-value	0.66	0.015[α]	0.331	0.12	0.146	0.099

CO: camelina oil; SECM: solvent extracted camelina meal

[α] $p<0.05$ in ANOVA, but Tukey-Kramer test did not show significant differences.

There was no treatment effect on MF, SNV, GC and SM in the hindgut at week 16 (Table 3.11). The mean scores of MF, SNV, GC and SM were all under 2.5. The higher score represented worse conditions in gut. Some moderate morphological changes were observed, but there was no sign of severe inflammation. In the lamina propria, the control (1.7) was significantly lower than 15% (2.3) and 20% SECM (2.5) ($p<0.05$), but was significantly higher than 50% CO (1.2) ($p<0.05$). There was no difference between CO groups ($p \geq 0.05$).

Among the SECM groups, 5%SECM was significantly lower than 20% SECM, 10% SECM was significantly lower than 15 and 20% SECM ($p<0.05$).

Table 3.11 The scores (mean±SD) of different histological parameters in Atlantic salmon parr hindgut (n=18 per treatment).

	MF	SNV	GC	LP	SM
Control	1.4±0.7	1.6±0.7	1.7±0.8	1.7±0.5	1.0±0
50%CO	1.6±0.6	2±0.8	1.7±0.9	1.2±0.4	1.0±0
100%CO	1.8±0.9	2±1.0	2.1±0.8	1.4±0.6	1.0±0
5%SECM	1.8±0.9	1.6±0.8	1.8±0.8	1.8±0.9	1.0±0
10%SECM	1.8±0.9	1.6±0.6	2.1±0.7	1.9±0.6	1.0±0
15%SECM	2.3±1.1	2.2±0.9	1.8±0.4	2.3±0.5	1.0±0
20%SECM	1.9±0.7	2.1±0.5	2.1±0.5	2.6±0.8	1.0±0
Kruskal-Wallis Test	0.148	0.094	0.234	0.000	1.000
Mann-Whitney test					
Control vs 50%CO	ns	ns	ns	0.015	ns
Control vs 100%CO	ns	ns	ns	NS	ns
Control vs 5%SECM	ns	ns	ns	NS	ns
Control vs 10%SECM	ns	ns	ns	NS	ns
Control vs 15%SECM	ns	ns	ns	0.001	ns
Control vs 20%SECM	ns	ns	ns	0.0003	ns
50%CO vs 100%CO	ns	ns	ns	NS	ns
5%SECM vs 10%SECM	ns	ns	ns	NS	ns
5%SECM vs 15%SECM	ns	ns	ns	NS	ns
5%SECM vs 20%SECM	ns	ns	ns	0.0248	ns
10% SECM vs 15%SECM	ns	ns	ns	0.0148	ns
10% SECM vs 20%SECM	ns	ns	ns	0.0023	ns
15%SECM vs 20%SECM	ns	ns	ns	NS	ns

CO: camelina oil; SECM: solvent extracted camelina meal
MF: mucosal fold; SNV: superanuclear vacuoles; GC: goblet cell; LP: lamina propria; SM: sub-mucosal
NS: no significant difference

3.7 Discussion

3.7.1 Growth Performance

The result supported a previous study indicating that CO could efficiently replace 100% of fish oil in fish diets without compromising growth of rainbow trout (Hixson et al., 2014a). The differences in weight gain and feed consumption between CO and SECM groups (Table 3.6) demonstrated that weight gain was positively associated with the feed consumption. Mawson et al. (1993) indicated that the reduction of palatability in the diet was due to the increased dose of glucosinolates, and the palatability was improved by adding low glucosinolate rapeseed meal (1-5 μmol/g glucosinolates) at 30% in the diet for calves and dairy cows. The feed consumption between the control and the SECM diets was statistically similar. Therefore, the range of glucosinolates, in the current diets between 1.8-7.4 μmol/g did not reduce feed consumption of Atlantic salmon parr. The similar FCR demonstrated that fish could metabolize camelina diets equally as control diet.

Atlantic salmon parr (initial weight: 5.5 g) showed lower final weights when fed plant protein blends (soybean protein concentrate, wheat gluten meal and corn gluten meal) substitute for 50, 66, and 87% fish meal in the diets (Burr et al., 2012). In the current study, SECM was added at an inclusion rate in the diet, but was partial replacement of fish meal. It accounted for up to 23% replacement of fish meal (32% in the control diet) in 20% SECM, which was much lower than 50% replacement of fish meal stated by Burr et al. (2012). In a later trial (Burr et al, 2012), the growth of Atlantic salmon (initial weight: 31.5 g) fed diets where fish meal was completely replaced by plant protein blends or land animal protein blends was as good as control fed fish. These different results demonstrated that late stage juvenile salmon could tolerate non-fish meal protein better than the early juvenile stage. By contrast, Refstie et al. (2000) indicated that 44% reduction of body weight in large salmon (initial weight: 200g) was found, when 37% of fish meal was substituted by soybean meal in the diet. Canola protein concentrate could replace 13-16% of dietary protein fed to juvenile chinook salmon without inhibiting somatic growth (Higgs et al., 1982). In the current study, camelina meal comprised up to 17.7% of dietary protein in 20% SECM diet, and this test diet did not affect the SGR.

The amount of glucosinolate per kg fish body weight consumed per day was 87 μmol/kg /day (not listed in results) in the diet containing 20%SECM, following the calculation method of Burel et al.

(2000). This value was about two-fold greater than 30-47 μmol/kg fish/day, which compromised growth of rainbow trout in a 9-week trial (Burel et al., 2000). The growth reduction was induced by the glucosinolate breakdown products, and the threshold level of glucosinolates and their byproducts was suggested to be 3.7 μmol/g of diet (Burel et al., 2001). The level of glucosinolates byproducts in the present diets was unknown, but 15 and 20% SECM diets contained total glucosinolates over 3.7 μmol/g diet. Fish fed 15% SECM did show lower weight gain (week 13 to 16) and SGR compared to fish fed the control diet (SGR: 1.44 vs 1.58), but fish fed 20% SECM diet did not. The reason was not clear. Rainbow trout fed the diet containing 30% of rapeseed meal had similar growth to the fish fed the control diet for two months, because of the triiodothyronine (T_3) in plasma was compensated by Thyroxine (T_4) (Burel et al., 2001). It might be valid in Atlantic salmon parr, so total glucosinolates up to7.4 μmol/g in the diets did not trigger severe body weight reduction in 16 weeks in this study. A longer trial may need to justify the negative effects of high level of SECM. The proximate levels of other antinutritional factors can be estimated according to the values reported in the literatures (Chapter 2). In the 20% SECM diet, the proximate levels of tannins, sinapine and phytic acid in the diet were likely in the range 0.02-0.053%, 0.038~0.093% and 0.42% respectively. The concentration of tannins was much lower than 0.57-1.14%, which partially inhibited the growth of carp (Hossain and Jauncey, 1989). The differences among species should be taken into account. The detrimental level of sinapine remains unknown. The calculated phytic acid was lower than the safe level for Atlantic salmon parr at 0.47-1.0% (Denstadli et al., 2006).

3.7.2 Carcass Composition

Carcass protein and protein retention ratio were not affected by the concentration of SECM, which were in agreement with the results in other studies using plant meal inclusion diets for Atlantic salmon (Carter and Hauler, 2000; Espe et al., 2006; Overland et al., 2009). All treatment groups had a similar FCR, indicating they received adequate amounts of protein and deposited dietary protein in growing tissues without withdrawing protein from less vital tissues (Lall and Anderson, 2005). Although 50% and 100% CO groups showed similar levels of crude fat as the control, it was reported that CO reduced the long-chain PUFA levels and elevated 18:3n-3 and 18:2n-6 levels in Atlantic cod (Morais et al., 2012; Hixson et al., 2013).

3.7.3 Hindgut Histology

The structure of villi was not affected by feeding SECM or CO, but the structure grew as fish size increased. The length of simple villi in the fish fed control diet was lower than 1 mm in 550g salmon (Baeverfjord and Krogdahl, 1996), but higher than 0.26 mm in 108g salmon (Sanden et al., 2005). The length, width and area of complex villi increased after 16 weeks, but were highly variable (Table 3.10). The morphology of simple villi in Atlantic salmon was depressed by feeding 33% soybean meal (Baeverfjord and Krogdahl, 1996), but not by feeding 12.5% soybean meal or 12.1% maize (Sanden et al., 2005). It demonstrated that the morphological changes depended on the dose of plant ingredient. In the present study the gut morphology indicated 20% SECM inclusion rate was acceptable.

Fish gastro-intestinal tracts were collected after one day of fasting in the current study. Morphological changes can be initiated after two days of starvation (Baeverfjord and Krogdahl, 1996). In the fish fed control diet, mucosal fold (MF) or villi had slight shrinkage and bloating (mean score: 1.4), supranuclear vacuoles (SNV) with some size reduction (mean score: 1.6), goble cells (GC) with increased number and sparse distribution (mean score: 1.7), LP with increased size (mean score: 1.7) and sub-mucosa (SM) was normal (mean score: 1.0) (Table 3.11). Higher temperature (12 °C vs 8 °C) increased the metabolic rate in fish, and enhanced the soybean induced enteritis process in 20 days (Uran et al., 2008b). Camelina byproducts caused somewhat less moderate inflammation signs, but only fish fed 15% and 20% SECM diets differed from the fish fed control at 12 °C (Table 3.11). This study agreed with the dose-dependent theory (Krogdahl et al., 2003; Uran et al., 2009). Increased size of LP was present in fish fed 15% (2.3) and 20% SECM diets (2.6). There was no literature describing the effects of antinutritional factors in camelina on gut histology. The widened LP might be due to the infiltration of eosinophilic granular cells, macrophages and polymorphnuclear leucocytes into LP and the epithelial lining (Baeverfjord and Krodahl, 1996), and the increased levels of immunoglobulin M (*Ig* M) and neurophilic granulocytes in LP (Bakke-McKellep et al., 2000). When Atlantic salmon were under stress and infection conditions, neutrophils and eosinophils had high invasive capacity and activation reaction, as a result of penetration in the intestinal epithelium (reviewed by Rombout et al., 2011) and the LP was widened. Defense responses in hindgut epithelial cells and increased number and diversity of microbiota were reported in soybean induced enteritis (Bakke-McKellep et al., 2007). Surprisingly, fish fed 50% CO (1.2) showed better LP condition than fish fed the control diet (1.7)

($p<0.05$). This could not be explained on the basis of rudimentary state of knowledge in the published literature.

3.8 Conclusion

Fish fed test diets had similar growth performance to the fish fed control diet, except fish fed 15% SECM showed lower SGR. The glucosinolates at 1.8-3.3 μmol/g did not trigger significant reduction in feed consumption and growth performance. Carcass composition (protein, fat, ash and moisture as wet weight basis) and protein retention ratio in fish fed camelina byproduct diets were similar to the control at week 16. The microstructure (length, width and area) of hindgut villi were not altered by SECM or CO. However, lamina propria in the hindgut villi were widened in both of 15% SECM and 20% SECM. Further study on gut immunology in fish fed camelina byproducts is needed. This study recommended 50% CO, 100% CO, 5% and 10% SECM in parr diet, but 10% SECM should be used with caution.

Chapter 4 : The Effects of Camelina Oil and Solvent Extracted Camelina Meal on Growth Performance, Carcass Composition and Hindgut Histology in Atlantic Salmon (*Salmo salar*) Post-smolts in Seawater

4.1 Abstract

Atlantic salmon post-smolts (initial mean SD weight: 242±46 g) were fed fish meal and oil based control diets, three graded levels (8, 16, and 24%) of solvent extracted camelina meal (SECM), and four diets containing camelina oil (CO) replacing fish oil (100% CO, 100% CO+solvent extracted fish meal (SEFM), 100% CO+SEFM+10% SECM, and 100% CO+10% SECM) for 16 weeks. They were reared at 14 °C seawater. SGR and FCR were not affected by treatments. Weight gain was not compromised by feeding 100% CO (385±33 g) and 8% SECM (391±99 g) diets compared to the control group (508±12 g), but was lower in fish fed 16% SECM, 24% SECM, and complex 100% CO diets. Carcass compositions (fat, protein, ash and moisture) were similar among all treatments ($p \geq 0.05$). CO and SECM did not affect the villi length, width and area compared to controls ($p \geq 0.05$). However, severe diffusion of supranuclear vacuoles and thicker sub-mucosal layer were observed in 24% SECM. This study indicated 8% inclusion of SECM and up to 100% CO in practical diets for Atlantic salmon post-smolts. It indirectly reflexed that 2.6 μmol/g glucosinolates did not inhibit the growth.

4.2 Introduction

Feed cost accounts for up to 50% of total budget in a salmon sea-cage farm. Fish meal and fish oil are the two key ingredients in salmon feed, but the prices of these two ingredients have substantially increased in recent years. Alternative protein and lipid sources are required to achieve economic, sustainable salmon production. Full substitution of fish oil by camelina oil (CO) can be applied in Atlantic cod (Hixson et al., 2013), rainbow trout (Hixson et al., 2014a) and Atlantic salmon parr (Chapter 3) diets without negative growth response. Camelina oil is deficient in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), while rich in α-linolenic acid (18:3n-3). Salmonid species are able to convert 18:3n-3 fatty acid to DHA (Buzzi et al., 1997; Tocher et al., 1997 and Hastings et al., 2004), but the conversion rate was not clear. In addition, the fish oil residue in the fish meal supplies small amounts of EPA and DHA. The effect of a fish oil devoid diet on Atlantic salmon growth remains unknown. Thus, solvent extracted fish meal (SEFM), a fish oil-free meal, was used to replace the regular fish meal. The effects of SECM, CO or the combination of both on the growth of Atlantic salmon post-smolt are not clear. Up to 20% SECM did not reduce the final body weight of parr (Chapter 3). Atlantic salmon post-smolt have lower dietary protein requirement than parr (40 vs. 44-48%) (NRC, 2011), thus a higher level of SECM (24 vs. 20% in Chapter 3) was tested in this study due to differences in the two life stages.

The carcass composition of parr was independent of dietary treatments (Chapter 3), but the effects of camelina byproducts on carcass composition of post-smolts remain unknown. Widened lamina propria was evident in the parr fed 15 and 20% SECM diets. This condition was considered as hindgut enteritis (Chapter 3). The histological evaluation for the parr study should be conducted at post-smolts. Post-smolts had decreased paracellular permeability in the posterior intestine, and transcellular pathway became dominant in directing water flow in post-smolts (Sundell et al., 2003). Therefore, post-smolts fed diets containing camelina byproducts may show different responses in the hindgut histology.

The growth result including CO diets has been published by Hixson et al. (2014b), the statistical method was a three-way nested ANOVA in a General Linear Model (individual fish as a unit) or two-way ANOVA (tank as a unit). In current study, the growth data of fish fed CO diets and SECM diets were analysed by repeated measures in a Mixed Model (tank as a unit). Carcass analysis and the evaluation of hindgut histology were the unique parts of this study.

4.3 Objectives

This study quantified the growth performance, carcass composition (moisture, protein, fat and ash) and gut histology of Atlantic salmon smolts fed eight practical diets: Control containing local fish oil and fish meal, 100% CO+solvent extracted fish meal (SEFM), 100% CO+10 %SECM+SEFM, 100% CO+ fish meal (FM), 100% CO+10% SECM+FM, 8% SECM, 16% SECM, 24% SECM.

4.4 Hypothesis

The growth of fish fed 100% CO+SEFM, 100% CO+10% SECM+SEFM and 24% SECM diets would be compromised. Fish fed the other test diets would show similar growth response to fish fed the control diet.

The carcass composition (moisture, protein, fat and ash) would be similar among all treatments.

Diets containing 16% and 24% SECM would have negative impact on the gut histology in fish.

4.5 Materials and Methods

4.5.1 Preparation of Test Ingredients and Feed Formulations

The study used a variety of camelina called Calena. The preparation of camelina oil (CO) and solvent extracted camelina meal (SECM) were the same as described the trial on parr (Chapter 3). Diets were formulated to be isonitrogenous (44%) and isocaloric (4400 Kcal/kg, Table 4.1)

according to the nutrient requirements for Atlantic salmon (NRC, 2011). The control diet did not contain any camelina byproduct and solvent extracted fish meal (SEFM). The amount of fish meal, fish or camelina oil and wheat were adjusted to achieve balanced diets. These formulations included two parts: 100% camelina oil (100% CO) and graded levels of solvent extracted camelina meal (SECM) basis. The abbreviation '100% CO' means all fish oil was replaced by CO. SEFM represented defatted fish meal, but still contained 2.58% crude fat as fed basis, while FM contained 7.94% crude fat. The approximate fish oil content were 0.84% as fed basis in 100% CO+SEFM

Table 4.1 The formulations of eight practical diets for Atlantic salmon smolts (% as fed basis).

Ingredients	Control	100% Camelina oil				SECM		
		SEFM	10%SECM +SEFM	FM	10%SECM +FM	8%	16%	24%
Wheat Gluten Meal	15	15	15	15	15	15	15	15
Empyreal 75®	5	5	5	5	5	5	5	5
D/L Methionine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin Mineral Premix [α]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Special Premix [β]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline Chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Whey	5	5	5	5	5	5	5	5
Pregelatinized starch	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
FM	34.9	-	-	34.9	31.2	31.9	28.9	25.9
SEFM	-	32.9	29.3	-	-	-	-	-
SECM	0	0	10	0	10	8	16	24
Fish Oil	14	0	0	0	0	16.5	19	21.4
Camelina Oil	0	17.8	20.5	14	17.1	0	0	0
Ground wheat	22.4	20.7	11.6	22.4	13.1	15	7.5	0.1
Total	100	100	100	100	100	100	100	100

FM: fish meal; SEFM: solvent extracted fish meal; SECM: solvent extracted camelina meal;

[α] Vitamin and mineral premix: zine 77.5 mg, manganese 125 mg, iron 84 mg, copper 2.5 mg, iodine 7.5 mg, vitamin A 5000 IU, vitamin D 4000 IU, vitamin K 2mg, vitamin B12: 4 µg, thiamin 8mg, riboflavin 18 mg, pantothenic acid 40mg, niacin 100mg, folic acid 4 mg, biotin 0.6 mg, pyridoxine 15mg, inositol 100mg, ethoxyquin 42mg, wheat shorts 1372mg (per kg of diet).

[β] Special Premix: selenium 0.22 mg, vitamin E 250 mg, vitamin C 200 mg, astaxanthin 60 mg, and wheat shorts 1988mg (per kg of diet).

diet, and 2.77% in 100% CO+FM diets. When 10% of SECM was included in the diet, it was described as 10% SECM.

All diets were mixed according to the formulations, then steam pelleted by a California pellet mill at Chute Animal Nutrition Centre, Faculty of Agriculture, Dalhousie University, dried at 54 °C, cooled down, bagged and stored at frozen (-20 °C) until used. Pellet sizes used were 4mm and

6mm depending on fish size. The calculated essential amino acids in each diet met the requirements of NRC, 2011 (Table 4.2).

Table 4.2 The calculated nutrients in practical diets for Atlantic salmon smolts (% as fed basis).

		100%CO				SEFM			
	Control	SEFM	10%SECM+SEFM	FM	10%SECM+FM	8%	16%	24%	NRC[α]
CP (%)	44	44	44	44	44	44	44	44	44
DE (4400 kcal/kg)	4400	4400	4400	4400	4400	4400	4400	4400	4400
Amino acids:									
Arginine	2.18	2.1	2.21	2.18	2.29	2.27	2.36	2.45	1.6-2.2
Histidine	1.1	1.06	1.07	1.1	1.11	1.11	1.11	1.12	0.7
Isoleucine	1.77	1.71	1.69	1.77	1.75	1.75	1.73	1.71	1
Leucine	3.45	3.34	3.33	3.45	3.43	3.43	3.42	3.41	1.6
Lysine	2.67	2.56	2.51	2.67	2.61	2.62	2.58	2.53	2.0-2.2
Methionine	1.18	1.14	1.12	1.18	1.15	1.16	1.14	1.12	0.7
Methionine +Cysteine	1.51	1.46	1.48	1.51	1.53	1.52	1.53	1.55	1.1
Phenylalanine	1.93	1.87	1.89	1.93	1.94	1.94	1.94	1.95	1.7
Phenylalanine +Tyrosine	2.95	2.84	2.85	2.95	2.94	2.95	2.94	2.93	1.8
Threonine	1.43	1.36	1.39	1.43	1.44	1.44	1.45	1.47	1.1
Tryptophan	0.47	0.46	0.45	0.47	0.46	0.46	0.46	0.45	0.1-0.3
Valine	2.02	1.95	1.95	2.02	2.02	2.02	2.02	2.01	0.8-1.6

CO: camelina oil; FM: fish meal; SEFM: solvent extracted fish meal; SECM: solvent extracted camelina meal; CP: crude protein; DE: digestible energy

[α] Nutrients requirements for Atlantic salmon, National Research Council (NRC, 2011).

4.5.2 Experimental Fish

Atlantic salmon (Saint John River stock) were transferred from the hatchery (freshwater), Cooke Aquaculture (St. Alban's NL, Canada) to the Joe Brown Aquatic Research Building (JBARB) (seawater), Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NL, Canada. The post-smolts (1200 fish) were randomly assigned to 24 tanks (500L), with 50 fish per tank. Each diet was randomly assigned to triplicate tanks. One week acclimation for the control diet was provided prior to initial sampling. The trial commenced on August 2, 2012. The initial

weight was about 242 g per fish. All fish were reared in a flow through system with filtered seawater (14.3±0.7 °C, 12 L/min), and supplied with dissolved oxygen (>10 mg/L). The post-smolts were fed to apparent satiation under a photoperiod of 12 h for 16 weeks. All feed fed to the fish was recorded every week. This experiment was conducted in according with Animal care protocol (12-50-Mar) approved by the Institutional Animal Care Committee of Memorial University of Newfoundland using the guidelines of the Canadian Council on Animal Care (2005).

4.5.3 Sampling

Fish were deprived of feed for 24 hours before sampling. Fish body weight, fork length, viscera weight were measured on individual fish at week 0 (n=3 per tank), week 8 (n=7 per tank) and week 16 (n=12 per tank). Each fish was euthanized by an overdose of buffered tricaine methane sulfonate (TMS). At week 16, viscera was removed from three fish per tank, the carcasses were stored at minus 20 °C. Hindgut samples were collected from three fish per tank, and stored in 10% neutral buffered formalin.

4.5.4 Calculations

The weight gain, feed consumption, specific growth ratio (SGR), feed conversion ratio (FCR), condition factor and viscera index (VI) were calculated at week 16.

SGR = $\frac{\ln Mf - \ln Mi}{t} * 100$; M_f and M_i are the mean final and initial individual masses, t refers to time

FCR = $\frac{\text{Total feed fed to tank}}{\text{tank biomass gain}} * 100$

Condition factor (CF) = $\frac{\text{body weight (g)}}{\text{fork length (cm)\^3}} * 100$

Viscera index (VI) = $\frac{\text{viscera weight}}{\text{body weight}} * 100\%$

4.5.5 Carcass Analysis

Each fish carcass without viscera was ground using a meat grinder. The ground carcass was weighed and put in a foil plate, then stored at -20 °C for at least 24 hours. The samples were dried in a Modulyod freeze dryer (Source: Thermo Fisher Scientific) for 24 hours to constant weight and weighed again. The loss of weight was the moisture content in each carcass sample.

In the preparation step for further analysis, the freeze dried samples were ground using a small coffee grinder (Brand: Interek). Dry matter in prepared samples was measured following AOAC procedure (method no. 934.01, 2011). LECO was used to analyse crude protein (N×6.25) (AOAC, 2011; method no. 992.15). Crude fat was extracted by ANKOM XT15 extraction system and defatted samples were dried in ANKOMRD dryer (AOAC, 2011; method no. 920.39). The difference of weight represented the fat level in each sample. Ash was the residue after 550 °C incineration for at least 2 hours (AOAC, 2011; method no. 942.05). All samples were analysed in duplicate and reanalysed if greater than 3% difference occurred between duplicates.

4.5.6 Hindgut Histology Evaluation

Gut samples were fixed in 10% neutral buffered formalin. They were cut into 0.5 μm cross sections, stained with haematoxylin and eosin (H&E) and fixed on slides by the Animal Health Laboratory, Agriculture and Food Operations Branch, Nova Scotia Dept. of Agriculture as described in Chapter 3. Nikon Coolscan 4000ED was used to scan slides in the image lab, Haley Institute, Faculty of Agriculture, Dalhousie University. The length, width and area of a villus were measured by SigmaScan Pro 5 program. Nine simple villi and one complex villus were selected clockwise in each slide. A semi-quantitative scoring system (Uran et al., 2008b) was adopted to evaluate supranuclear vacuoles (SNV), lamina propria (LP), goblet cells (GC), and sub-epithelial mucosa (SM). The score ranged from 1 to 5, half point was given if necessary. The enteritis condition deteriorates as the score increases.

4.5.7 Statistical Analysis

The experiment was a completely randomized design, and dietary treatment was the fixed effect. The mean of sampled fish represented the value of each tank. When time was a factor, the parameters including weight, fork length, condition factor, and viscera index (VI) were analysed by repeated measurement in the Mixed model of SAS 9.3 (Littell et al., 2006). The type of covariance structure was CS. When the interaction of time and treatment was significant ($p<0.05$), the data were sliced into each time point (week 0, 8 and 16) or period (week 0-8 and week 9-16). When ANOVA was significant ($p<0.05$), Tukey-Kramer test (Hayter, 1984) was applied to differentiate the means. The parameters including total weight gain, total feed consumption, total feed conversion rate (FCR) and carcass compositions (moisture, fat, protein and ash) were

analysed by one-way ANOVA, Tukey-Kramer test in mixed model (SAS, 9.3). The models were the same as described in Chapter 3.

The length, width and area of villi were analysed in one-way ANOVA, Tukey-Kramer test (Minitab 16.2.2). The histological scores were analysed in Kruskal-Wallis test first (Minitab 16.2.2). If the p-value was less than 0.05, Mann-Whitney U-test would be used for pairwise comparison. Significant difference was addressed between two groups when p-value was less than 0.05. If the Kruskal-Wallis test showed the p-value greater or equal to 0.05, then interpretation stopped as there were no significant differences among all treatments.

4.6 Results

4.6.1 Experimental Diets

The analysed crude protein levels in all diets were up to 3% lower than the expected levels at 44% (Table 4.3), but the levels ranged from 41.0 to 42.8% among treatments.

Table 4.3 Chemical analysis of the practical diets fed to Atlantic salmon post-smolts (% as fed basis).

	Control	100% Camelina oil				SECM		
		SEFM	10%SECM +SEFM	FM	10%SECM +FM	8%	16%	24%
Dry matter	91.2	89.9	90.7	90.8	92.1	93	92.1	92.3
Crude protein	42.7	41	41	42.5	42.7	42.8	41.5	42.1
Crude fat	17.9	20	22.6	17.5	20.6	19.9	22.3	24.6
Calcium (%)	1.7	1.4	1.3	1.5	1.5	1.5	1.4	1.3
Total phosphorus (%)	1.1	1	1	1.1	1.1	1.1	1.1	1
Sodium (%)	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3
Potassium (%)	0.4	0.5	0.6	0.5	0.6	0.5	0.6	0.6
Magnesium (%)	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.2
Manganese (mg/kg)	131.4	154.7	132.8	139.8	141.3	159.1	147.1	156.2
Copper (mg/kg)	6.1	7.4	6.4	6.6	7.6	8.7	9.2	9.6
Zinc (mg/kg)	131.4	125.6	119.1	121.8	133.7	135.5	133.4	137
Glucosinolates (μmol/g)[α]	0	0	3	0	4	2.6	5.1	8.7

SECM: solvent extracted camelina meal.
Feed (n=2) was analysed at Nova Scotia Agriculture quality evaluation division laboratory services, 176 College Road, Harlow Institute, Truro, NS, Canada.
[α] The glucosinolates (n=2) were analysed by HPLC at Agriculture and Agri-Food Canada, Saskatoon,.

The difference of crude fat was up to 7% among all diets, 24% SECM diet was the highest one. All diets contained similar levels of minerals with marginal variation (Table 4.3). The level of glucosinolates increased from 2.6 μmol/g in 8%SECM diet to 8.7 μmol/g in 24% SECM diet, and it was 3.0 μmol/g in 100%CO+10%SECM+FM and 4.0 μmol/g in 100%CO+10%SECM+SEFM respectively (Table 4.3).

4.6.2 Growth Performance

All treatments had statistically similar initial mean body weight (mean±SD) and fork length at 242±46 g and 26.9±1.8 cm, respectively. However, the large variation should be concerned, and it was due to the limited fish quantity for this experiment. The body weight of fish was similar among all treatments at week 8 ($p \geq 0.05$, Table 4.4). Fish fed 8% SECM diet grew to 637±95 g in 16 weeks, which was statistically similar to the fish fed the control diet (738±13 g) (Table 4.4). The final mean weight of fish fed the other test diets (546-622 g) were significantly lower than the controls ($p<0.05$), and there were no significant differences among fish fed seven test diets. The fork length of fish fed 100% CO+10% SECM+SEFM (32.8±0.5 cm) was significantly shorter than that of fish fed the control diet (36.2±0.3 cm) ($p<0.05$). However, the condition factor was not affected by the treatments (~1.5). During 16 weeks, fish fed 100% CO+FM (385±33 g) and 8% SECM (391±99 g) diets gained similar weight to the fish fed the control diet (508±12 g) ($p \geq 0.05$), while the fish fed other test diets gained less weight (298-359 g) than the control group ($p<0.05$). However, fish fed 100% CO+FM diet had statistically lower final mean body weight (622±25 g) to the control. Fish consumed a similar amount of 100%CO+FM diet (436±11 g/fish) to that of the control diet (508±12 g/fish), and consumed lower amounts of the other test diets (384-420 g). Viscera index (VI), specific growth rate (SGR) and feed conversion ratio (FCR) were similar throughout the trial among all treatments.

Table 4.4 The growth performance, feed consumption and conversion ratio, and viscera index of Atlantic salmon post-smolts fed eight experimental diets for 16 weeks.

	Control	100% Camelina oil				SECM			p-value
		SEFM	10%SECM +SEFM	FM	10%SECM +FM	8%	16%	24%	
Initial body weight (g)[α]	230±1	231±27	247±18	236±32	255±27	246±56	250±29	241±9	0.9865
Initial fork length (cm)[α]	26.2±0.3	26.3±1.5	27.6±0.4	26.8±0.6	27.3±0.9	26.9±1.9	27.5±1.0	26.8±0.9	0.4794
Week 8 body weight (g) [α]	467±8	393±17	398±38	399±20	390±51	438±25	365±44	407±51	0.0501
Final body weight (g) [α]	738±13 a	590±32 b	546±11 b	622±25 b	608±60 b	637±95 ab	595±26 b	577±25 b	<0.001
Final fork length (cm) [α]	36.2±0.3 a	34.1±0.6 ab	32.8±0.5 b	34.5±0.5 ab	33.9±1.2 ab	34.8±1.4 ab	34.0±0.3 ab	33.9±0.4 ab	0.0048
Final condition factor [β]	1.55±0.03	1.48±0.01	1.53±0.04	1.50±0.05	1.54±0.02	1.47±0.08	1.50±0.02	1.47±0.02	0.1081
Weight gain (g) [β]	508±12 a	359±56 b	298±24 b	385±33 ab	353±58 b	391±99 ab	345±44 b	336±18 b	0.0057
Feed consumption (g) [β]	515±8 a	400±29 b	381±46 b	436±11 ab	391±15 b	420±57 b	384±33 b	391.3±20 b	0.0016
VI (%) [α]	9.8±0.5	11.1±0.4	12.0±0.3	11.2±0.4	11.1±0.6	10.8±0.3	11.2±0.2	11.3±0.7	0.2195
SGR (% day^{-1}) [β]	1.04±0.01	0.84±0.15	0.71±0.07	0.87±0.12	0.78±0.11	0.86±0.23	0.78±0.12	0.78±0.02	0.112
FCR [β]	1.01±0.02	1.12±0.12	1.27±0.09	1.14±0.12	1.12±0.14	1.10±0.17	1.12±0.11	1.16±0.02	0.326

SECM: solvent extracted camelina meal; VI: viscera index; SGR: specific growth rate; FCR: feed conversion ratio

Initial measurements: n=3 per tank. Week 8 measurements: n=7 per tank; Final measurements: n=12 per tank.

[α] repeated measurement analysis

[β] one-way ANOVA analysis

a-b: mean±SD, tank as a unity, triplicate tanks per treatment. Different letters in each row means significant differences ($p<0.05$).

4.6.3 Carcass Composition

Carcass composition was similar ($p>0.05$) among all treatments at week 16 (Table 4.5). The carcass from fish fed control diet contained 68.2% moisture, 19.3% crude protein, 10.3% crude lipid, and 2.0% ash on wet weight basis.

Table 4.5 The carcass compositions (moisture, crude protein, crude lipid and ash on dry matter basis) of Atlantic salmon smolts fed eight experimental diets for 16 weeks.

Treatments	Moisture %	Crude Protein %	Crude Lipid %	Ash %
Control (n=5)	68.2±1.4	19.3±0.3	10.3±0.7	2.0±0.3
100% CO+SEFM (n=5)	67.4±0.9	20.0±0.2	10.8±0.9	1.8±0.1
100% CO+10% SECM+SEFM (n=5)	67.4±0.5	20.1±0.4	10.6±0.6	2.0±0.3
100% CO+FM(n=6)	68.2±1.4	19.9±0.4	9.3±1.7	2.1±0.2
100% CO+10% SECM+FM (n=5)	67.0±0.7	20.0±0.2	11.2±0.5	2.0±0.2
8% SECM (n=6)	67.1±0.7	19.6±0.6	10.8±1.0	2.1±0.3
16% SECM (n=6)	67.7±1.0	19.6±0.4	10.5±1.0	2.1±0.2
24% SECM (n=4)	66.8±0.6	19.8±0.5	10.9±0.9	2.1±0.3
p-value	0.2888	0.0702	0.1439	0.4975

SEFM: solvent extracted fish meal; SECM: solvent extracted camelina meal; FM fish meal; CO: camelina oil
Values were mean±SD, one-way ANOVA, Tukey-Kramer test.

4.6.4 Hindgut Histology

Villi length, width and area of the fish fed test diets were similar to the fish fed the control (Table 4.6). Fish fed 16% SECM diet had longer simple villi (1.07±0.27 mm) than the fish fed 100% CO+FM (0.69±0.18 mm) and 100% CO+10% SECM+FM (0.63±0.06 mm) diets. The width of simple villi in the fish fed 8%SECM (0.15±0.02 mm) was wider than that of fish fed 100% CO+SEFM (0.12±0.01 mm) and 24% SECM (0.12±0.02 mm) diets. The area of simple villi in fish fed 8% SECM diet was larger (0.17±0.03 mm^2) than that of fish fed any diet containing 100% CO (~0.10 mm^2). Larger area of simple villi was seen in fish fed 16%SECM (0.16±0.03 mm^2) compared to fish fed 100% CO+10% SECM+FM and 100% CO+SEFM diets. The length (4.75-8.70 mm), width (0.79-1.33 mm) and area (4.36-7.33 mm^2) of complex villi were similar among all treatments (Table 4.6).

Table 4.6 The quantitative measurements of length, width and area of simple villi and complex villi in the hindgut of Atlantic salmon post-smolts fed eight experimental diets for 16 weeks.

Treatments	Simple villi			Complex villi		
	length(mm)	width (mm)	area(mm^2)	length(mm)	width (mm)	area(mm^2)
Control	0.87±0.01 ab	0.14±0.01 ab	0.14±0.03 abc	7.21±4.41	0.79±0.25	6.33±2.85
100%CO+SEFM	0.70±0.10 ab	0.12±0.01 b	0.10±0.02 c	7.73±3.12	0.82±0.14	7.38±3.34
100%CO+10%SECM+SEFM	0.70±0.13 ab	0.13±0.01 ab	0.10±0.03 bc	6.14±2.32	0.94±0.37	5.20±1.39
100%CO+FM	0.69±0.18 b	0.12±0.01 ab	0.11±0.03 bc	4.75±1.42	0.87±0.11	4.36±1.50
100%CO+10%SECM+FM	0.63±0.06 b	0.12±0.01 ab	0.09±0.01 c	7.72±2.83	0.99±0.19	7.33±1.57
8%SECM	0.9±0.14 ab	0.15±0.02 a	0.17±0.03 a	5.59±1.45	1.33±0.53	6.63±2.67
16%SECM	1.07±0.27 a	0.13±0.02 ab	0.16±0.03 ab	8.70±4.45	0.89±0.27	6.99±4.72
24%SECM	0.87±0.31 ab	0.12±0.02 b	0.12±0.04 abc	6.29±2.18	1.05±0.42	6.26±3.14
One-way ANOVA						
p-value	0.013	0.018	0.000	0.500	0.249	0.585

SEFM: solvent extracted fish meal; SECM: solvent extracted camelina meal; FM: fish meal; CO: camelina oil
a-c: mean±SD. Values with different letters in each column referred to significant differences.
Number of fish per treatment: Control (n=5), 100% CO+SEFM (n=6), 100% CO+10% SECM+SEFM (n=6), 100% CO+FM (n=6), 100% CO+10% SECM+FM (n=3), 8% SECM (n=5), 16% SECM (n=3), 24% SECM (n=4)

Fish fed 24% SECM diet showed higher score of SNV (4.0±0.6) than fish fed the control diet (2.5±0.4), but there were no significant differences among fish fed other test diets and control diet (Table 4.7). The number of GC and the width of LP were not affected by the diet. The score of SM was higher in fish fed 24% SECM (2.3±1.5) than that in fish fed the control diet (1.0±0.0).

Table 4.7 The scores of histological parameters (SNV, GC, LP and SM) in the hindgut of Atlantic salmon post-smolts fed eight experimental diets for 16 weeks.

Treatments	SNV	GC	LP	SM
Control	2.5±0.4 b	2.4±1.1	1.8±0.8	1.0±0.0 b
100%CO+SEFM	2.8±1.3 ab	2.0±0.6	3.0±1.5	1.2±0.4 b
100%CO+10%SECM+SEFM	2.5±0.5 b	2.0±0.7	2.4±0.5	1.0±0.0 b
100%CO+FM	3.2±0.8 b	1.5±0.5	3.3±0.8	1.0±0.0 b
100%CO+10%SECM+FM	3.3±1.0 b	2.0±0.8	3.5±1.0	1.5±0.6 b
8%SECM	2.5±0.4 b	2.3±0.8	2.7±0.5	1.0±0.0 b
16%SECM	2.4±0.5 b	1.6±0.9	2.4±0.9	1.0±0.0 b
24%SECM	4.0±0.6 a	3.8±1.8	3.2±1.0	2.3±1.5 a
Kruskal-Wallis test				
p-value	0.042	0.203	0.129	0.006

SNV: supranuclear vacuoles, GC: goblet cell, LP: lamina propria, SM: sub-mucosa
a-b: mean±SD. Values with different letters in each column referred to significant differences
Control (n=5), 100% CO+SEFM (n=6), 100% CO+10% SECM+SEFM (n=6), 100% CO+FM (n=6), 100% CO+10% SECM+FM (n=4), 8% SECM (n=6), 16% SECM (n=5), 24% SECM (n=6)

4.7 Discussion

4.7.1 Growth

The means of the growth data in the current study were slightly different from the results reported by Hixson et al. (2014b), because the mean of body weight was calculated on different numbers of fish at the final sampling point (n=12 vs n=48-66), and subsequently the other growth parameters associated with body weight differed between these two studies. Both statistical methods (repeated measures vs three-way nested ANOVA) showed lower final weight of fish fed the diets containing 100% CO,

but similar weight gain between the fish fed 100% CO+FM diet and the control diet. Considering other growth parameters including final condition factor, feed conversion ratio and specific growth rate. Atlantic salmon post-smolts fed 100% CO+FM diet had similar growth performance to the fish fed control diet for 16 weeks, which was in accordance with the results of CO replacing full fish oil in the diets for various species (Morais, et al., 2012; Hixson et al., 2014a).

Both the final mean weight and weight gain among fish fed 100% CO+SEFM diet (free of fish oil) was significantly lower than the fish fed the control diet, but 100% CO+FM was better than 100% CO+SEFM. The process of solvent extraction did not affect the growth, since the growth of the fish fed the test diets were similar. The difference between these two test diets was 1.9% fish oil. The calculated fish oil level in 100% CO+FM (2.77%) was higher than 100% CO+SECM (0.84%). Therefore, this small amount of residual oil from fish meal was the key to ensure growth. It is well known that fish oil contains long chain omega-3 fatty acids (DHA and EPA), which are not present in vegetable oils. Although salmonids can convert 18:3n-3 to DHA (Tocher et al., 1997; Bell et al., 2001; Hixson et al., 2014a), this capacity to elongate and desaturate was not enough for the fish fed the 100% CO+SEFM diet. Erucic acid (22:1n-9) should be considered, since it reduced growth and impaired heart health of animals (Sauer and Kramer, 1983; Corner, 1983). 3% erucic acid in the diet depressed the growth of coho salmon (Hendricks, 2002). The range of erucic acid in the diets containing 100% CO was from 0.39 to 0.57% as fed basis, 100% CO+FM diet contained the lowest level (Hixson et al., 2014b). Whether this range of erucic acid is harmful for Atlantic salmon post-smolts remains unclear. Lower final mean body weight and weight gain were found in the fish fed the diets containing 100% CO plus SECM or SEFM or both. Together these results suggested fish growth was not affected by a single factor including 100% CO, SEFM, and 10% SECM, but perhaps a combination of these factors.

The growth of fish fed 8% SECM diet was similar compared to the fish fed the control diet, whereas growth of fish fed 16% and 24% SECM diets was suppressed. This may be due to a decrease in the digestibility of crude protein, since inhibition of somatic growth was evident among Atlantic salmon post-smolts fed diets containing 14-24% plant proteins, such as soybean, sunflower, rapeseed and oats (Aslaksen et al., 2007). However, Burr et al. (2012) indicated that Atlantic salmon parr could tolerate zero fish protein without inhibiting somatic growth.

Based on the current study, the antinutritional factors should be taken into account. The glucosinolates levels in all SECM diets were higher than the maximum recommended level at 1.4 μmol/g (Burel et al., 2000; NRC, 2011). However, up to 7.4 μmol/g of glucosinolates did not show an adverse impact on the growth and feed intake among Atlantic salmon parr over 16 weeks (Chapter 3). By contrast, post-smolts tolerated 2.6 μmol/g glucosinolates (8% SECM diet) without compromising growth. The contrast in the growth response between these two studies might be due to the different life stages and environment (freshwater vs seawater). Both Atlantic salmon parr and post-smolts in the present study showed a higher tolerance of glucosinolates compared to rainbow trout (Burel et al., 2000). Aside from species differences, another reason for the contrasting growth responses might be the difference in the glucosinolate content of the diets. The major component of glucosinolates in camelina meal is 10-methyl-sulfinyl-decyl-glucosinolate (Mathaus and Zubr, 2000), which was different from rapeseed containing progoitrine and gluconapine (Burel et al., 2000). Its metabolic pathway in fish is unclear, but it could possibly be degraded into isothiocyanates, nitriles, thiocyanates or oxazolidithione (Rask et al., 2000) via the intestinal microflora (Rask et al., 2000; Vaughn and Berhow, 2005; reviewed by Tripathi and Mishra, 2007). These metabolic byproducts could interrupt the thyroid function in animals (Lakshmy et al., 1995; Burel et al., 2000; Burel et al., 2001), and subsequently reduce somatic growth. Rainbow trout fed diets containing 12 μmol/g glucosinolates grew to similar body weight to the control group in two months, but the growth was affected adversely in six months (Burel et al., 2001). This safe period was 8 weeks in the current study, since the

mean body weight decreased after 8 weeks. In addition, glucosinolates impaired the palatability of feed and subsequently reduce feed intake (Korsrud and Bell, 1967; Mawson et al., 1993). It was reflected in the current study, where feed consumption of all diets containing SECM was lower than that of control diet (Table 4.4). However, the negative effect of glucosinolates was not dose-dependent, since the feed consumption was similar in the fish fed 8, 12% 24% SECM diets. In addition, other antinutritional factors, such as phytic acid, sinapine, tannins and non-starch polysaccharides (NSP) also changed, and therefore should be considered. Sinapine reduces the palatability of feed (McCurdy and March, 1992). Tannins decrease the digestibility of nutrients via forming complexes proteins, starch and digestive enzymes (Reed, 1995). Phytic acid inhibites the digestibility of zinc and magnesium (Denstadli et al., 2006). NSP reduces the digestibility of nitrogen and fat in Atlantic salmon (initial weight 600g and in seawater) (Refstie et al., 1999). The antinutritional factors in camelina diets need to be investigated in the future.

4.7.2 Carcass Composition

Although the crude lipid level in the carcass was not affected by the diets containing 100% CO, the lipid profile in salmon flesh was different from the control treatment (Hixson et al., 2014b). This result was in accordance with other studies feeding diet containing vegetable oil to Atlantic salmon post-smolts caused a decrease in the EPA and DHA content of the meat, while the level of ALA increased (Tocher et al., 2002; Bell et al., 2003; Torstensen et al., 2004). Robin et al. (2003) indicated that the fatty acid composition in the diet altered the fatty acid profile in the tissues of brown trout (*Salmon trutta*) and turbot (*Psetta maxima*), due to multiple factors including various metabolic factors, initial fatty acid content, cumulative intake of dietary fatty acids, growth rate and duration. A turn over period (12-24 weeks) by feeding a fish oil finishing diet could restore EPA and DHA in Atlantic salmon post-smolts (Bell et al., 2003; 2004). This strategy can improve the levels of EPA and DHA in the salmon fed a vegetable oil diet for a long term, and subsequently benefit the health of consumers. The consumption of long chain omega-3 polyunsaturated fatty acid, particularly EPA

and DHA, can prevent cardiovascular disease, inflammatory conditions and the common form of dementia-Alzheimer's disease, and EPA and DHA are important in fetal development (Connor, 2000; Ruxton and Derbyshire 2009; Swanson, et al., 2012).

4.7.3 Hindgut Histology

The microstructure (length, width and area) of intestinal villi in Atlantic salmon post-smolts in seawater was not influenced by the camelina oil or meal. This result agreed with the findings of the trial on Atlantic salmon parr in freshwater (Chapter 3). Fish were fasted for 24 hours before sampling in the current study. The changes in supranuclear vacuoles (SNV) was observed in post-smolts in seawater on second day starvation, and other histology parameters deteriorated following three weeks starvation (Baeverfjord and Krogdahl, 1996). When fish were in seawater, Na^+/K^+-ATPase activity increased in the posterior intestine, transcellular pathway gradually became dominant compared to the paracellular route (Sundell et al., 2003). The starvation and physiological changes might explain that post-smolts fed the control diet showed less moderate scores of histology parameters, which were higher than that of the parr fed control diet (Chapter 3). The hindgut in fish fed 24% SECM diet showed some characteristics similar to soybean induced enteritis in Atlantic salmon (Baeverfjord and Krogdahl, 1996; Uran et al., 2009), significantly diffused SNV and thickened SM with infiltration of inflammatory cells. The disappearance of SNV was due to the blockage of endocytosis, but the causative substances were unclear (Uran et al., 2008a). Knudsen et al. (2007 and 2008) reported that soya saponins increased the intestinal permeability, and subsequently induced inflammation combining with other factors, such as antigenic soybean proteins or the intestinal gut microflora. Camelina meal did not contain any soyasaponins, therefore there may be other components influencing the permeability and inducing inflammation in the hindgut. The solvent residue should not be a problem, because its concentration was only 2.1 ppm in the SECM (Biosciences, Saskatoon, SK, Canada), and the level was diluted when SECM was included in the diet. The glucosinolates were an important antinutritional factor in camelina meal, but there is no published data on the specific effects of glucosinolates on the intestinal histology of

either teleosts or any other animals. Rapeseed (*Brassica napus)*, also contained glucosinolates, but did not impair the gut histology of Atlantic salmon post-smolts when it was included at 18% in diet (Aslaksen et al., 2007). In the present study, inclusion of SECM under 16% of the diet did not induce severe inflammations, but abnormalities were evident among fish fed 24% SECM diet. By contrast, parr fed 15% and 20% SECM diets exhibited widened lamina propria (Chapter 3). Glucosinolates might only have minor effect on the gut histology, and this effect was dose-dependent, and depended on stage of development and salinity. Higher temperature (14 vs. 12 °C) might increase the development of enteritis (Uran et al., 2008b). Phytic acid did not affect the morphology of the distal intestine in Atlantic salmon parr (Denstadli et al., 2006). The level of mucilage in SECM was not clear, but should be greater than the 6% level in camelina seeds. Mucilage forms gel in water (Mazza and Biliaderis, 1989), which may be difficult to be digested and absorbed, resulting in the residual mucilage are transferred with digesta to the hindgut. The viscosity of mucilage can be significantly reduced by 2 ppt NaCl at 25 °C (Mazza and Biliaderis, 1989). The effect of mucilage may be ameliorated, since Atlantic salmon post-smolts drink seawater, but at 14 °C. The sticky matter may block the epithelium of mucosal fold in the hindgut, subsequently causing the reduced size of SNV. The causative agents of camelina-induced enteritis need further investigation.

4.8 Conclusion

The body weight of Atlantic salmon post-smolts in seawater fed various experimental diets were similar at week 8. The growth performance was not affected by either 100% CO or 8% SECM after 16 weeks. The combination of CO, SEFM, and SECM depressed the growth. It indirectly reflected that 2.6 μmol/g glucosinolates did not inhibit the growth. Multiple antinutritional factors contributed to the negative effects. The carcass composition in all fish were independent of the diets. The enteritis in the hindgut was evident in the fish fed 24%SECM, but the reason was unknown.

Chapter 5 : The Effects of High Oil Residue Camelina Meal on Growth Performance, Carcass Composition and Hindgut Histology of Atlantic Salmon (*Salmo salar*) Smolts in Freshwater

5.1 Abstract

Atlantic salmon smolts (61.8±9.1 g) were reared at 11.9±0.1 °C in freshwater for 16 weeks. Experimental diets included control, 8%, 16% and 24% high oil residue camelina meal (HOCM). The final weight, weight gain, feed consumption, specific growth rate, and condition factor decreased as the level of HOCM increased in the diets ($p<0.05$). The feed conversion ratio and hepatosomatic index had increasing trend ($p<0.05$). Carcass composition and protein retention ratio were not affected by 8%HOCM diet. The carcass protein, lipid and protein retention ratio decreased in fish fed higher level of HOCM diets, but the carcass moisture increased, and carcass ash was unchanged ($p<0.05$). The width and area of simple villi and the thickness of intestinal wall in fish fed 24%HOCM diet were reduced. The length and area of complex villi in fish fed 16% and 24%HOCM diets decreased. The number of goblet cells increased as the level of HOCM increased. Enterocytes separated from lamina propria at the apical of villi, which was found in all treatments. The causative agents of enteritis and the cracks within the villi were unknown.

5.2 Introduction

Since 1985, there has been a decreasing trend of fish meal usage in salmonid feed (Tacon, 2005). The content of fish meal in the commercial salmonid feeds has been reduced to 18% in 2013 (EWOS Reporting Centre). It is predicted to be 8% by 2020 (Tacon and Metian, 2008). In this case, more alternative protein sources are required by feed manufacturers to ensure cost efficient feed. Camelina meal is a potential replacement, it has a similar protein level and amino acid composition to canola meal (Bell and Keith, 1991).

In two previous trials, solvent extracted camelina (SECM) was readily accepted up to 10% in the diet for Atlantic salmon parr (Chapter 3) but only 8% for post-smolts (Chapter 4). The production cost of high oil residue camelina meal (HOCM) is lower than SECM. The growth of rainbow trout was not inhibited by 20% HOCM (Bullerwell and Anderson, 2012), indicating that HOCM can be a potential ingredient in aquafeeds. However, an optimal level for Atlantic salmon has not been estimated. The HOCM used in this study contained 35.9 μmol/g glucosinolates (AAFC, Saskatoon, SK, Canada) and 0.57% phytic acid (University of Manitoba, Winnipeg, MN, Canada). Negative

effects of antinutritional factors on the growth performance were seen in post-smolts fed 16% and 24% SECM diets (Chapter 4). Some enteritis characteristics in the hindgut were observed in high level of camelina meal in the two previous trials, but the causative agent was unknown. Hindgut histology evaluation was conducted in this study.

5.3 Objectives

This study investigated a satisfactory level of HOCM (8%, 16% and 24%) in the diet of Atlantic salmon smolts in freshwater by evaluating the growth performance, carcass composition, hindgut histology.

5.4 Hypothesis

The fish fed 8% HOCM diet would show similar growth and carcass composition to that of control, but poorer results would be found in fish fed higher levels of HOCM.

The hindgut histological parameters would show HOCM dose-dependent responses, fish fed 16% and 24% HOCM diets would show significant histological changes in the hindgut.

5.5 Materials and Methods

5.5.1 Preparation of Test Ingredients and Feed Formulations

Oil was pressed out of camelina seeds (007 line) by using an EGON KELLER KEK expeller-press at Atlantic Oilseed Processing, Ltd (Summerside, PEI, Canada). The residue meal was ground with a hammer mill (screen size 8mm) into meal at Atlantic Oilseed Processing, Ltd. This meal product was named high oil residue meal (HOCM) in the current study. It contained 30.6% crude protein and 10.3% crude fat as fed basis (Nova Scotia Agriculture quality evaluation division laboratory services, 176 College Road, Harlow Institute, Truro, NS, Canada). The composition of HOCM was in Appendix B. Diets were formulated to be isonitrogenous (44%) and isocaloric (4400 Kcal/kg, Table 5.1) levels to meet the nutrient requirements for Atlantic salmon (NRC, 2011).The level of fish meal was set as 15% in all diets. Fish oil, poultry meal and wheat were used to balance the diets. All diets were mixed according to the formulations, and

steam pelleted using a 2.5mm or 3mm die by a California pellet mill at Chute Animal Nutrition Centre, Faculty of Agriculture, Dalhousie University, then dried at 54 °C, then cooled, and stored at -20 °C. The essential amino acids, minerals and antinutrient factors in each diet were calculated according to ingredient composition listed in

Table 5.1 The formulation of four practical diets with graded levels of high oil residue camelina meal (HOCM) fed to Atlantic salmon smolts (% as fed basis).

		HOCM (%)		
Ingredient of diet	Control	8	16	24
Wheat Gluten Meal	5.00	5.00	5.00	5.00
Feather Meal	8.00	8.00	8.00	8.00
Blood Meal	6.00	6.00	6.00	6.00
Empyreal 75®	8.00	8.00	8.00	8.00
D/L Methionine	0.17	0.17	0.17	0.17
Vitamin Mineral Premix [α]	0.20	0.20	0.20	0.20
Special Premix [β]	0.25	0.25	0.25	0.25
Choline Chloride	0.50	0.50	0.50	0.50
Whey	5.00	5.00	5.00	5.00
Pregelatinized starch	2.50	2.50	2.50	2.50
Fish Meal	15.00	15.00	15.00	15.00
Fish Oil	17.29	17.87	18.46	19.04
Poultry Meal	13.38	10.89	8.40	5.91
Wheat	18.71	12.62	6.52	0.43
HOCM	0.00	8.00	16.00	24.00
Total	100.00	100.00	100.00	100.00

[α] Vitamin and mineral premix: zine 77.5 mg, manganese 125 mg, iron 84 mg, copper 2.5 mg, iodine 7.5 mg, vitamin A 5000 IU, vitamin D 4000 IU, vitamin K 2mg, vitamin B12: 4 µg, thiamin 8mg, riboflavin 18 mg, pantothenic acid 40mg, niacin 100mg, folic acid 4 mg, biotin 0.6 mg, pyridoxine 15mg, inositol 100mg, ethoxyquin 42mg, wheat shorts 1372mg (per kg of diet).

[β] Special Premix: selenium 0.22 mg, vitamin E 250 mg, vitamin C 200 mg, astaxanthin 60 mg, and wheat shorts 1988mg (per kg of diet).

nutrient requirements of fish and shrimp (NRC, 2011) (Table 5.2; Table 5.3). No obvious nutrient deficiency in the experimental diets was detected based on the calculation.

Table 5.2 Calculated nutrients contents in smolts experimental diets (% as fed basis).

	Control	HOCM (%)			Requirements[α]
		8	16	24	
CP (%)	44	44	44	44	44
DE (4400 kcal/kg)	4400	4400	4400	4400	4400
Amino acids:					
Arginine	2.30	2.34	2.39	2.44	1.6-2.2
Histidine	1.07	1.08	1.09	1.10	0.70
Isoleucine	1.65	1.65	1.66	1.67	1.00
Leucine	4.00	4.01	4.02	4.02	1.60
Lysine	2.23	2.24	2.25	2.26	2.0-2.2
Methionine	0.81	0.82	0.82	0.83	0.70
Methionine+Cysteine	1.57	1.59	1.61	1.63	1.10
Phenylalanine	1.96	2.00	2.04	2.07	1.70
Phenylalanine+Tyrosine	3.14	3.12	3.11	3.09	1.80
Threonine	1.61	1.63	1.66	1.68	1.10
Tryptophan	0.34	0.36	0.38	0.40	0.1-0.3
Valine	2.26	2.26	2.25	2.25	0.8-1.6

HOCM: high oil residue camelina meal

[α] Nutrients requirements for Atlantic salmon, National Research Council (NRC, 2011)

Table 5.3 Calculated minerals and antinutrient factors in smolts diets (% as fed basis).

	Control	HOCM (%)			Requirements[β]
		8	16	24	
Calcium (%)	1.2	1.2	1.2	1.2	-
Total phosphorus (%)	0.9	0.9	0.9	0.9	0.8
Sodium (%)	0.3	0.3	0.3	0.3	-
Potassium (%)	0.6	0.6	0.6	0.6	-
Magnesium (%)	0.16	0.16	0.17	0.17	0.04
Manganese (mg/kg)	157.2	153.2	149.2	145.2	10
Copper (mg/kg)	12.3	11.7	11.0	10.4	5
Zinc (mg/kg)	152.9	153.3	153.7	154.1	37
Antinutrient factors[α]					
Sinapine mg/g	0.00	0.14	0.28	0.42	
Phytic acid % as fed	0.00	0.05	0.09	0.14	
Glucosinolates umol/g	0.00	2.87	5.74	8.62	

HOCM: high oil residue camelina meal

[α] Antinutrient factor content were calculated from the analysed content in HOCM.

[β] Nutrients requirements for Atlantic salmon, National Research Council (NRC, 2011)

- Not available

5.5.2 Experimental Fish and Rearing Conditions

Atlantic salmon (Saint John River stock) were transferred from Big Falls Fish Grower Ltd., Wolfville, NS, Canada to Aquaculture Centre, Faculty of Agriculture, Dalhousie University, NS, Canada. Fish were graded by weight in the range of 61.8±9.1g. These fish were randomly assigned to 24 tanks, with 35 fish per tank. Tank size is 65cm*100cm (height*diameter), and the volume is 500 L. The tanks were divided into six blocks (Figure 5.1). Each diet was randomly assigned to one of the four tanks in each block. This experiment was conducted in according with animal care protocol (File: 2013-019) approved by animal care committee in Faculty of Agriculture, Dalhousie University using the guidelines of the Canadian Council on Animal Care (2005).

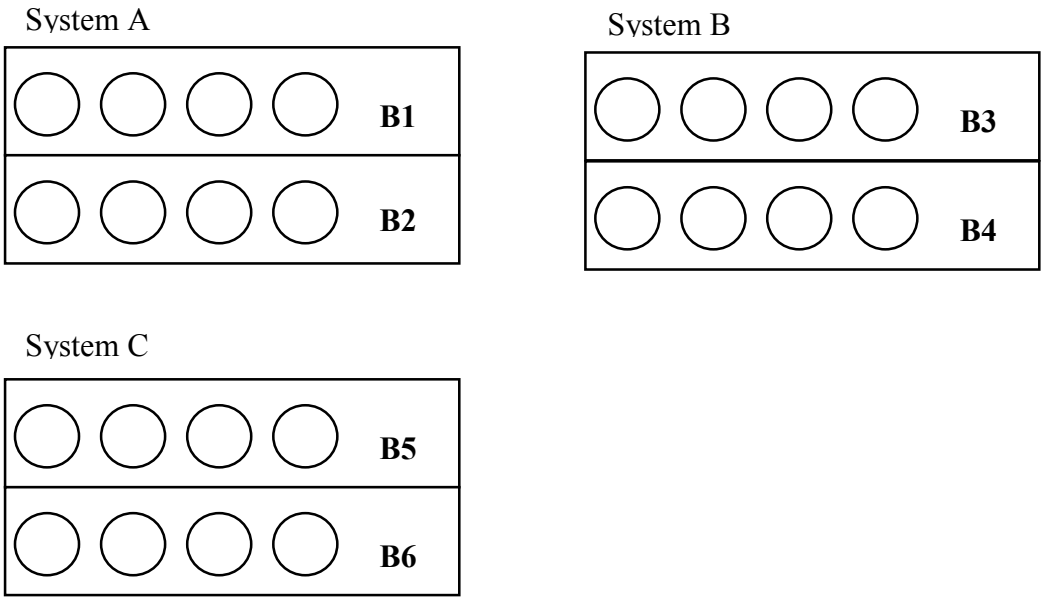


Figure 5.1 The diagram of rearing tanks in six blocks.

Fish were reared in freshwater in three identical recirculation systems. The temperature was 11.9±0.1 °C and the oxygen level was about 114±9%. The proximate total ammonia (Ammonia nitrogen test kit, 0.2-3.0 ppm, LaMotte) and nitrite (Nitrite nitrogen kit, 0.05-0.8 ppm, LaMotte) were monitored weekly, the average levels during 16 weeks were 0.2 ppm and 0.1 ppm respectively. Fish were fed with a commercial diet for one week, then were offered experimental diets twice per day for 16 weeks. The experiment

stated on August 3, 2013. All the tanks were purged twice per day to remove solid waste. Due to a fungus infection, fish in each tank were treated three times (August 15-18) with formalin (36.5% formaldehyde solution + 10-15% methanol) bath (1:4000). To reduce the risk of further fungal infections, the salinity was elevated to 2 ppt at week 5 until the end of the trial.

5.5.3 Sampling

Fish mean body weight was measured in batches in each tank at the beginning of the trial and subsequently every four weeks. Feed consumption was measured weekly. Eight fish from the fish population (week 1), three fish per tank (week 8) and six fish per tank (week 16) were euthanized by an overdose of buffered tricaine methane sulfonate (TMS). The body weight and fork length of each fish were measured individually. Hindgut samples were collected from these fish, and stored in 10% neutral buffered formalin. The weight of liver was measured in triplicate in each tank at week 16. The carcass of each fish without viscera and kidney, was weighed and stored at -20 °C.

5.5.4 Calculations

The weight gain, feed consumption, specific growth ratio (SGR), and feed conversion ratio (FCR) were calculated at weeks 4, 8 and 16. Condition factor (CF) at was calculated at week 16. Viscera index (VI) and Hepatosomatic index (HSI) were calculated at week 16. Protein retention ratio (PRR) was calculated at week 16.

SGR = $\frac{\ln Mf - \ln Mi}{t} * 100$; M_f and M_i are the mean final and initial individual masses, t refers to time

FCR = $\frac{\text{Total feed fed to tank}}{\text{tank biomass gain}} * 100$

CF = $\frac{\text{body weight}}{\text{fork length\^3}} * 100$

Viscera index (VI) = $\frac{\text{viscera weight}}{\text{body weight}} * 100\%$

HSI = $\frac{\text{liver weight}}{\text{body weight}} * 100$

$$PRR = \frac{(\text{final body weight} * \text{final body protein content as wet weight basis}) - (\text{initial body weight} * \text{initial protein content as wet weight basis})}{\text{total feed intake} * \text{protein content in diet}}$$

5.5.5 Carcass Analysis

Eight fish were collected for carcass analysis at the beginning, two fish carcasses were pooled as one sample. Each fish carcass (week 8 and week 16) was ground individually using a manual meat grinder. Two fish carcasses from week 16 were pooled as one sample. The ground carcass was weighed and stored at -20 °C for at least 24 hours. The loss of weight measured after 24 hours of freeze drying was calculated as the moisture content in the carcass. The procedures were described in Chapter 4. All samples were analysed in duplicate for each procedure

5.5.6 Hindgut Histology Evaluation

The detail of slide preparation was described in Chapter 3. Nikon Coolscan 4000ED was used to scan the slides in the image analysis lab, Haley Institute, Faculty of Agriculture, Dalhousie University. Five simple villi and one complex villus were selected clockwise in each slide. SigmaScan Pro 5 program was used to measure the length, width and area of each villus and the thickness of the intestine wall. A semi-quantitative scoring system (Uran et al., 2008b) was adopted to evaluate mucosal fold (MF), supranuclear vacuoles (SNV), lamina propria (LP), goblet cells (GC), and sub-epithelial mucosa (SM). The scale of score ranged from 1 to 5, half point was given if necessary. An increased score reflected an increased inflammation process.

5.5.7 Post Mortem Evaluation

Two fish per tank were randomly sampled at the end of the trial, all fish were euthanized by overdose of buffered tricaine methane sulfonate (TMS). These samples were stored in a cooler with ice and sent to Nova Scotia Department of Fisheries and Aquaculture for post mortem evaluation on the same day. Evaluation included necropsy, bacteriology (kidney), histopathology (liver), and gill and skin wet mounts.

5.5.8 Statistical Analysis

This experiment was a randomized complete block design with diet as the main effect, block as a random effect, and tank as the experimental unit. When time was a factor, the parameters were analysed by repeated measures in the mixed model of SAS 9.3 (Littell et al., 2006), comparisons of means were adjusted by Tukey-Kramer test as a conservative method (Hayter, 1984), if ANOVA indicated differences ($p<0.05$). The type of covariance structure was compound symmetry (CS). When time was not a factor, the parameters were analysed by one-way ANOVA, Tukey-Kramer test in the mixed model of SAS 9.3. The normality of residuals were checked. If the data was not normal, an appropriate transformation was done (e.g. ^0.5 or log).

Model for a single time point:

$$Y_{ij} = \mu + T_i + \alpha_j + \varepsilon_{ij}$$

Y_{ij} - response

μ - the mean

T_i - the effect of treatment (i=1-4)

α_j - the effect of block (j=1-6)

ε_{ij} – the random error

Model for repeat measures:

$$Y_{ijk} = \mu + T_i + \beta_j + (T\beta)_{ij} + \alpha_g + \varepsilon_{ijk}$$

Where: Y = the response

μ - the mean

T_i - the effect of treatment (i=1-4)

β_j - the effect of time (j=1-5 or 1-2)

α_g – the effect of block g (g=1-6)

$(T\beta)_{ij}$ - the interactive effect of treatment and time

ε_{ijk} - the random error

The histological scores were analysed by Kruskal-Wallis test firstly (Minitab 16.2.2). If the p-value was less than 0.05, Mann-Whitney U-test was used for pairwise comparison. When p-value was less than 0.05, it was significantly different.

5.6 Results

5.6.1 Experimental Diets

The protein content in each diet (Table 5.4) ranged from 44.6-45.5%. The crude fat increased as the HOCM level increased in the diets. The mineral content were similar among all diets (Table 5.4). The levels of zinc and manganese were about five-fold and thirteen-fold respectively as the requirements, but the toxic levels were unknown. This occurred in the two previous studies, but no toxic symptoms were found. The increased level of glucosinolates reflected the graded level of HOCM in the diet, but 8% HOCM contained much higher level than expected (4.9 vs 2.87 μmol/g).

Table 5.4 Nutrient analyses of Atlantic salmon smolt experimental diets (% as fed basis).

		HOCM (%)			Requirements[β]
	Control	8	16	24	
DM	92.8	94.0	93.6	94.1	-
Crude Protein	44.6	45.2	45.1	45.5	44
Crude Fat	18.6	21.3	22.9	22.6	-
Calcium (%)	1.5	1.3	1.2	1.2	-
Total phosphorus (%)	1.0	0.9	0.9	1.0	0.8
Potassium (%)	0.5	0.6	0.6	0.7	-
Magnesium (%)	0.1	0.1	0.1	0.2	0.04
Sodium (%)	0.3	0.2	0.2	0.2	-
Manganese (mg/kg)	128.1	135.7	132.9	143.7	10
Copper (mg/kg)	8.4	7.5	8.4	10.3	5
Zinc (mg/kg)	178.2	173.9	184.8	183.2	37
Glucosinolates (μmol/g)[α]	0.0	4.9	5.7	8.7	

HOCM: high oil residue camelina meal, - not available

Feed was analysed at Nova Scotia Agriculture quality evaluation division laboratory services, 176 College Road, Harlow Institute, Truro, NS, Canada.

[α] The glucosinolates were analysed by HPLC at Agriculture and Agri-Food Canada, Saskatoon.

[β] Nutrients requirements for Atlantic salmon, National Research Council (NRC, 2011)

5.6.2 Mortality

Fungus (*Saprolegnia sp.*) on skin was first observed on one fish during week 2, and a 7.9% mortalities as whole population subsequently occurred in the next two weeks

(Table 5.5). The *Saprolegnia* problem was controlled by elevating the salinity to 2 ppt at week 5. Only a few fish died in the next week, and the fungus problem never re-occurred during the remaining period of the experiment. The mortalities of fish fed HOCM diets were higher than that of fish fed the control diet.

Table 5.5 The number of mortalities of Atlantic salmon due to fungal infection in each dietary treatment at different salinity during the first 5 weeks of the experiment.

Salinity	Control	High oil residue camelina meal (%)		
		8	16	24
0 ppt (week 1-4)	4	21	23	18
2 ppt (week 5)	2	5	7	10
Total number	6	26	30	28

5.6.3 Growth Performance

The initial weight was similar among all treatments (Table 5.6). A significant reduction of feed consumption occurred during week 1 in fish fed HOCM diets (3.5-7.1 vs 9.1 g/fish). The weight gain was strongly correlated ($R^2 = 0.98$) with feed consumption. The final weight, weight gain, specific growth rate (SGR) and feed consumption (FC) showed a decreasing trend as the HOCM level increased in the diets ($p<0.05$, Table 5.6). However, the mean body weight was not different in fish fed the control diet and 8%HOCM diet at week 4. Fish fed the control and 8% HOCM diets had similar feed conversion ratios (FCR) at 1.00, and the fish fed 24% HOCM diets had the worst FCR at 1.61. The fork length and condition factor in fish fed control and 8% HOCM diets were superior to the fish fed 16% and 24%HOCM diets. The viscera index and hepatosomatic index (HSI) in fish increased as HOCM level increased. The protein retention ratio was not significantly affected by 8% HOCM diet, but was adversely reduced by higher level of HOCM diets.

The block effect was significant on final weight, weight gain and feed consumption (Table 5.6). However, the weight and feed consumption had the same patterns in each block (Figure 5.2 and Figure 5.3). Weight gain was calculated based on the weight data, so it was in the same case. It illustrated that the block design was useful to minimize the variation between the blocks without interrupting the effects of the treatments.

Table 5.6 The growth performance, viscera index, hepatosomatic index, and protein retention ratio (mean/fish) of Atlantic salmon smolts in freshwater fed four experimental diets for 16 weeks.

	Control	High oil residue camelina meal (%)			p-value	
		8	16	24	block	trt
Initial body weight (g/fish) [β]	61.2±1.6	61.4±0.8	61.4±1.1	61.9±1.1	0.0538	0.1902
Body weight at week 4 [α]	116.2±5.3 a	107.6±1.9 a	95.72±2.7 b	82.6±1.2 c	<.0001	<.0001
Final weight (g/fish) [α]	439.4±31.7 a	321.3±30.2 b	191.4±6.9 c	130.3±8.2 d	<.0001	<.0001
Weight gain (g/fish) [β]	378.2±31.8 a	259.8±30 b	129.0±6.1 c	68.4±9.1 d	0.0312	<.0001
SGR (% day^{-1}) [β]	1.65±0.07 a	1.39±0.08 b	0.94±0.02 c	0.62±0.07 d	0.0872	<.0001
FC (g/fish) during week 1 [β]	9.1±1.1 a	7.1±0.5 b	4.3±0.3 c	3.5±0.4 c	0.4386	<.0001
FC (g/fish) [β]	378.5±33.5 a	273.0±32 b	154.6±9.8 c	110.1±18 d	0.0044	<.0001
FCR [β]	1.00±0.04 c	1.05±0.10 bc	1.20±0.08 b	1.61±0.14 a	0.4977	<.0001
Final fork length (cm) [β]	33.0±1.0 a	31.2±1.1 a	26.8±1.5 b	24.0±1.4 c	0.4132	<.0001
Condition factor [α]	1.19±0.04 a	1.12±0.04 a	0.98±0.04 b	0.89±0.04 c	0.8484	<.0001
Viscera index (%) [β]	11.8±0.5 c	13.4±0.6 b	13.8±0.5 ab	14.8±0.7 a	0.7478	<.0001
HSI (%) [β]	1.21±0.04 b	1.26±0.06 ab	1.28±0.08 ab	1.37±0.09 a	0.4693	0.0099
PRR [β]	42.2±2.1 a	40.8±5.1 a	32.9±1.8 b	23.8±2.0 c	0.062	<.0001

SGR: specific growth rate;
FC: feed consumption;
FCR: feed conversion ratio;
HSI: hepatosomatic index;
PRR: protein retention ratio
a-d: mean±SD. Tank as a unit, n=3 per treatment. Means with different letters in each row are significantly different.
[α] repeated measurement analysis
[β] one-way ANOVA analysis

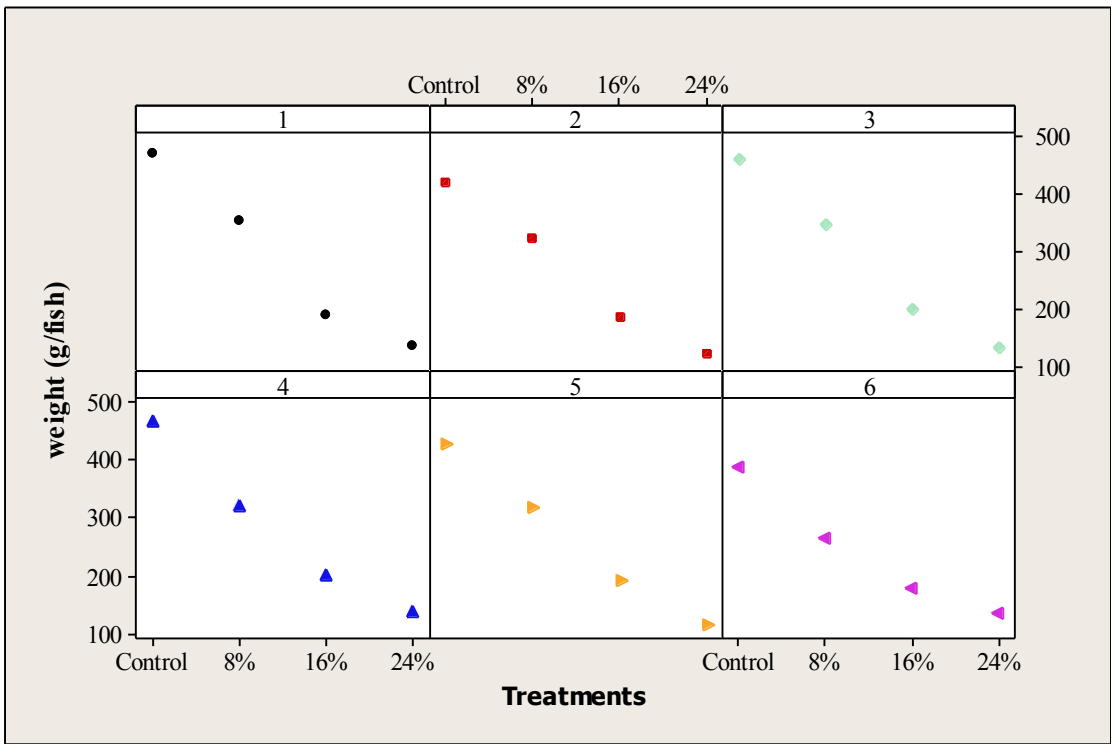


Figure 5.2 The relationship between final mean body weight of Atlantic salmon smolts and dietary treatments were compared with each of the six blocks at week 16 (panel variable: block).

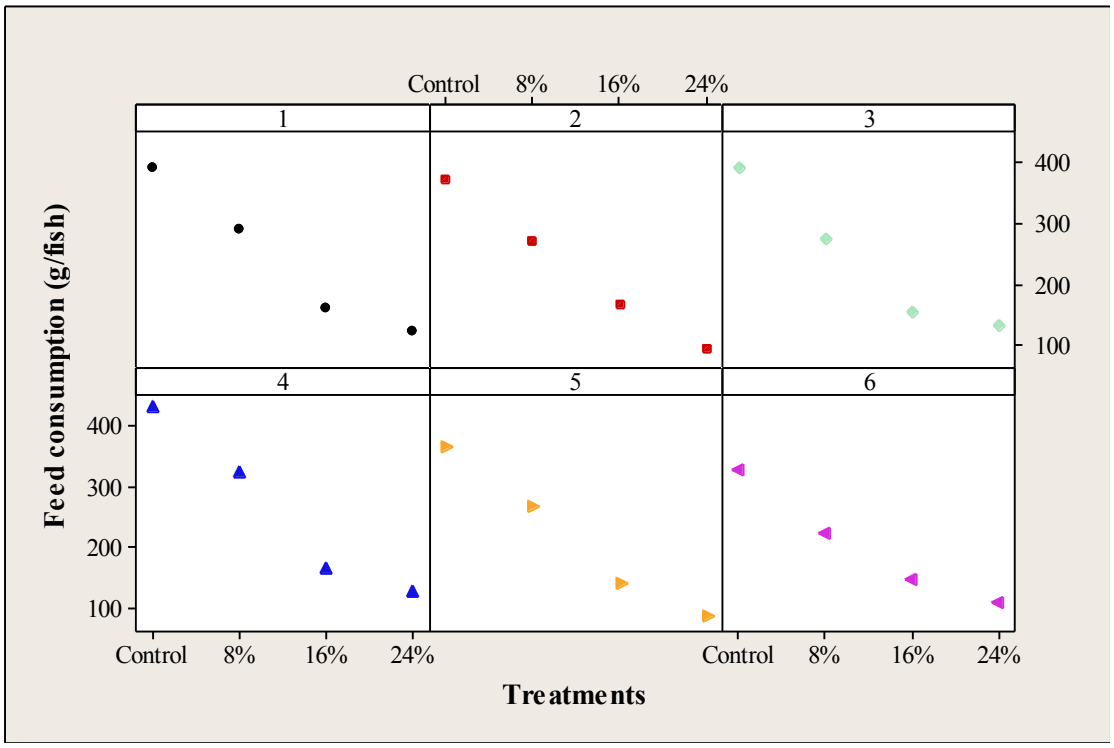


Figure 5.3 The relationship between feed consumption of Atlantic salmon smolts and dietary treatments were compared with each of the six blocks at week 16 (panel variable: block).

5.6.4 Carcass Composition

Fish carcass initially contained 17% crude protein (Table 5.7). There was significant interaction effect on carcass protein ($p<0.05$). The carcass protein content was similar in all fish at week 8, and the average was 17.3%. There was a decreasing trend at week 16. Fish fed the control diet had higher crude protein (18.6%) than the fish fed 16% and 24% SECM diets (~17.5%), but was similar to the fish fed 8% SECM (18.3%). The initial crude lipid content was 4.7% (Table 5.7). It was affected by the interaction of time and treatments ($p<0.05$). The carcass lipid content decreased as the level of HOCM increased at both week 8 and week 16. Fish fed the control (11.1%) and 8% SECM (10.4%) diets contained similar lipid level, and they were higher than the fish fed 16% (8.4%) and 24% SECM (6.0%) diets. Carcass ash content did not change by time, with initial ash 2.2% (Table 5.7), but the mean by treatment was higher in the fish fed 16% and 24% SECM diets compared to the fish fed the control and 8% SECM diets. Carcass moisture content was highest at the beginning with 75.5% (Table 5.7). It was affected by the interaction of time and treatment ($p<0.05$). An increasing trend was seen at either week 8 or week 16. The final carcass moisture level was lowest in the fish fed control diet (67.5%), and highest in the fish fed 24% SECM (74%).

Table 5.7 The carcass composition (crude protein, crude lipid, ash and moisture) of Atlantic salmon smolts fed four experimental diets at initial day, week 8 and week 16 (% on wet weight basis).

		High oil residue camelina meal				p-value		
	Control	8%	16%	24%	Mean by time	time*trt	trt	time
Crude protein								
Initial	17.0±0.4							
Week 8	17.5±0.3 c	17.2±0.2 c	17.4±0.5 c	17.2±0.5 c	17.3±0.4	0.0078	0.0002	<0.0001
Week 16	18.6±0.6 a	18.3±0.5 ab	17.6±0.3 bc	17.3±0.3 c	18.0±0.7			
Mean by trt	18.1±0.7	17.8±0.7	17.5±0.4	17.3±0.4				
Crude lipid								
Initial	4.7±0.4							
Week 8	9.2±0.6 bc	8.3±0.8 cd	7.4±0.8 d	5.7±0.8 e	7.6±1.5	0.0092	<0.0001	<0.0001
Week 16	11.1±0.2 a	10.4±0.6 ab	8.4±0.5 cd	6.0±1.0 e	9.0±2.1			
Mean by trt	10.2±1.1	9.4±1.3	7.9±0.8	5.9±0.9				
Ash								
Initial	2.2±0.4							
Week 8	2.1±0.3	2.3±0.2	2.3±0.2	2.3±0.2	2.2±0.2	0.3905	0.001	0.5640
Week 16	2.0±0.2	2.2±0.1	2.4±0.2	2.6±0.3	2.3±0.3			
Mean by trt	2.0±0.2 b	2.2±0.2 ab	2.3±0.2 a	2.4±0.2 a				
Moisture								
Initial	75.5±0.9							
Week 8	70.6±0.6 d	71.7±0.8 cd	72.8±0.9 bc	74.5±0.9 a	72.4±1.7	0.0002	<0.0001	<0.0001
Week 16	67.5±0.2 e	68.4±0.7 e	70.9±0.6 d	74.0±1.0 ab	70.2±2.7			
Mean by trt	69.0±1.7	70.1±1.9	71.8±1.2	74.3±1.0				

Initial: n=8; week 8: n=18 per treatment; week 16: n=36 per treatment.
a-e: mean±SD. Means with different letters were significantly different in each panel.

5.6.5 Hindgut Histology

A few abnormal villi were observed in some hindgut slides (Figure 5.4) across all treatments. At the apices of villi, the absorptive cells were separated from lamina propria, and formed an empty space. This abnormal observation was not described in the semi-quantitative scoring system, and it was common in all treatments. Therefore, it was not included in the scoring parameters. The other histological parameters looked normal, such as lamina propria, supranuclear vacuoles and goblet cells.

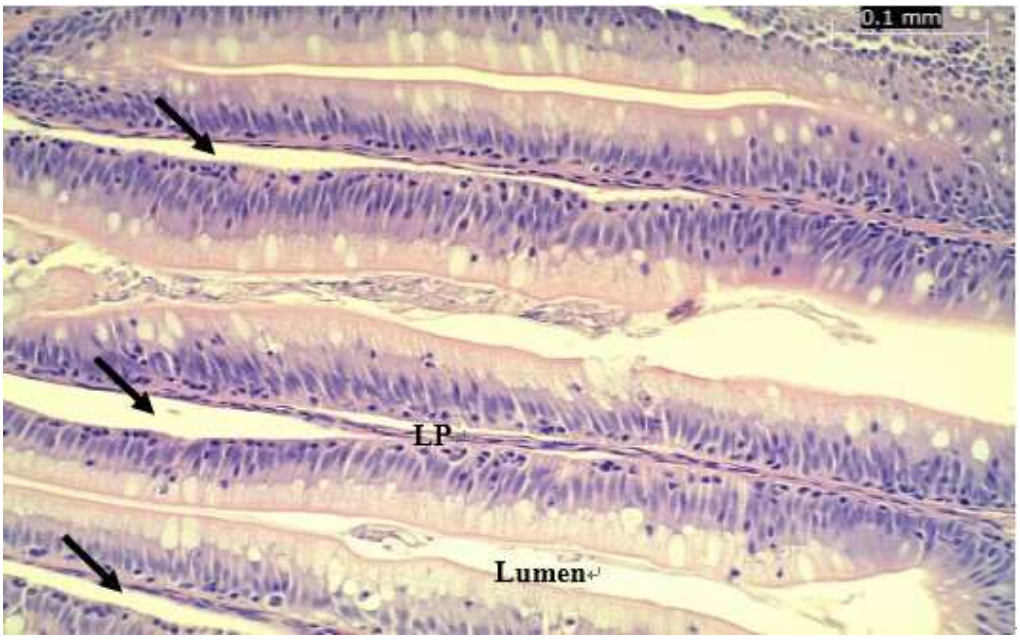


Figure 5.4 An example of abnormal villi in the hindgut of Atlantic salmon smolts in freshwater fed various experimental diets. Various degree of cracks beside lamina propria (LP) at the apical of simple villi (arrows), but it was normal at the base of villi.

There was no difference on the length of simple villi among all treatments (Table 5.8). However, the width and area of simple villi were reduced in fish fed 24% HOCM diet. For complex villi, the width did not change due to the treatment, but the length and area decreased in fish fed 16% and 24% HCOM diets. The intestinal wall was thinner in fish fed 24% HOCM compared to that of fish fed other diets.

Table 5.8 The quantitative measurements of villi and intestine wall in hindgut of Atlantic salmon smolts fed various experimental diets for 16 weeks.

		Control	High oil residue camelina meal (%)			p-value	
			8	16	24	block	trt
Simple villi	Length (mm)	0.677±0.115	0.672±0.165	0.665±0.0.185	0.635±0.156	0.000	0.659
	Width (mm)	0.167±0.027 a	0.155±0.026 a	0.155±0.020 a	0.134±0.018 b	0.191	0.000
	Area (mm)	0.111±0.032 a	0.104±0.041 a	0.102±0.036 ab	0.083±0.027 b	0.000	0.003
	Sample number	33	35	35	34		
Complex villi	Length (mm)	5.933±3.083 a	5.083±3.087 ab	3.167±1.884 c	3.760±2.029 bc	0.415	0.000
	Width (mm)	0.222±0.051	0.276±0.134	0.293±0.170	0.259±0.131	0.310	0.123
	Area (mm)	5.563±3.019 a	4.426±2.427 ab	3.013±2.011 c	3.125±2.034 bc	0.425	0.000
	Sample number	33	32	35	32		
Intestinal wall (mm)		0.165±0.032 a	0.168±0.032 a	0.162±0.027 ab	0.145±0.028 b	0.891	0.005
Sample number		33	35	35	34		

a-c: mean±SD. Means with different letters in each row are significantly different

The sub-mucosa (SM), supranuclear vacuoles (SNV) and lamina propria (LP) were similar among all treatments (Table 5.9). Both SNV and LP had moderate changes in fish fed all types of diets. The number of goblet cell (GC) increased due to higher level of HOCM in the diets.

Table 5.9 The scores of histological parameters (sub-mucosa, supranuclear vacuoles, goblet cells, and lamina propria) in hindgut of Atlantic salmon smolts fed four experimental diets for 16 weeks.

	Control	HOCM			p-value
		8%	16%	24%	
SM	1.0±0.0	1.0±0.2	1.0±0.2	1.0±0.2	0.802
SNV	2.8±0.6	2.7±0.7	2.6±0.6	2.4±0.6	0.098
GC	1.9±0.6 c	2.6±0.9 b	2.7±0.6 ab	2.9±0.7 a	0.000
LP	2.3±0.5	2.2±0.6	2.4±0.5	2.5±0.6	0.064

SM: sub-mucosa, SNV: supranuclear vacuoles, GC: goblet cells, LP: lamina propria
N= 36 per treatment.
a-c: mean±SD. Means with different letters in each row were significantly different.

5.6.6 Post-mortem Evaluation

There were no significant findings on external and internal necropsy examination among all 48 fish, and there were no infectious diseases. Fish fed 16% and 24% HOCM contained less peri-cecal fat and had a thinner body than fish fed 8% HOCM and control diets. There were no significant findings in the bacteriology of kidney, gill and skin wet mounts. Two fish with white lesions on the liver were found during collection of gut samples, was diagnosed as low grade mycobacterial infection in the liver. This problem was not evident in the 48 post-mortem fish. Fish were not infected with any pathogenic disease. The image of fish body size in various groups is attached as Appendix H.

5.7 Discussion

5.7.1 Experimental Diets and Fish Growth

Bullerwell and Anderson (2012) reported that 20% of HOCM did not inhibit the growth of rainbow trout. By contrast, the growth of Atlantic salmon smolts was reduced by 8% of

HOCM in the current study. The effects of HOCM on fish growth may be different in various species. Body weight gain was driven by the rate of amino acid deposition (Dumas et al., 2007). The lower levels of protein retention ratio in the fish fed 16% and 24% HOCM diets was correlated with the decreased body weight gain. Lall and Anderson (2005) indicated that the availability of amino acids in plant protein was not as high as that in fishmeal. Although the calculated amino acids met the requirements of Atlantic salmon, the actual availability of amino acids may decrease due to the increased level of HOCM in the diets. The various levels of poultry byproduct meal (<15%) in the experimental diets should not cause the reduction of growth, since poultry byproduct meal can be effectively included up to 20% in the salmonid diet (Fowler, 1991; Steffens, 1994). The fish fed 8% HOCM had similar protein retention ratio to the fish fed control, but lower weight gain. This result revealed that there must be other factors inhibiting the growth.

The potential antinutrional factors in HOCM included tannins, sinapine, phytic acid, and glucosinolates. The harmful levels of tannins and sinapine for Atlantic salmon are unknown. The phytic acid level in all diets was under the safe upper limit level of 1% as fed (Denstadli et al., 2006). The level of glucosinolates in HOCM diets (4.9-8.7μmol/g) was over the safe upper limit level of 1.4 μmol/g reported for rainbow trout fed rapeseed meal (Burel et al., 2000; NRC, 2011), and also higher than 2.6 μmol/g recommended in Chapter 4. In addition, Burel et al (2000) reported 30 μmol/ kg fish body weight/day glucosinolates impaired the feed efficiency, and it was found at 35μmol/kg fish body weight/day of glucosinolates (8%HOCM diet, not reported in the results section) in the current study. The variation might be due to the significantly different composition of glucosinolates between rapeseed and camelina, different fish species and interaction with other antinutrient factors. In general, glucosinolates impair the growth of animals via two ways, one is the reduction of feed intake due to the bitterness, and another is the interruption of thyroid function. The decrease of feed consumption was evident in rats, dairy cows, and pigs (Korsrud and Bell, 1967; Mawson et al., 1993). In the current study, fish consumed a significantly lower amounts of HOCM diets compared to control diet, and weight gain was highly dependent on the feed consumption over the time (R^2=0.98). This result indicated that glucosinolates may be reducing the feed palatability to fish, and subsequently decreased weight gain. Glucosinolates induced hypothyroid condition was reported by several workers (Hardy and

Sullivan, 1983; Burel et al., 2000; 2001). The levels of triiodothyronine (T_3) and thyroxine (T_4) in plasma decreased, associated with hyperactivity in the thyroid tissue. This is because thiocyanate anions, metabolic byproducts from glucosinolates, compete for iodine (Burel et al., 2001). Self-compensation of T_3 could ensure a normal growth of rainbow trout in two months, when the fish fed a diet containing 7.3 μmol/g of glucosinolates (Burel et al., 2000; 2001). T_4 can partly convert to T_3 via desiodination, and the level of T_3 was unchanged (Burel et al., 2001). In the current study, the weight of fish fed 8% HOCM (4.9 μmol/g of glucosinolates) was reduced after four weeks. In addition, other antinutritional factors in camelina meal, such as non-starch polysaccharides (NSP), phytic acid, sinapine and tannins could contribute to the poor voluntary feed consumption and growth. This issue needs further studies to isolate the effect of each factor on salmon.

5.7.2 Carcass Composition

The carcass composition (DM basis) of rainbow trout fed HOCM diets was similar (Pan et al., 2011). However, it was affected by the dietary treatments in the current study, which was associated with severe reduction in the feed consumption and final weight. For growing fish, fish size, life cycle stage and energy intake play primary role in proximate composition in fish (Shearer, 1994b). It seems that the dietary treatments affected the growth firstly, and then altered the carcass composition in the current study. The fast growing fish tended to increase the lipid deposition in their body, because the addition energy intake promoted the lipid deposition and fat tissue growth when the protein deposition reached the maximal level (Dumas et al., 2007). On the contrary, the fish fed 16% and 24% SECM diets had poor feed consumption and growth, thus the protein and lipid deposition rates in these fish were relatively low.

5.7.3 Hindgut Histology

The hindgut in fish fed the control diet showed moderate changes in supranuclear vacuoles (SNV), goblet cells (GC), and lamina propria (LP). The starvation (Baeverfjord and Krogdahl, 1996) and acute stress (Olsen et al., 2005) contributed some degree of histological changes in the fish hindgut. The cracks between absorptive cells and lamina propria among all treatments in present study were not described in the soybean induced enteritis in Atlantic salmon (Baeverfjord and Krodahl, 1996; Bakke-McKellep et al., 2007;

Uran et al., 2008b, 2009). The cause was undetermined. It might be caused by the multiple stresses, such as fungal infections, formalin bath, and frequently inevitable handlings in the early period of experiment.

Goblet cells (GC) play an important role by secreting mucus as part of the innate defense system (Marchetti et al., 2006). Supranuclear vacuoles (SNV) and GC were believed to be the first locations exhibiting enteritis features, and villi could not recover when Atlantic salmon were continually fed with soybean meal (Uran et al., 2008b). Both abnormalities of GC and SNV were evident in Atlantic salmon fed full fat soybean meal (Van den Ingh et al., 1991). The increased number of GC when graded levels of HOCM were fed implied that enteritis was initiated. The hindgut was a major site of endocytosis of intact proteins in teleosts (Stroband and Van der veen, 1981; Rombout, et al., 1985). The soybean induced enteritis caused reduction of microvilli, which resulted in exposure of enterocyte tight junctions, and led to the loss of the protective barrier on the intestinal epithelium in rainbow trout (Merrifield et al., 2009). Uran et al. (2009) found the damage on microvilli, and subsequently the disappearance of SNV reflected that the endocytosis was completely blocked (Uran et al., 2008a). Mucilage in camelina may be responsible for the decreased size of SNV as discussed in Chapter 4. In the diet formulation, pregelatinized starch was added at 2.5% as a binding agent. Wheat also has a binding function, and its content decreased from 12.62 to 0.43% as HOCM level increased in the diet (Table 5.1). NSP exist in these ingredients, and the soluble NSP increase the viscosity of digesta (NRC, 2011). This may explain the observation of diffused size reduction of SNV was independent with the dietary treatments. The higher level of poultry meal in control diet should be considered, but the effects of poultry meal on the gut histology of Atlantic salmon has not been described in the scientific literature. Plant lipid also increased the number of GC (Caballero et al., 2002; Merida et al., 2010), but it did not occur in fish fed CO diets in previous two studies (Chapter 3 and Chapter 4). Overall, the enteritis was developing in fish fed HOCM diets during the experiment, and it was dose dependent. Theoretically, the following changes in the hindgut of Atlantic salmon might have occurred in the present study: the enteritis was accompanied with reduction of microvilli and shrinkage of villi (Uran et al., 2009), reduction of digestive enzyme activity (Krogdahl et al., 2003), increased permeability of hindgut epithelium, inhibition of nutrient absorption (Knudsen et al., 2008;

Nordrum, et al., 2000), and changes in intestinal microbiota (Merrifield et al., 2009; Green et al., 2013). The hindgut was not considered a major site for uptake of nutrients, but exhibited immune function in Atlantic salmon (reviewed by Rombout et al., 2011). Nevertheless, the decrease of villi area due to HOCM in the present study implied that the absorptive area decreased, and it might be associated with the decreased mean body weight.

5.7.4 Mortality

Atlantic salmon smolts were reared in freshwater in the first four weeks at 12 °C. Those fish were supposed to lose some smolt characteristics, such as decrease in Na^+/K^+-ATPase activity (Duston et al., 1991). A large number of dead fish due to fungus were seen in the first five weeks. One reason might be the weakened immune system during the reversal of physiology. Furthermore, some inadequate essential nutrients could weaken the immune system, for example the compromise of physical barriers including skin and mucus (Lall, 2000). However, current diets were nutritionally balanced, the problem related to mortality maybe due to factors in camelina other than inadequate nutrients. It indicated that it is unsafe to use this formulation when Atlantic salmon are at stressed/delicate state. The salinity was adjusted from 0 to 2 ppt, which was not considered to impair the growth (Duston, 1994). In addition, the low salinity succeeded in inhibiting the growth of fungus (*Saprolegnia sp.*) on the fish.

5.8 Conclusion

The growth of Atlantic salmon smolts in freshwater was impaired by high oil residue camelina meal due to low feed consumption. Carcass protein, lipid and protein retention ratio were not compromised by 8%HOCM diet, but was reduced by higher level HOCM. The onset of enteritis was observed in fish fed diets containing HOCM. An investigation of a satisfactory level of HOCM less than 8% is needed. The causative agents of enteritis and the cracks between lamina propria and enterocytes in the hindgut villi need further study. This study suggests a study with lower levels of HOCM from 0 to 10% in the diet.

Chapter 6 : Conclusion

6.1 Outcomes of Current Study

The camelina byproducts in the present studies included camelina oil (CO), solvent extracted camelina meal (SECM) and high oil residue camelina meal (HOCM). The life stages of Atlantic salmon tested were parr (initial weight: 8.4 g) and smolts (initial weight: 61.8 g) and post-smolts (initial weight: 242 g).

In the parr study, experimental diets were control, 50% CO, 100% CO, and 5, 10, 15 and 20% SECM. Camelina oil was successful in replacing 50% and 100% fish oil. Ten percent SECM could be included in the diet without compromising the growth, carcass composition, and hindgut histology of Atlantic salmon parr in 16 weeks.

In the post-smolt study in seawater, control, 8%, 16%, 24% SECM, 100% CO+SEFM, 100% CO+10% SECM+SEFM, 100% CO+FM, and 100% CO+10% SECM+FM diets were tested. Only 100% CO and 8% SECM diets were found satisfactory by evaluating the growth, carcass composition, and hindgut histology. The combination of 100% CO and 10% SECM inhibited the growth of fish. The supranuclear vacuoles tended to disappear, and the sub-mucosa increased in fish fed 24% SECM.

In the smolt trial in freshwater, fish were fed control, 8%, 16% and 24% HOCM diets. Growth performance was inhibited by HOCM in a dose-dependent manner. The reduced growth was caused by the poor feed intake. Carcass composition was affected by HOCM. The protein retention ratio, carcass protein and fat decreased as the level of HOCM increased. The area of hindgut villi decreased in fish fed 24% HOCM diet. The number of goblet cells increased by dietary treatments. Overall, HOCM was not accepted, even at 8% of the diet.

In all the three experiments, CO could effectively replace 100% of fish oil in the diet without compromising the growth of Atlantic salmon parr and smolts, μbut it did not show the same response as the combination with camelina meal. The variation of growth performance in fish fed SECM diets between different experiments may have been due to various factors, such as life stage, rearing conditions, variety of camelina seed, and differences in the diet formulation. In general, the acceptable level of SECM for Atlantic

salmon was up to 8-10% in the diet. Multiple antinutritional factors induced the reduction in feed intake and growth, the effect of each single factor needs to be addressed in the future. The effect of glucosinolates was suspected to be one of the major antinutritional factors and the adverse effects were discussed in this thesis. The safe range of glucosinolates was 1.8 to 2.6 μmolg/g in the diet. The effect of camelina meal on the carcass composition was not consistent in three experiments. The enteritis in distal intestine was not observed in fish fed diets containing CO and the combination of CO and SECM, but various degrees of enteritis were found in fish fed the diets with more than 15% of SECM and the diets with more than 8% of HOCM. The causative agents of enteritis need to be identified in the future.

6.2 Future Directions

In general, there are three directions for future studies: the optimal levels of camelina byproducts for the various life stages/fish size that the current studies have not covered, the efficient procedures to reduce antinutritional factors in camelina meal, and the causative agents of hindgut enteritis in Atlantic salmon fed camelina meals.

The investigation of camelina byproducts fed to first feeding salmon has been conducted. In commercial production, the parr are reared to 80-100g in the hatchery, and transferred to the sea cages. The size of fish in the first experiment (Chapter 3) reached 50 g after 16 weeks. A similar design, but in a longer feed period maybe needed to cover the entire parr stage. A graded levels of HOCM up to 8% need to be investigated. Once the optimal level of HOCM for Atlantic salmon smolts was found, it should be evaluated on the large post-smolts (>500 g fish). The effects of 10% SECM and 100% CO on the large post-smolts need to be investigated, since the period of grow-out is about 1.5 years, and current studies are restricted to the early part of this period. How long does a fish oil diet restore the levels of EPA and DHA in Atlantic salmon flesh remains unknown, since the EPA and DHA levels were reduced due to the 100% CO. The camelina protein concentrate with higher protein content should be developed and tested in salmon diets.

Comparisons of pre-treated camelina meal fed to Atlantic salmon are needed. Factors such as heat, solvent extraction, and enzyme treatments need to be evaluated. The changes of thyroid morphology and thyroid hormones in Atlantic salmon fed camelina need to be investigated, regarding the negative effects of glucosinolates.

References

Anderson, J. S., Lall, S. P., Anderson, D. M. and McNiven, M. A., 1995. Availability of amino acids from various fish meals fed to Atlantic salmon (*Salmo salar*). Aquaculture. **138**: 291-301.

Anderson, D. M., MacPherson, M. J. and MacIsaac, P. F., 2008. Feeding full-fat oilseeds as partial replacement for fish meal and fish oil in practical diets for rainbow trout fingerlings. Can. J. Anim. Sci. **88**: 166.

AOAC International. (2011). Official methods of analysis of AOAC International. 18th Edition, Revision 4. Arlington, Va: AOAC International.

Aslaksen, M. A., Kraugerud, O. F., Penn, M., Svihus, B., Denstadli, V., Jorgensen, H. Y., Hillestad, M., Krogdahl, A. and 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.

Aziza, A. E., Quezada, N. and Cherian, G., 2010. Feeding *Camelina sativa* meal to meat-type chickens: effect on production performance and tissue fatty acid composition. J. Appl. Poultry. Res. **19**: 157-168.

Baeverfjord, G. and 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. J. Fish. Biol. **19**: 375-387.

Bakke-McKellep, A. M., Press, C. M., Baeverfjord, G., Krogdahl, A. and 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. J. Fish. Biol. **23**: 115-127.

Bakke-McKellep, A. M., Penn, M. H., Salas, P. M., Refstie, S., Sperstad, S., Landsverk, T. Ringo, E. and Krogdahl, A., 2007. Effects of dietary soyabean meal, inulin and oxytetracycline on intestinal microbiota and epithelial cell stress, apoptosis and proliferation in the teleost Atlantic salmon (*Salmo salar* L.). Brit. J. Nutr. **97**:699-713.

Bell, J. M., and Keith, M. O., 1991. A survey of variation in the chemical composition of commercial canola meal produced in Western Canadian crushing plants. Can. J. Anim. Sci.**71**: 469-480.

Bell, M. V., Dick, J. R. and Porter, A. E. A., 2001. Biosynthesis and tissue deposition of docosahexaenoic acid (22:6n-3) in rainbow trout (*Oncorhynchus mykiss*). Lipids. **36**: 1153-1159.

Bell, J. G., McGhee, F., Campbell, P. J. and Sargent, J. R., 2003. Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar L.*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil "wash out". Aquaculture. **218**: 515-528.

Bell, J. G., Henderson, R. J., Tocher, D. R. and Sargent, J. R., 2004. Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet. Lipid. **39**: 223-232.

Bell, J. G., Pratoomyot, J., Strachan, F., Henderson, R. J., Fontanillas, R., Hebard, A., Guy, D. R., Hunter, D. and Tocher, D. R., 2010. Growth, flesh adiposity and fatty acid composition of Atlantic salmon (*Salmo salar*) families with contrasting flesh adiposity: Effects of replacement of dietary fish oil with vegetable oils. Aquaculture. **306**: 225-232.

Bransden, M. P., Carter, C. G., and Nichols, P. D., 2003. Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (*Salmo salar L.*): effect on growth performance, tissue fatty acid composition and disease resistance. Comp. Biochem. Phys. B, **135**: 611-625.

Budin, J. T., Breene, W. M. and Putnam, D. H., 1995. Some compositional properties of camelina (*Camelina sativa L.* Grantz) seeds and oils. J. Am. Oil. Chem. Soc., **72**: 309-315.

Bullerwell, C. and Anderson, D. M., 2012. Performance of rainbow trout (*Oncorhynchus mykiss*) fed camelina sativa seeds and high oil residue meal. Proc. Aquaculture Association of Canada Annual Conference, Charlottetown, PEI. pp:137.

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

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

Burr, G. S., Wolters, W. R., Barrows, F. T., and Hardy, R. W., 2012. Replacing fishmeal with blends of alternative proteins on growth performance of rainbow trout (*Oncorhynchus mykiss*), and early or late stage juvenile Atlantic salmon (*Salmo salar*). Aquaculture. **334-337**: 110-116.

Burr, G. S., Wolters, W. R., Barrows, F. T. and Donkin, A. W., 2013. Evaluation of a canola protein concentrate as a replacement for fishmeal and poultry by-product meal in a commercial production diet for Atlantic salmon (*Salmo salar*). Int. Aquat. Res. **5**: 5.

Buzzi, M., Henderson, R. J. and Sargent, R., 1997. Biosynthesis of docosahexaenoic acid in trout hepatocytes proceeds via 24-carbon intermediates. Comp. Biochem. Physiol. **116B**: 263-267.

Caballero, M. J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M. and Lzquierdo, M. S., 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. Aquaculture. **214**: 253-271.

Canadian Council on Animal Care (CCAC), 2005. Guidelines on: the care and use of fish in research, teaching and testing.

Canola Council of Canada. Health benefits of canola oil. http://www.canolacouncil.org/oil-and-meal/canola-oil/health-benefits-of-canola-oil/. Accessed on March 30, 2014.

Cao, L., Wang, W. M., Yang, C. T., Yang, Y., Diana, J., Yakupitiyage, A., Luo, Z. and Li, D. P., 2007. Review: Application of microbial phytase in fish feed. Enzyme. Microb. Tech. **40**: 497-507.

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

Carey, J. B. and McCormick, S. D., 1998. Atlantic salmon smolts are more responsive to an acute handling and confinement stress than parr. Aquaculture. **168**: 237-253.

Cherian, G., Campbell, A. and Parker, T., 2009. Egg quality and lipid composition of eggs from hens fed *Camelina sativa*. J. Appl. Poultry. Res. **18**: 143-150.

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

Connor, W. E., 2000. Importance of n-3 fatty acids in health and disease. Am. J. Clin. Nutr. **71** (suppl): 171s-175s.

Corner, A. H., 1983. Cardio pathology associated with the feeding of vegetable and marine oils. In Kramer, J. K. G. (ed.), Sauer, F. D. (ed.) and Pigden, W. J. (ed.). High and low erucic acid rapeseed oils. Pp: 293-313. Academic Press.

Crowley, J. G. and Frohlich, A., 1998. Factors affecting the composition and use of camelina. ISBN 19001138666. Crops Research Centre, Teagasc. Dublin, Ireland.

Daxenbichler, M. E., Vanetten, C. H. and Brown, F. S., 1964. Oxazolidinethiones and volatile isothiocyanates in enzyme-treated seed meals from 65 species of Cruciferae. J. Agr. Food. Chem. **12**: 127-130.

Deng, Q., Huang, F., Huang, Q, Xu, J. and Liu, C., 2011. Lipid-lowering evaluation of cold-pressed *Camelina sativa* oil. J. Food. Agric. Environ. **9**: 157-162.

Denstadli, V., Skrede, A., Krogdahl, Sahlstrom, S. and Storebakken, T., 2006. Feed intake, growth, feed conversion, digestibility, enzyme activities and intestinal structure in Atlantic salmon (*Salmo salar L.*). Aquaculture, **256**: 365-376.

Downey, R. K., 1971. Agricultural and genetic potentials of cruciferous oilseed crops. J. Am. Oil. Chem. Soc. **48**: 718-722.

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

Dumas, A., De Lange, C. F. M., France, J. and Bureau, D. P., 2007. Quantitative description of body composition and rates of nutrient deposition in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. **273**: 165-181.

Duston, J., 1994. Effect of salinity on survival and growth of Atlantic salmon (*Salmo salar*) parr and smolts, 1994. Aquaculture. **121**: 115-124.

Duston, J., Saunders, R. L. and Knox, D. E., 1991. Effects of increases in freshwater temperature on loss of smolt characteristics in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **48**: 164-169.

Eidhin, D. H., Burke, J. and O'Beirne, D., 2003. Oxidative stability of ω3-rich camelina oil and camelina oil-based spread compared with plant and fish oils and sunflower spread. J. Food. Sci. **68**: 345-353.

Ellis, A. E., Roberts, R. F. and Tyler, P., 1978. Chapter 2: The anatomy and physiology of teleosts In Roberts, R. J. (ed.). Fish pathology. Pp: 37. London: Bailliere Tindall.

Espe, M., Lemme, A., Petri, A. and EI-Mowafi, A., 2006. Can Atlantic salmon (*Salmo salar*) grow on diets devoid of fish meal? Aquaculture. **255**: 255-262.

European Food Safety Authority (EFSA), 2010. Scientific opinion on the safety of allyl isothiocyanate for the proposed uses as a food additive. EFSA journal. **8**:1943.

EWOS Reporting Centre, 2013. http://www.reporting.ewos.com/sustainability-reports/gri-report-2013/performance/?id=ew-i08. Accessed on August 13, 2014.

Food and Agriculture Organization (FAO), Fisheries and Aquaculture Department, 2010. The state of world fisheries and aquaculture. Food and Agriculture organization of the United Nations, Rome. Pp: 3, 38-44.

Food and Agriculture Organization (FAO), Fisheries and Aquaculture Department, 2012. The state of world fisheries and aquaculture. Food and Agriculture organization of the United Nations, Rome. Pp: 3, 65, 81 172-177.

Food and Agriculture Organization (FAO), 2014. The state of world fisheries and aquaculture: opportunities and challenges. Food and Agriculture organization of the United Nations, Rome. Pp: 61-62, 201.

Fowler, L. G., 1991. Poultry by-product meal as a dietary protein in fall chinook salmon diets. Aquaculture. **99**: 309-321.

Frame, D. D., Palmer, M. and Peterson, B., 2007. Use of *Camelina sativa* in the diets of young turkeys. J. Appl. Poultry. Res. **16**: 381-386.

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

Francis, C. M. and Campbell, M. C., 2003. New high quality oil seed crops for temperate and tropical Australia. RIRDC Publication NO 03/045. Rural Industries Research and Development Corporation.

Green, T. J., Smullen, R. and Barnes, A. C., 2013. Dietary soybean protein concentrate-induced intestinal disorder in marine farmed Atlantic salmon, *Salmo salar* is associated with alterations in gut microbiota. Vet. Microbiol. **166**: 286-292.

Gugel, R. K. and Falk, K. C., 2006. Agronomic and seed quality evaluation of *Camelina sativa* in western Canada. Can. J. Plant. Sci. **86**: 1047-1058.

Handeland, S. O. and Stefansson, S. O., 2002. Effects of salinity acclimation on pre-smolt growth, smolting and post-smolt performance in off-season Atlantic salmon smolts (*Salmo salar* L.). Aquaculture. **209**: 125-137.

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

Hastings, N., Agaba, M. K., Tocher, D. R., Zheng, X. Z., Dickson, C. A. 2004. Molecular cloning and functional characterization of fatty acyl desaturase and elongase cDNAs involved in the production of eicosapentaenoic and docosahexaenoic acids from α-linolenic acid in Atlantic salmon (*Salmo salar*). Mar. Biotechnol. **6**: 463-474.

Hayter, A. J., 1984. A proof of the conjecture that the Tukey-Kramer multiple comparisions procedure is conservative. Ann. Stat. **12**: 61-75.

Hendricks, J. D., 2002. Adventitious toxins. In Halver, J. E. (ed.) and Hardy, R. W. (ed.) Fish Nutrition, 3rd Edition. Pp: 602-041. Academic Press.

Higgs, D. A., McBride, J. R., Markert, J. R., Dosanjh, B. S., Plotnikoff, M. D., and Clarke, W. C., 1982. Evaluation of tower and candle rapeseed (canola) meal and Bronowski rapseed protein concentrate as protein supplements in practical dry

diets for juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture. **29**: 1-31.

Hixson, S. M., Parrish, C. C. and Anderson, D. M., 2013. Effect of replacement of fish oil with camelina (*Camelina sativa*) oil on growth, lipid class and fatty acid composition of farmed juvenile Atlantic cod (*Gadus morhua*). Fish. Physiol. Biochem. **39**: 1441-1456.

Hixson, S. M., Parrish, C. C. and Anderson, D. M., 2014a. Changes in tissue lipid and fatty acid composition of farmed rainbow trout in response to dietary camelina oil as a replacement of fish oil. Lipids. **49**: 97-111.

Hixson, S. M., Parrish, C. C. and Anderson, D. M., 2014b. Full substitution of fish oil with camelina (*Camelina sativa*) oil, with partial substitution of fish meal with camelina meal, in diets for farmed Atlantic salmon (*Salmo salar*) and its effect on tissue lipids and sensory quality. Food. Chem. **157**: 51-61.

Hixson, S. M., Parrish, C. C. and Anderson, D. M., 2014c. Use of camelina oil to replace fish oil in diets for farmed salmonids and Atlantic cod. Aquaculture. **431**: 44-52.

Hossain, M. A., and Jauncey, K., 1989. Nutritional evaluation of some Bangladeshi oilseed meals as partial substitutes for fish meal in the diet of common carp, *Cyprinus carpio* L. Aquacult. Fish. Manage. **20**: 255-268.

Jiang, Y. F., Caldwell, C. D. and Falk, K. C., 2014. Camelina seed quality in response to applied nitrogen, genotype and environment. Can. J. Plant. Sci. **94**: 971-980.

Jobling, M., 1993. Nutrition, diet formulation and feeding practices. In Heen, K. (ed.), Monahan, R. L. (ed.), and Utter, F. (ed.). Salmon Aquaculture. Oxford: Fishing News Books. pp: 83-126.

Johnson, A., personal communication. Analytical services supervisor, POS Bio-Science, 118 Veterinary Road, Saskatoon, SK, Canada. S7N 2R4.

Kjaer, A., Gmelin, R. and Jensen, R. B., 1956. Isothiocyanates XXI. (-)-10-methylsulphinyldecyl isothiocyanate, a new mustard oil present as a glucoside (Glucocamelinin) in *Camelina* species. Acta Chemica Scandinavica. **10**: 1614-1619.

Knudsen, D., Uran, P. A., Arnous, A., Koppe, W. and Frokiaer, H., 2007. Saponin-containing subfractions of soybean molasses induce enteritis in the distal intestine of Atlantic salmon. J. Arg. Food. Chem. **55**: 2261-2267.

Knudsen, D., Jutfelt, F., Sundh, H., Sundell, K., Koppe, W. and Frokiaer, 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.). Brit. J. Nutr. **100**: 120-129.

Korsrud, G. O. and Bell, J. M., 1967. The feeding value of solvent-extracted camelina meal effects of levels fed, heat treatments and flavor supplements. Can. J. Anim. Sci. **47**: 109-114.

Korsrud, G. O., Keith, M. O. and Bell, J. M., 1978. A comparison of the nutritional value of crambe and camelina seed meals with egg and casein. Can. J. Anim. Sci. **58**: 493-499.

Krogdahl, A., Bakke-McKellep, A. M., Roed, K. H. and Baeverfjord, G., 2000. Feeding Atlantic salmon *Salmo salar* L. soybean produts: effects on disease resistance (*furunculosis*), and lysozyme and IgM levels in the intestinal mucosa. Aquacult. Nutr. **6**: 77-84.

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

Lakshmy, R., Rao, P. S., Sesikeran, B., and Suryaprakash, P., 1995. Iodine metabolism in response to goitrogen induced altered thyroid status under conditions of moderate and high intake of iodine. Horm. Metab. Res. **27**: 450-454.

Lall, S. P., 2000. Nutrition and health of fish. In: Cruz -Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Olvera-Novoa, M.A. y Civera-Cerecedo, R., (Eds.). Avances en Nutrición Acuícola V. Memorias del V Simposium Internacional de Nutrición Acuícola. 19-22.

Lall, S. P. and Anderson, S., 2005. Amino acid nutrition of salmonids: Dietary requirements and bioavailability. In Monter, D. (ed.), Basurco, B. (ed.), Nengas, I. (ed.), Alexis, M. (ed.), and Izquierdo, M. (ed.). Mediterranean fish nutrition. **63** (pp: 73-90). International centre for Advanced Mediterranean Agronomic Studies (CIHEAM).

Landero, J. L., Beltranena, E., Cervantes, M., Araiza, A. B. and Zijlstra, R. T., 2012. The effect of feeding expeller-pressed canola meal on growth performance and diet nutrient digestibility in weaned pigs. Anim. Feed. Sci. Technol. **171**: 240-245.

Littell, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R. D. and Schabenberger, O., 2006. SAS for mixed models (2nd ed). Cary, NC, USA: SAS institute.

Lokka, G., Austbo, L., Falk, K., Bjerkas, I. and Koppang, E. O., 2013. Intestinal morphology of the wild Atlantic salmon (*Salmo salar*). J. Morphol. **274**: 859-876.

Mandal, S. and Ghosh, K., 2010. Inhibitory effect of *Pistia* tannin on digestive enzymes of Indian major carps: an in vitro study. Fish. Physiol. Biochem. **36**: 1171-1180.

Marchetti, L., Capacchietti, M., Sabbieti, M. G., Accili, D., Materazzi, G. and Menghi, G., 2006. Histology and carbohydrate histochemistry of the alimentary canal in the rainbow trout *Oncorhynchus mykiss*. J. Fish. Biol. **68**: 1808-1821.

Matthaus, B., 1997. Antinutritive compounds in different oilseeds. Lipid/Fett. **99**: 170-174.

Matthaus, B. and Zubr, J., 2000. Variability of specific components in *Camelina sativa* oilseed cakes. Ind. Crop. Prod. **12**: 9-18.

Mawson, R., **Heaney, R. K., Zdunczyk. Z. and Kozlowska, H., 1993.** Rapeseed meal-glucosinolates and their antinutritional effects. Part 2. Flavour and Palatability. Molecular Nutrition. **37**: 336-334.

Mazza, G and Biliaderis, C. G., 1989. Functional properties of flax seed mucilage. J. Food. Sci. **54**: 1302-1305.

McCormick, S. D., and Saunders, R. L., 1987. Preparatory physiological adaptation for marine life of salmonids: Osmoregulation, Growth, and Metabolism. Am. Fish. Soc. Symp. **1**: 211-229.

McCormick, S. D., Shrimpton, J. M., Moriyama, S. and Bjornsson, B.T., 2007. Differential hormonal responses of Atlantic salmon parr and smolt to increased daylength: A possible developmental basis for smolting. Aquaculture. **273**: 337-344.

McCurdy, S. M. and March, B. E., 1992. Processing of canola meal for incorporation in trout and salmon diets. J. Am. Oil. Chem. Soc. **69**: 213-220.

Merida, S. N., Vidal, A. T., Llorens, S. M., and Cerda, M. J., 2010. Sunflower meal as a partial substitute in juvenile sharpsnout sea bream (*Diplodus puntazzo*) diets: amino acid retention, gut and liver histology. Aquaculture. **298**: 275-281.

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

Merrifield, D. L., Rolf, E. O., Myklebust, R. and Ringo, E., 2011. Dietary effect of soybean (*Glycine max*) products on gut histology and microbiota of fish. In El-Shemy, H. (ed.) Soybean and Nutrition, Chapter 12 (231-250). ISBN 978-953-307-536-5: InTech.

Miller, R. W., VanEtten, C. H., McGrew, C., Wolff, I. A., and Jones, Q., 1962. Seed meal amino acids, amino acid composition of seed meals from forty-one Species of Cruciferae. J. Agr. Food. Chem. **10**, 426-430.

Mithen, R. F., Dekker, M., Verkerk, R., and Rabot, S., 2000. Review: The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. J. Sci. Food. Agr. **80**: 967-984.

Morais, S., **Edvardsen, R. B., Tocher, D. R. and Bell, J. G., 2012.** Transcriptiomic analyses of intestinal gene expression of juvenile Atlantic cod (*Gadus morhua*) fed diets with camelina oil as replacement for fish oil. Comp. Biochem. Physiol. **161**: 283-293.

National Research Council (NRC, U.S.), 2011. Nutrient requirements of fish and shrimp. Washington, D.C: National Academies Press. Pp: 70, 236-237, 327-347.

Newkirk, R. W., Classen, H. L., Scott, T. A. and Edney, M. J., 2003. The digestibility and content of amino acids in toasted and non-toasted canola meals. Can. J. Anim. Sci. **83**: 131-139.

Nordrum, S., Bakke-McKellep, A. M., Krogdahl, A. and 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*). Comp. Biochem. Physiol. B: Biochem Mol Biol. **125**:317-335.

Olli, J. J. and Krogdahl, A., 1995. Alcohol soluble components of soybeans seem to reduce fat digestibility in fish-meal-based diets for Atlantic salmon, *Salmo salar* L. Aquac. Res. **26**: 831-835.

Olin, P. G., Smith, J., and Nabi, R., 2010. Regional review on status and trends in aquaculture development in North American: Canada and the United States of American. FAO Fisheries and Aquculture Circular NO. 1061/2, Pp 9-11.

Olsen, R. E., Sundell, K., Mayhew, T. M., Myklebust, R. and Ringo, E., 2005. Acute stress alters intestinal function of rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture. **250**: 480-495.

Overland, M., Sorensen, M., Storebakken, T., Penn, M., Krogdahl, A. and Skrede, A., 2009. Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon, (*Salmo salar*)-effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. Aquaculture. **288**: 305-311.

Pan, W., Xie, W., Caldwell, C. D. and Anderson, D. M., 2011. Growth performance and carcass composition of rainbow trout (*Oncorhynchus mykiss*) fed practical diets containing graded levels of high fat residue *Camelina* meal. Can. J. Anim. Sci. **91**: 484.

Peiretti, P. G., **Mussa, P. P., Prola, L. and Meineri, G., 2007a.** Use of different levels of false flax (*Camelina sativa L.*) seed in diets for fattening rabbits. Livest. Sci. **107**: 192-198.

Peiretti, P. G., Mussa, P. P., Meineri, G. and Perona, G., 2007b. Apparent digestibility of mixed feed with increasing levels of false flax (*Camelina sativa L.*) seeds in rabbit diets. J. Food. Agric. Environ. **5**: 58-88.

Plessers, A. G., McGregor, W. G., Carson, R. B. and Nakoneshny, W., 1962. Species trials with oilseed plants: II Camelina. Can. J. Plant. Sci. **42**: 452-458.

Putnam, D. H., Budin, J. T., Field, L. A. and Breene, W. M., 1993. Camelina: A promising low-input oilseed. Pp: 314-322. In: J. Janick and J.E. Simon (eds.), New crops. Wiley, New York.

Rask, L., Andreasson, E., Ekbom, B., Eriksson, S., Pontoppidan, B. and Meijer, J., 2000. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. Plant. Mol. Biol. **42:** 93-113.

Redovnikovic, I. R., Glivetic, T. J., Delonga, K. and Vorkapic-Furac, J., 2008. Glucosinolates and their potential role in plant. Period Biol. **110**: 297-309.

Reed, J. D., 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci. **73**: 1516-1528.

Refstie, S., Svihus, B., Shearer, K. D., and 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., Korsoen, O. J., Storebakken, T., Baeverfjord, G., Lein, I. and 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.

Richardson, N. L., Higgs, D. A., Beames, R. M. and McBride, J. R., 1985. Influence of dietary calcium, phosphorus, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile chinook salmon (*Oncorhynchus tshawytscha*). J. Nutr. **115**: 553-567.

Robin, J. H., Regost, C., Arzel, J. and Kaushik, S. J., 2003. Fatty acid profile of fish following a change in dietary fatty acid source: model of fatty acid composition with a dilution hypothesis. Aquaculture. **225**: 283-293.

Robinson, R. G., 1987. Camelina: A useful research crop and a potential oilseed crop. Minnesota Agricultural Experiment Station. **579**: 1-12.

Rombout, J. H. W. M., Lamers, C. H. J., Helfrich, M. H., Dekker, A. and Taverne-Thiele, J. J., 1985. Uptake and transport of intact macromolecules in the intestinal epithelium of carp (*Cyprinus carpio* L.) and the possible immunological implications. Cell. Tissue. Res. **239**: 519-530.

Rombout, J. H. W. M., Abelli, L., Picchietti, S., Scapigliati, G. and Kiron, V., 2011. Teleost intestinal immunology. Fish. Shellfish. Immun. **31**: 616-626.

Ruxton, C. H. S. and Derbyshire, E., 2009. Latest evidence on omega-3 fatty acids and health. Nutr. Food. Sci. **39**: 423-438

Sanden, M., Berntssen, M. H. G., Krogdahl, A., Hemre, G-I.and Bakke-McKellep, A-M., 2005. An examination of the intestinal tract of Atlantic salmon, *Salmo salar L*., parr fed different varieties of soy and maize. J. Fish. Biol. **28**: 317-330.

Sauer, F. D. and Kramer, J. K. G., 1983. The problems associated with the feeding of high erucic acid rapeseed oils and some fish oils to experimental animals. In Kramer, J. K. G., Sauer, F. D. and Pigden, W. J. (eds.). High and low erucic acid rapeseed oils. Pp: 253-291. Academic Press.

Schuster, A. and Friedt, W., 1998. Glucosinolate content and composition as parameters of quality of *Camelina* seed. Ind. Crop. Prod. **7**: 297-302.

Shafaeipour, A., Yavari, V., Falahatkar, B., Maremmazi, J. G. H. and Gorjipour, E., 2007. Effects of canola meal on physiological and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*). Aquacult. Nutr. **14**: 110-119.

Shapiro, T. A., Fahey, J. W., Wade, K. L., Stephenson, K. K. and Talalay, P., 2001. Chemoprotecitve glucosinolates and isothiocyanates of broccoli sprouts: metabolism and excretion in Humans. Cancer. Epidem. Biomar. **10**: 501-508.

Shearer, K. D., Asgard, T., Andorsdottir, G. and Aas, G. H., 1994a. Whole body elemental and proximate composition of Atlantic salmon (*Salmo salar*) during the life cycle. J. Fish. Biol. **44**: 785-797.

Shearer, K. D., 1994b. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture. **119**: 63-88.

Stefansson, S. O., Bjornsson, B. T., Ebbesson, L. O. E. and McCormick, S. D., 2008. Smoltification. In Finn, R. N. and Kappor, B. G. (Eds). Fish larval physiology. Enfield, NH: Science Publishers. Pp: 640-681.

Stefansson, S. O., Bjornsson, B. T., Sundell, K., Nyhammer, G. and McCormick, S. D., 2003. Physiological characteristics of wild Atlantic salmon post-smolts during estuarine and coastal migration. J. Fish. Bio. **63**: 942-955.

Steffens, W., 1994. Replacing fish meal with poultry by-product meal in diets for rainbow trout, *Oncorhynchus mykiss*. Aquaculture. **124**: 27-34.

Stobiecki, M., and Makkar, H. P. S., 2004. Recent advances in analytical methods for identification and quantification of phenolic compounds. In Muzquiz, M., Hill, G. D., Cuadrado, C., Pedrosa, M. M. and Burbano, C., (Eds). Recent advances of research in antinutritional factors in legume seeds and oilseeds: Proceedings of the Fourth International Workshop on Antinutritional Factors in Legume Seeds and Oilseeds, Toledo, Spain, 8-10 March 2004. Wageningen, Netherlands: Wageningen Academic Publishers. Pp 11-28.

Storebakken, T., 1985. Binders in fish feeds: 1. Effect of alginate and guar gum on growth, digestibility, feed intake and passage through the gastrointestinal tract of rainbow trout. Aquaculture. **47**: 11-26.

Stroband, H. W. J. and Van der veen, F. H., 1981. Localization of protein absorption during transport of food in the intestine of the grasscarp, *Ctenopharyngodon idella* (Val.). J. Exp. Zool. **218**: 149-156.

Sundell, K., Jutfelt, F., Agustsson, T., Olsen, R. E., Sandblom, E., Hansen, T. and Bjomsson, B. J., 2003. Intestinal transport mechanisms and plasma cortisol levels during normal and out-of-season parr-smolt transformation of Atlantic salmon, *Salmo salar*. Aquaculture. **222**: 265-285.

Swanson, D., Block, R. and Mousa, S. A., 2012. Omega-3 fatty acid EPA and DHA: health benefits throughout life. Adv. Nutr. **3**: 1-7.

Tacon, A. G. J., 2005. Salmon aquaculture dialogue: State of Information on salmon aquaculture feed and the environment**.** International Aquafeed. **8**: 22-37.

Tacon, A. G. J., and Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture. **285**: 146—158.

Tacon, A. G. J., Hasan, M. R. and Metian, M., 2011. Demand and supply of feed ingredients for farmed fish and crustaceans - trends and prospects. FAO Fisheries and Aquculture Technical paper. **564**: 24, 54, 61-62.

Talalay, P. and Fahey, J. W., 2001. Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. J. Nutr. **131**:3027S-3033S.

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

Tocher, D. R., Bell, J. G., Dick, J. R. and Sargent, J. R. 1997. Fatty acyl desaturation in isolated hepatocytes from Atlantic salmon (*Salmo salar*): Stimulation by dietary borage oil containing γ-linolenic acid. Lipids. **32**: 1237-1247.

Tocher, D. R., Madrigal, J. F., Bell, J. G., Dick, J. R., Henderson, R. J. and Sargent, J. R., 2002. Effects of diets containing linseed oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes in Atlantic salmon (*Salmo salar*). Fish. Physiol. Biochem. **26**: 157-170.

Tocher, D. R., 2003. Metabolism and function of lipids and fatty acids in teleost fish. Reviews in Fisheries Science. **11**. 107-184.

Tocher, D. R., Bell, J. G., McGhee, F, Dick, J. R. and Madrigal, J. F., 2003. Effects of dietary lipid level and vegetable oil on fatty acid metabolism in Atlantic salmon (*Salmo salar* L.) over the whole production cycle. Fish. Physiol. Biochem. **29**: 193-209.

Torstensen, B. E., Froyland, L. and Lie, O., 2004. Replacing dietary of fish oil with increasing levels of rapeseed oil and olive oil – effects on Atlantic salmon (*Salmo salar* L.) tissue and lipoprotein lipid composition and lipogenic enzyme activities. Aquacult. Nutr. **10**: 175-192.

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

Tzeng, Y. M., Diosady, L. L. and Rubin, L. J., 1990. Production of canola protein materials by alkaline extraction, precipitation, and membrane processing. J. Food. Sci. **55**: 1147-1156.

Uran, P. A., Aydin, R., Schrama, J. W., Verreth, J. A. J. and Rombout, J. H. W. M., 2008a. Soybean meal-induced uptake block in Atlantic salmon *Salmo salar* distal enterocytes. J. Fish. Biol. **73**: 2571-2579.

Uran, P. A., Schrama, J. W., Rombout, J. H. W. M., Obach, A., Jensen, L., Koppe, W. and Verreth, J. A. J., 2008b. Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar* L.) at different temperatures. Aquacult. Nutr. **14**: 324-330.

Uran, P. A., Schrama, J. W., Rombout, J. H. W. M., Taverne-Thiele, J. J., Obach, A., Koppe, W. and Verreth, J. A., 2009. Time-related changes of the intestinal morphology of Atlantic salmon, *Salmo salar* L., at two different soybean meal inclusion levels. J. Fish. Biol. **32**: 733-744.

Van den Ingh, T. S. G. A. M., Krogdahl, A., Olli, J. J., Hendriks, H. G. C. J. M. and Koninkx, J. G. J. F., 1991. Effects of soybean-containning diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. Aquaculture. **94**: 297-305.

Van den Ingh, T. S. G. A. M., Olli, J. J. and Krogdahl, A. 1996. Alcohol-soluble components in soybeans cause morphological changes in the distal intestine of Atlantic salmon, *Salmo salar* L. J. Fish. Biol. **19**: 47-53.

Varsamos, S., Nebel, C. and CharmantieR, G., 2005. Ontogeny of osmoregulation in postembryonic fish: A review. Comp. Biochem. Phys. A. **141**: 401-429.

Vaughn, S. and Berhow, M. A., 2005. Glucosinolate hydrolysis products from various plant sources: pH effects, isolation, and purification. Ind. Crop. Prod..**21**: 193-202.

Vogtmann, H., Clandinin, D. R. and Hardin, R. T., 1974. The influence of high and low erucic acid rapeseed oils on the productive performance of laying hens and on the lipid fraction of egg yolk. Can. J. Anim. Sci. **54**: 403-410.

Wanasundara, P. K. J. P. D. and Shahidi, F., 1997. Removal of flaxseed mucilage by chemical and enzymatic treatments. Food. Chem, **59**: 47-55.

Wilson, J. M. and Castro, L. F. C., 2010. Morphological diversity of the gastrointestinal tract in fishes. Fish physiology (pp. 1-55) Academic Press. DOI: http: // dx.doi.org /10.1016 /S1 546-5098(10)03001-3.

Zubr, J., 1997. Oil-seed crop: *Camelina sativa*. Ind. Crop. Prod. **6**: 113-119.

Zubr, J. and Matthaus, B., 2002. Effects of growth conditions on fatty acids and tocopherols in *Camelina sativa* oil. Ind. Crop. Prod. **15**: 155-162.

Zubr, J., 2003a. Qualitative variation of *Camelina sativa* seed from different locations. Ind. Crop. Prod. **17**: 161-169.

Zubr, J., 2003b. Dietary fatty acids and amino acids of *Camelina sativa* seed. J. Food Quality. **26**: 451-462.

Zubr, J., 2010. Carbohydrates, vitamins and minerals of *Camelina sativa* seed. Nutr. Food. Sci. **40**: 523-531.

Appendix A. The Composition of Solvent Extracted Camelina Meal (SECM)

SECM (Calena cultivar) Typical analysis (% as fed basis)			
Crude lipid	3.0	ADF	25.4
Crude protein	39.0	ANF	39.9
Moisture	7.5	Gross energy (kcal/kg)	4320

Amino acids	% protein	% As-is
ASP	8.21	3.2
THR	4.12	1.61
SER	4.83	1.89
GLU	16.93	6.60
PRO	5.32	2.08
GLY	4.92	1.92
ALA	4.26	1.66
CYS	2.08	0.81
VAL	4.45	1.73
MET	1.60	0.62
ILE	3.11	1.21
LEU	6.21	2.42
TYR	2.48	0.97
PHE	3.88	1.52
HIS	2.88	1.12
LYS	4.55	1.78
ARG	8.37	3.27
TRP	0.96	0.37

Appendix B. The Composition of High Oil Residue Camelina Meal (HOCM)

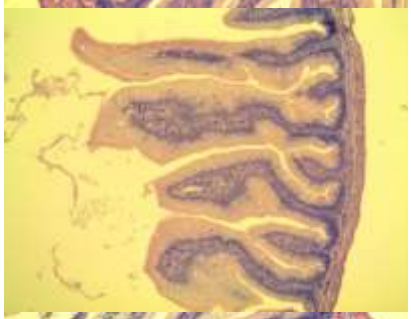
HOCM (007 line)			
Typical analysis (% as fed basis)			
Crude lipid	10.3	ADF	25.21
Crude protein	30.6	NDF	37.71
Moisture	12.3	Gross energy (kcal/kg)	5092.5

Amino acids	% protein	% As-is
ASP	7.00	2.14
THR	3.38	1.04
SER	3.96	1.21
GLU	13.69	4.19
PRO	4.13	1.26
GLY	4.14	1.27
ALA	3.48	1.07
CYS	1.73	0.53
VAL	3.67	1.12
MET	1.34	0.41
ILE	2.50	0.77
LEU	4.96	1.52
TYR	2.01	0.61
PHE	3.16	0.97
HIS	2.33	0.71
LYS	3.79	1.16
ARG	6.58	2.01
TRP	0.82	0.25

Appendix C. The Scores of Mucosal Fold (MF) in Semi-quantitative Scoring System

1

Normal MF

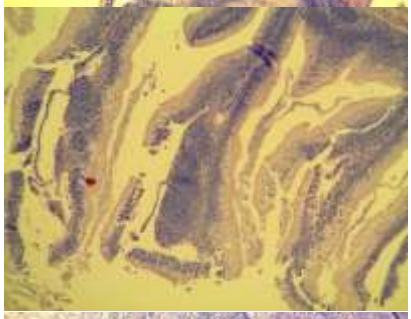


2

Some shrinkage and bloating

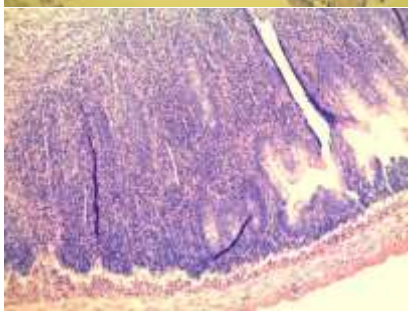
3

On set of MF disruption



4

MF disruption

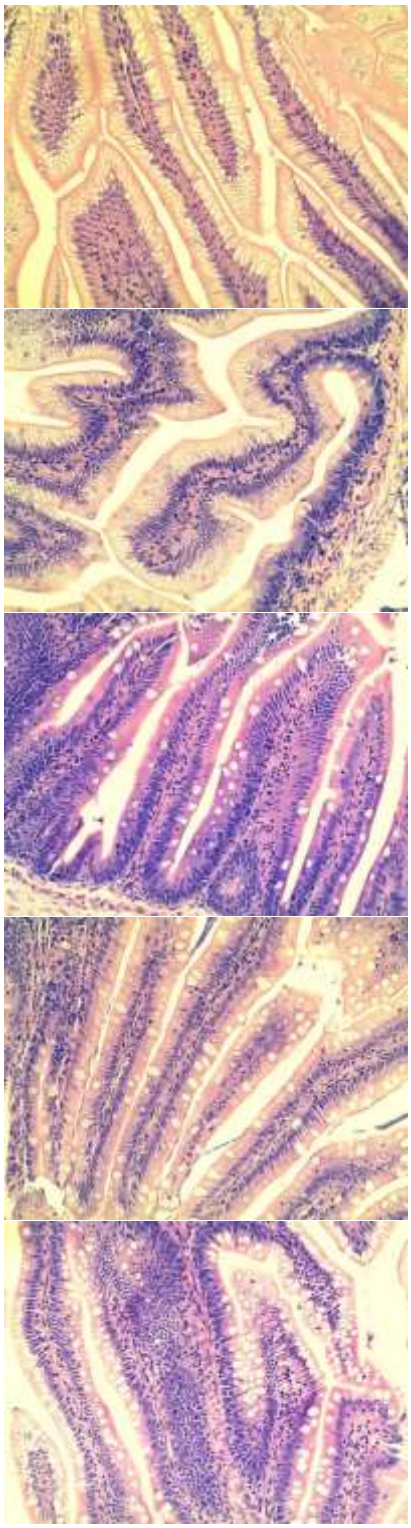


5

Total disruption

Bar is 0.2 mm

Appendix D. The Scores of Goblet Cells (GC) in Semi-quantitative Scoring System



Bar is 0.1 mm

1

Scattered cells

2

Increased number and sparsely distributed

3

Diffused number widely spread

4

Densely grouped cells

5

Highly abundant and tightly-packed cells

Appendix E. The Scores of Supranuclear Vacuoles (SNV) in Semi-quantitative Scoring System

1

Normal SNV

2

Some size reduction

3

Diffused size reduction

4

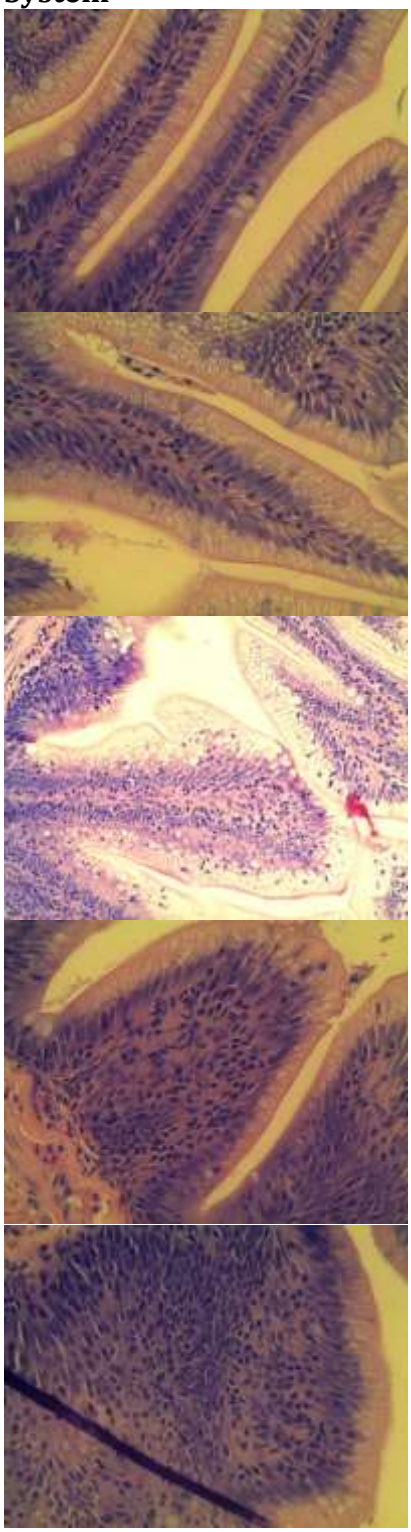
Onset of extinction

5

No SNV

Bar is 0.05 mm

Appendix F. The Scores of Lamina Propria (LP) in Semi-quantitative Scoring System



1

Normal LP

2

Increased size

3

Medium size

4

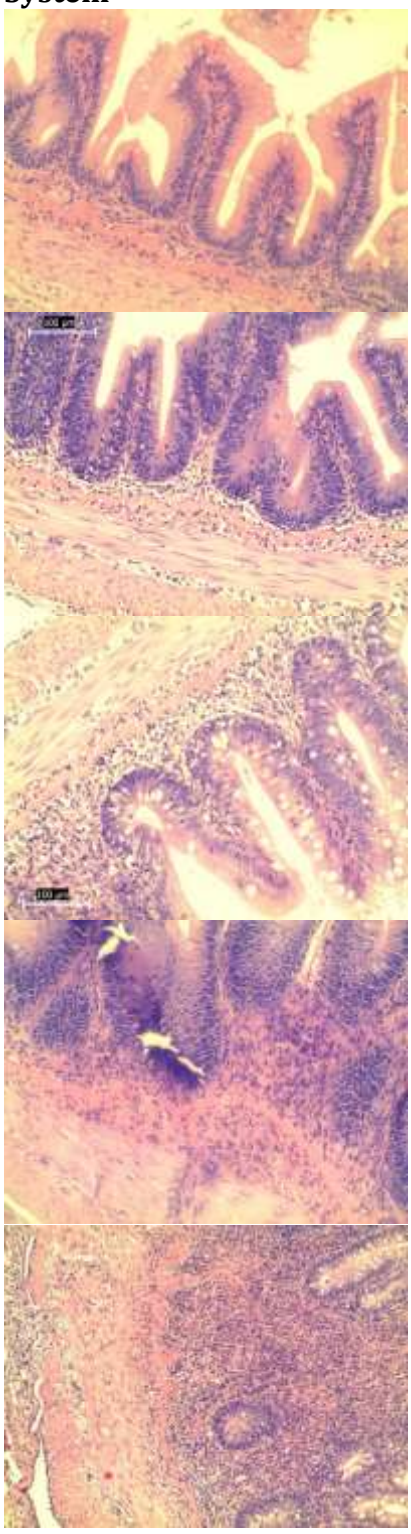
Large LP

5

Largest LP

Bar is 0.05 mm

Appendix G. The Scores of Sub-mucosal (SM) in Semi-quantitative Scoring System



1

Normal SM

2

Increased size of SM

3

Medium size of SM

4

Large SM

5

Largest SM

Bar is 0.1 mm

Appendix H. The Image of Post-mortem Evaluation

(a)Atlantic salmon smolts fed control diet. (b) fish fed 8%HOCM. (c) fish fed 16%HOCM. (d) fish fed 24%HOCM.