USE OF OIL, SOLVENT-EXTRACTED MEAL AND PROTEIN CONCENTRATE FROM CAMELINA (*CAMELINA SATIVA* L. CRANTZ) FOR SALMONIDS AT THE EARLY LIFE STAGE

By

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

at

Dalhousie University
Halifax, Nova Scotia
June 2018

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Abstract

Studies were conducted to evaluate the effects of using camelina products including camelina oil (CO), solvent-extracted camelina meal (SECM) and camelina protein concentrate (CPC) as feed ingredients for salmonid fish at early life stages. Replacing up to 100% fish oil with CO had no adverse effect on growth performance of first feeding rainbow trout (0.105 g), Atlantic salmon fry (1.1 g), and rainbow trout fry (1.0 g). Diet with the highest SECM inclusion rate tested, 18%, significantly increased feed consumption and subsequently weight gain of first feeding trout. Both salmon and trout fry performed well on 18%SECM diet ($p>0.05$). The 18%CPC treatment resulted in over 50% mortality rate of first feeding trout and significantly poorer growth after 56 days. The highest level of CPC tested, 12%, in trout fry diet did not lead to any difference in growth when compared to remaining treatments.

List of Abbreviations Used

ALA	α-linolenic acid
ADC	apparent digestibility coefficient
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AOM	Active oxygen method
CF	Condition factor
CFIA	Canadian Food Inspection Agency
CO	Camelina oil
CPC	Camelina protein concentrate
DHA	Docosahexaenoic acid
DP	Digestible protein
dph	Day post hatch
DE	Digestible energy
EPA	Eicosapentaenoic acid
N/A	Not available
NRC	National Research Council
NSD	No significant difference
FAO	Food and Agriculture Organization of United Nations
FCR	Feed conversion ratio
HSI	Hepato-somatic index
HORM	High oil residue camelina meal

PUFA	Polyunsaturated fatty acid
PER	Protein efficiency ratio
SAS	Statistical Analysis System
SD	Standard deviation
SECM	Solvent-extracted camelina meal
VSI	Viscera-somatic index

Acknowledgements

I would like to express my sincere gratitude and appreciation to my supervisor Dr. Derek Anderson for allowing me to become one of his graduate students and his guidance, support and encouragement throughout the years. I would like to thank my committee members, Dr. Jim Duston, Dr. Santosh Lall and Dr. Sean Tibbetts for their insightful comments and kind words. A special thank you to Dr. Stephanie Collins for being such an inspiration and giving me the extra push when needed.

Many thanks to Audrey-Jo McConkey, Janice MacIsaac, Jamie Fraser, Margie Hartling, Mike McConkey, Scott Jeffrey and Paul MacIsaac for their help in the Aquaculture center, nutrition lab and feedmill. Thank you to Cara Kirkpatrick and Marilyn Roberts for the organizational support. Thank you to all the students who helped in the trials.

Thank you to the funders, Atlantic Canada Opportunities Agency and Genome Atlantic for their generous financial support.

Finally, love and thanks to Youwei Chen and my parents for the continuous support and encouragement during the journey.

Chapter 1

Introduction

Salmon and trout production account for 62 and 5%, respectively, of the total Canadian aquaculture production (200, 565 tonnes) in 2016 (Department of Fisheries and Oceans, 2018). Salmonid diets have been well studied, and dietary feed composition has gone through tremendous changes in the past few decades. Traditionally, salmon farming was heavily reliant on ingredients of marine origin. In 1990, about 90% of Norwegian salmonid feeds were sourced from marine ingredients, with 66 and 24% being fish oil and fishmeal respectively (Ytrestøyl et al., 2015). Due to the pressure of constantly increasing prices and sustainability issues for production from wild-caught pelagic fish, the search for alternatives to the finite supply of marine ingredients has intensified. By 2000, feed manufacturers in Norway were able to reduce marine ingredients in the commercial salmonid diet to about 30%, with fish oil accounting for 11% and fishmeal accounting for 18%. About 19% plant oil and 37% plant protein were used in the diet to fill the gap (Ytrestøyl et al., 2015). Most terrestrial plants, compared to fish oil and fishmeal, are low in n-3 fatty acids and are completely devoid of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (National Research Council (NRC), 2011). Increased use of terrestrial plants possibly lowers the dietary level of total n-3 fatty acids, and leads to a lower n-3/n-6 fatty acid ratio in fish flesh. Farmed Atlantic salmon fillets analyzed in 1987 had an n-3/n-6 fatty acid ratio of 6:1 (van Vliet and Katan, 1990), which was double the ratio (2.9:1) in 2012 (Strobel et al., 2012). The n-3/n-6 fatty acid ratio in the fillet of wild salmon was consistent over the same time, with 11:1 and 12.4:1 reported in 1990 and 2000 respectively (van Vliet and Katan, 1990; Strobel et al., 2012). Significant

reductions of EPA and DHA contents in farmed salmonid fillets has been observed over time. Farmed Scottish Atlantic salmon contained 1.36 g of EPA+DHA per 100 g fillet in 2015, about 50% lower than the same product nine years earlier (2.75 g per 100 g fillet) (Sprague et al., 2016). To maintain the nutritional value of salmon products, and the health benefits of consuming them, the search for alternative ingredients is greatly needed.

The oilseed crop, *Camelina sativa* (L.) Crantz, potentially can become a valuable ingredient in salmonid diets. The plant itself has a low nutritional requirement and grows well on marginal lands (Canadian Food Inspection Agency (CFIA), 2014). It is resistant to drought, frost, diseases, and insects (Zubr, 1997). The seedlings of some varieties can survive low temperatures at -11 ºC (CFIA, 2014). Most importantly, camelina oil (CO) has a high content of n-3 alpha-linolenic acid (ALA) at 36.2-39.4% (Gugel and Falk, 2006). Rainbow trout and Atlantic salmon might still possess the ability to use dietary ALA as the precursor for EPA and DHA synthesis through desaturation and elongation (Collins et al., 2011; Lazzarotto et al., 2015; Hixson et al., 2014a and b). With a high level of ALA, CO has an n-3/n-6 ratio at 1.6:1. This ratio much lower than fish oil including anchovy and herring oil (range from 9.8:1 to 11.6:1), but it is higher than most commercially available plant oils from rapeseed (0.5:1), soybean (0.1:1) and sunflower (<0.1:1) (Sprague et al., 2016). Camelina products have been tested in salmonid diets. Atlantic salmon and rainbow trout at juvenile and grow-out stages both demonstrated up to 100% tolerance to CO, however limited acceptability to different camelina meals ≤ 20% (Hixson et al., 2014a, b, c; 2015 a and b; 2017; Hixson and Parish, 2014; Ye et al., 2016; Bullerwell et al., 2016).

Feed accounts for 40-48% of total production cost (Marine Harvest, 2015), with most feed consumed juveniles through grow-out to harvest size. Hence, fish at these stages are the main area of research for investigations on alternative feed ingredients. In recent years, a new approach has been developed to expose fish to plant ingredients at younger life stages when they have zero or little experience with non-fish oil and fishmeal-based diets. The purpose is to improve their tolerance of the same plant ingredients in later growth stages. A three-year trial demonstrated that feeding rainbow trout with a 100% plant-based diet (blend of vegetable oils and plant proteins in replacement of 10.5 and 43.4% of fish oil and fishmeal respectively) from first feeding throughout the whole life cycle did not negatively affect their growth performance (Lazzarotto et al., 2015). Rainbow trout receiving 100% plant-based diet for the first 21 days of exogenous feeding showed a better acceptance of the same diet at the grow-out stage, compared to fish that had never been exposed to the plant-based diet (Geurden et al., 2013). The current study is the first to investigate the effects of feeding CO, a residue meal from oil extraction called solvent extracted camelina meal (SECM), or a high protein product made from the residue meal called camelina protein concentrate (CPC) to first feeding rainbow trout, trout fry and Atlantic salmon fry.

The evaluation of early acceptance to enhance inclusion levels in older fish has not been investigated to date. The first step, and the principal objective of this thesis, was to determine the acceptability of plant ingredients including CO, SECM and CPC at early life stage of rainbow trout and Atlantic salmon. Results will be compared to earlier works

evaluating camelina products on salmonids at later life stages. Results from current study were used for the application to CFIA for approval of CO and its co-products as feed ingredients for salmonid fish in Canada. Acceptance by CFIA is a key requirement for inclusion of camelina products as recognized feed ingredients suitable for inclusion in diets for salmonid fish in Canada. Results from current study was part of the application to CFIA for approval of CO as a feed ingredients in Atlantic salmon and rainbow trout diet. In 2017, CO was approved as a feed ingredient in Atlantic salmon feed.

Chapter 2

Literature Review

2.1 Camelina and Potential Products

Camelina (commonly referred to as false flax or gold-of-pleasure) is an oilseed crop from the *Brassica* family that includes mustards, rapes, canola, crambe, radish, turnip, broccoli, cabbage, kale, cauliflower, and many weeds (Robinson, 1987). Camelina is an ancient crop that was probably first domesticated as an oil seed crop in Europe and Asia in the late Neolithic period, but its production considerably declined during the Middle Ages associated with the development of other crops (Knörzer, 1978). The introduction of camelina seed to North America was likely due to contamination in flax seeds (Knörzer, 1978). In the late 1990s, the commercial production of camelina seed increased in North America as a sustainable and economical feedstock for biodiesel production and high-quality lipid and protein for nutrition (CFIA, 2014).

The oil and crude protein content of camelina seed from western Canada varied from 38 to 43% and 27 to 32% (dry matter basis), respectively (Gugel and Falk, 2006). These levels are comparable to canola, the primary oilseed in Canada, with about 48% of oil content and 23% of crude protein (dry matter basis; Canadian Grain Commission, 2017). The oil extraction of camelina seed separates CO from the meal (Figure 2.1), and the residue cake with relatively high oil content (about 10%) is described as the high-oil residue meal (HORM) after grinding. The oil left in HORM can be further reduced to as low as 6% by solvent extraction and this product is described as solvent-extracted meal. Both HORM and SECM can be used to produce protein concentrate (Bilgi and Celik,

2004). The process elevates the protein content, decreases the carbohydrate content and greatly increases its potential economic value. The CPC processed from HORM in Saskatoon by POS Bio-Science has about 52.5% crude protein (as fed basis), while the CPC processed from SECM at Dalhousie University, Faculty of Agriculture by the author has 69% crude protein (as fed basis).

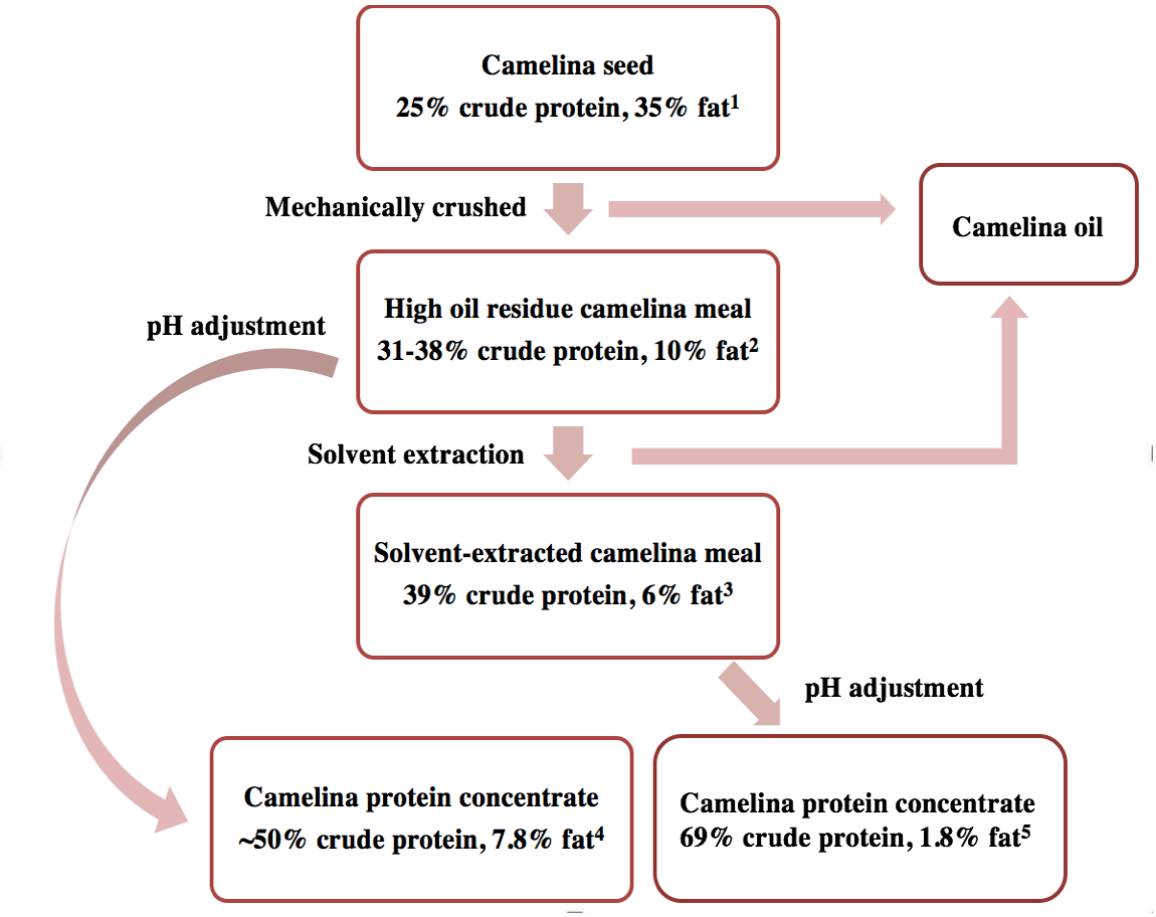

Figure 2.1 Camelina seed and its products (as fed basis).
[1]Bullerwell et al., 2016; [2]Ye, 2014; Fraser, 2016; [3]Hixson et al., 2015a; [4]Camelina protein concentrate contains 52.5% crude protein was prepared by POS Bio-Science, Saskatoon, Canada, [5]Camelina protein concentrate contains 69% crude protein was prepared by the author in Dalhousie University, Faculty of Agriculture, Truro, Nova Scotia, Canada.

2.2 Camelina Oil as a Lipid Source

2.2.1 Nutritional Profile of Camelina Oil

CO is highly unsaturated (>90%) (Zubr, 1997). CO is unique for its high ALA content (36.2-39.4% of total fatty acids; Gugel and Falk, 2006). The remaining major fatty acids are linoleic (16.3-17.2%), eicosenoic (14.0-15.5%), and oleic acid (12.8-14.7%) (Gugel and Falk, 2006). The level of ALA in CO is lower than flaxseed oil (53.3%; NRC, 2011) but far exceeds most other terrestrial plant oils including canola oil (12.0%; NRC, 2011). ALA is an important dietary n-3 fatty acid for mammals and many fish species including salmonids. The health benefit of ALA includes lowering the risk of fatal ischemic heart disease (Hu et al., 1999), vascular inflammation, and cardiovascular disease (Zhao et al., 2004). In mammals and birds, it can be used as the precursor for desaturation and elongation to produce the indispensable long chain poly-unsaturated fatty acids, EPA and DHA (Figure 2.2; Kaur et al., 2014; Sinclair et al., 2002 ; Burdge and Calder, 2005). But

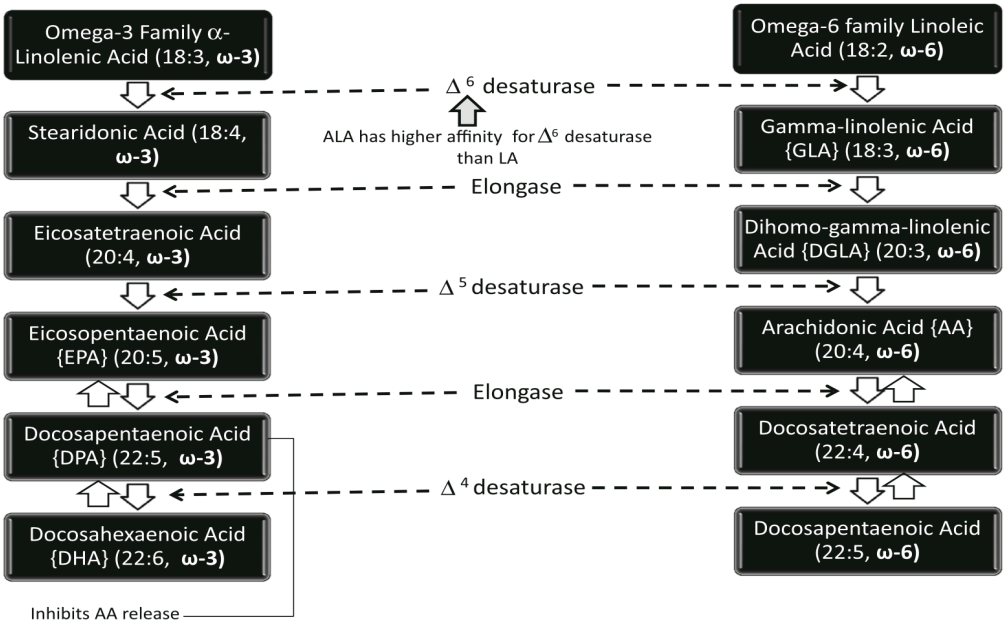

Figure 2.2 Metabolic steps involved in converting alpha-linolenic acid to eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3). Source: Kaur et al., 2014.

this ability is limited in fish. Rainbow trout were able to synthesize 27% of DHA from dietary ALA from CO, although this was not sufficient to compensate for the significantly loss of EPA and DHA in trout muscle when fed a diet with 100% added fish oil replaced with CO (Hixson et al., 2014a).

Replacing fish oil with terrestrial plant oils and animal fats in salmonid diets has been extensively studied. Full replacement of fish oil with plant oils such as soybean oil, palm oil or canola oil did not negatively affect the growth performance of salmon or trout (Turchini et al., 2009). However, the use of vegetable oils lacking either n-3 fatty acids or long-chain polyunsaturated fatty acids often resulted in the fish fillets having lower levels of n-3 fatty acids, imbalances in n-3/n-6 fatty acid ratio and reduction in EPA and DHA in fish fillets, and subsequently lower nutritional value for human consumption (Bell et al., 2002; Figueiredo Silva et al., 2005; Tocher, 2015). CO, which contains high levels of n-3 fatty acids, might be a better alternative to fish oil than more familiar plant oils. On the negative side, like most oils that contain significant levels of highly polyunsaturated fatty acids, CO is susceptible to lipid oxidation in daylight and may have a short shelf life if not adequately stabilized with natural or synthetic antioxidants (Ni Eidhin et al., 2003).

2.2.2 Camelina Oil as a Lipid Source in Animal Feeds

There are existing markets for CO in biofuel production and health food supplements (Shonnard et al., 2010; Bell et al., 2002; Figueiredo Silva et al., 2005; Tocher, 2015). Since 2010, cold-pressed unrefined CO has been approved as a human food in Canada by Health Canada (Health Canada, 2012). Numerous studies have aimed to CO as a high-

quality feed ingredient with benefits to enhance animal health or to produce high quality animal products from swine, poultry and finfish species (Habeanu et al., 2011; Pietras and Orczewska-Dudek, 2013; Hixson et al., 2014c; Morais et al., 2012).

CO has been successfully used to replace conventional sunflower oil in the diet of finishing pigs. Decreased cholesterol and triglyceride contents in the plasma, and significantly increased the level of n-3 fatty acids in pork were observed with the use of CO (Habeanu et al., 2011). CO can be incorporated into broiler diets, replacing traditional plant oils, without affecting growth performance and sensory qualities of the cooked meat (Pietras and Orczewska-Dudek, 2013; Jaskiewicz et al., 2014; Puzio et al., 2012). The broilers deposited more n-3 fatty acids, mainly ALA, and had an improved n-3/n-6 fatty acid ratio in their muscle compared to those fed soybean oil or rapeseed oil (Pietras and Orczewska-Dudek. 2013; Jaskiewicz et al., 2014). Moreover, their bone mineral content and bone density of broilers was improved by feeding CO (Puzio et al., 2012), which was possibly associated with stimulated bone metabolism by increased intake of n-3 fatty acid and a higher ratio of n-3/n-6 fatty acid (Weiss et al., 2005). Feeding a diet containing 5% CO to laying hens for three weeks enriched the nutritional composition of eggs; the total n-3 fatty acids and the DHA in the egg increased by 270% and 45% respectively, and the n-3/n-6 fatty acid ratio increased from 0.13 to 0.45 (Rokka et al., 2002).

In recent years, research on CO has been extended to finfish including Atlantic cod, Atlantic salmon and rainbow trout (summarized in Table 2.1). Small cod with an initial

Table 2.1 Feeding trials conducted to evaluate camelina products as a lipid source in diets of Atlantic cod, Atlantic salmon, and rainbow trout.

Experimental set up						Results				References
Species	Initial body weight (g)	Trial duration (week)	Water characteristics	Treatments	CO in the diet (% as fed basis)	Weight gain (g)	Feed consumption (g)	FCR	VSI	
Atlantic cod	1.4	12	9°C seawater	0, 33, 66 and 100% replacement of FO with CO	0, 2.6, 5.3 and 8.0	NSD	NSD	NSD	NSD	Morais et al., 2012
	14.4	13	10°C seawater	100% FO, 100% replacement of FO with CO (100CO), 100% replacement of FO +solvent extracted FM (100COSEFM), 100% replacement of FO +15% SECM inclusion (100CO15SECM)	0, 5.4, 9.7 and 6.0	FO>100CO= 100COSECM> 100CO15SECM	FO>100CO= 100COSECM> 100CO15SECM	FO=100CO= 100COSECM> 100CO15SECM	N/A	Hixson and Parish, 2014
	19	9	10°C seawater	0, 40 and 80% replacement of added FO with CO	0, 1.85 and 3.7	NSD	N/A	N/A	N/A	Hixson et al., 2013; Booman et al., 2014
	8.4	16	12°C freshwater	0, 50 and 100% replacement of FO with CO	0, 8, and 16	NSD	NSD	NSD	NSD	Ye et al., 2016
Atlantic salmon	242	16	14°C seawater	A control diet with FO (FO); 100% FO replacement with CO (100CO); 100% FO replacement with CO+ solvent extracted FM (100COSEFM);	0, 14 and 17.8	FO=100CO< 100COSEFM	FO<100CO< 100COSEFM	NSD	NSD	Hixson et al., 2014b; Xue et al., 2015
Rainbow trout	1	16	14°C freshwater	A control diet with FO; 100% FO replacement with CO+15% SECM inclusion	0 and 100	NSD	NSD	NSD	N/A	Bullerwell et al., 2016
	44.9	12	14°C freshwater	0, 50 and 100% replacement of FO with CO	0, 8.75 and 17.5	NSD	NSD	NSD	NSD	Hixson et al., 2014b

SECM= solvent extracted camelina meal; HORM= high oil residue meal; CS= camelina seed; CP= crude protein; CL= crude lipid; FM= fishmeal; FO= fish oil; VSI= visceral-somatic index; NSD = no significant difference; N/A= data not available.

weight of 1.4 g performed well on a diet with 8% added fish oil fully replaced with CO (Morais et al., 2012). Among larger cod (14 g), by contrast, weight gain, specific growth rate, feed intake, and FCR were significantly depressed by full replacement of the 5.4% of added fish oil with CO in the diet of cod with an initial weight of 14 g (Hixson and Parish, 2014). Smaller cod in the former trial of Morais et al. (2012) exhibited a higher tolerance to CO compared to larger cod in the study of Hixson and Parish (2014). The possible explanation could be the difference in fish size, or higher fishmeal and crude protein levels in the former diet compared to the latter diet (60 versus 50%, and 55 versus 45% respectively) masked the deleterious effects of the full oil replacement. In another study, cod with an initial weight of 19 g fed both fish oil based diet and a diet with 80% fish oil replaced with CO (the highest level tested) has similar growth performance ($p>0.05$; Hixson et al., 2013). In all three trials, n-3/n-6 fatty acid ratio in cod liver and muscle tissue decreased significantly when fish oil was replaced with CO. Replacing fish oil with CO elevated the dietary 18:2n-6 fatty acid content and subsequently high levels of that and total n-6 fatty acid were accumulated in both cod liver and muscle. Cod liver and muscle tissue had a higher level of ALA as CO was high in ALA, but the level was a little lower or just enough to compensate the loss in the other n-3 fatty acids, and therefore resulted in a slightly lower or similar level of total n-3 fatty acid. As a result of increased n-6 fatty acids and decreased n-3 fatty acids, the n-3/n-6 fatty acid ratio decreased in both liver and muscle tissue. In a parallel study to Hixson et al. (2013), cod fed a diet with up to 80% fish oil replaced with CO had similar immune response compared to those fed the fish oil diet (Booman et al., 2014). However, genes with the potential to alter cellular proliferation and death, and change structural properties of

intestinal muscle were negatively affected when 66% of fish oil was replaced with CO in the diet (Morais et al., 2012). Therefore, it is suggested that examination of intestinal morphology is needed in future oil replacement studies as intestine is not only the site of nutrient uptake but also a significant site for lipid metabolism (Morais et al., 2012).

The effect of dietary CO on Atlantic salmon was investigated at different life stages including parr and post-smolt with an initial body weight of 8.4, 242 and 256 g, respectively (Ye et al., 2016; Hixson et al., 2014b; Hixson et al., 2017). Parr (8.4 g) tolerated up to 100% fish oil replacement with CO with no deleterious effects on either growth performance or proximate carcass composition after 112 days (Ye et al., 2016). In the second study, full replacement of added fish oil with CO in diet of post-smolts with initial weight of 242 g did not affect their final weight gain. However, final weight and feed consumption were significantly reduced (Hixson et al., 2014b). Among post-smolts of 256 g fed diets with 0, 88 or 94% fish oil replaced with CO had similar weight gain or final weight (Hixson et al., 2017). Surprisingly, a diet with lower replacement level at 74% fish oil replaced with CO resulted in a significantly lower final weight compared to fish fed with 0, 88 or 94% replacement diets. The reason was not clear as the feed consumption, FCR and SGR were similar among all treatments. Hixson et al. (2017) also concluded that even the minimal level of replacement marine-based fatty acids with terrestrial fatty acids was enough to cause negative impacts on growth performance, according to regression analysis. Besides growth performance, using CO in salmon diet had no negative effect on hindgut morphology of Atlantic salmon parr (Ye et al., 2016). However, muscle tissue fatty acid composition was altered when dietary fish oil was

substituted with CO because muscle fatty acid composition usually resembles the fatty acid composition of the diet. Significant reduction in the n-3/n-6 fatty acid ratio from 3.9 to 1.5 when 100% CO was fed to the fish. Both EPA and DHA dropped about 60%, respectively, in salmon muscle tissue when 100% of fish oil was replaced with CO in salmon diet (Hixson et al., 2014b). Hixson et al. (2017) indicated that the n-3/n-6 fatty acid ratio (about 1.6) and DHA content in salmon muscle tissue was similar among fish fed diet with 0, 74, 88 and 94% of fish oil replaced with CO. However, EPA in salmon muscle decreased about 38% when the control diet was compared with any of the CO-containing diets ($p<0.05$). In the former study, fish oil (14%) and fishmeal (34.9%) accounted for 49% of the diet, while the control diet in the latter study had much lower levels of marine-sourced ingredients at 34% (24% of fish oil and 10% of fishmeal). Together, the results of the two studies suggest that in a traditional fish meal and oil based diet, CO is not adequate to compensate the losses of the key n-3 fatty acids favored by consumers due to decreased dietary level of fish oil. While in current salmon diets with relatively lower levels of fish oil and fishmeal, replacing fish oil with n-3 fatty acid rich-CO had less impact on the muscle fatty acid composition, especially when compared to canola oil and poultry fat (Hixson et al., 2017).

Use of CO in rainbow trout with initial body weight of 1.0 g and 44.9 g were investigated by Bullerwell et al. (2016) and Hixson et al. (2014a), respectively. Rainbow trout fed fish oil and fishmeal based control diet had about 25 and 20% higher weight gain and feed consumption, respectively, compared to fish fed a diet with 10% SECM inclusion and fish oil 100% replaced with CO after 112 days (76.3 versus 60.9 g; 70.0 versus 58.1 g; Bullerwell et al., 2016). However, the differences in both weight gain and feed intake

were not statistically significant. This might be due to an experimental design where data for all eight treatments (graded levels of camelina seed cake or high-oil residue meal inclusion, and a double substitution treatment with 10% SECM inclusion and 100% fish oil replaced with CO) were compared using the General Linear Model procedure of IBM SPSS 20.0 (SPSS Inc., Chicago, IL, USA). A paired two-sample t-test directly evaluating the weight gain or feed intake between trout fed control diet and the diet with 10% SECM inclusion and fish oil 100% replaced with CO might be more sensitive to detect the differences in this case. Feeding CO to trout did not change the FCR, PER or proximate carcass composition ($p>0.05$; Bullerwell et al., 2016). Morphology of midgut and hindgut measured by length, width and area of villus, crypt depth and intestinal wall thickness were similar among all treatments ($p>0.05$). Among larger rainbow trout (44 g), up to 100% fish oil replaced with CO had no adverse effects on mean body size in the 84-day trial (Hixson et al., 2014a). Fish deposited more 18:3n-3 and 18:2n-6 fatty acids in muscle and viscera when they received more of those fatty acids due to the increased level of CO in the diet. However, the increase in the 18:3n-3 fatty acid was not enough to make up or prevent the loss of other n-3 fatty acids including EPA and DHA. This subsequently contributed to a significantly lower level of total n-3 fatty acid in trout muscle when fed CO-containing diets. As a consequence of decreased dietary n-3 fatty acid and increased n-6 fatty acid in the CO feeds, the n-3/n-6 fatty acid ratio in trout muscle was significantly decreased. Fish fed both 50 and 100%CO diets had DHA contents doubled the amount offered in their diets, suggesting possible desaturation and elongation of ALA for DHA production.

2.3 Camelina Products as a Protein Source

2.3.1 Potential Camelina Protein Products

The utilization of by-products after oil extraction adds economic value to oilseed processing as a whole and is critical for their commercialization. The commercialization of other oilseed crops including soybean and canola provides a framework for camelina by-product identification. One of the applications for these protein-containing products has been their use as alternatives for high protein feedstuffs, such as fishmeal, or the commonly used plant protein such as canola meal, soybean meal and others in the aquaculture industry. Cost, availability, protein quality and anti-nutrients are the primary constraints.

The plant protein alternative to fishmeal can be classified into two product categories, low protein-containing products such as plant meals and high protein-containing products such as protein concentrates and isolates. Low protein-containing products, like soybean meal and canola meal are less expensive and widely available. Hence, they have been commonly used in salmon diets. Camelina meal, with a comparable crude protein content and amino acid profile, also has the potential to be used in aquaculture to replace these practical protein feeds (Table 2.2). However, these types of low protein-containing meals are usually incorporated in salmonid diets at low levels, typically less than 20% in salmonid grow-out diet. High level of inclusion is problematic for formulating diets to meet the high protein requirement of salmonids as carnivorous fish require 40-48% of dietary crude protein (NRC, 2011). Fishmeal contains 60-75% crude protein that is highly digestible (~95% apparent digestibility coefficient; Table 2.2). The crude protein in

fishmeal has a well-balanced amino acid profile in terms of requirements of carnivorous fish. It was the main ingredient of salmonid diets for decades. To effectively substitute fishmeal, the ideal alternative feedstuff should have a similar nutritional profile. Corn gluten meal with a minimum of 60% crude protein, corn protein concentrate with 75% crude protein, soy protein concentrate with approximately 70% crude protein, and wheat gluten meal with 85% crude protein have been commonly used as feed ingredients for carnivorous fish. However, limited amounts can be included in the diet. Corn products are high in xanthophyll carotenoids, which can impart an undesirable yellowish color to the fillets (Skonberg et al., 1998). Although about 90% of xanthophyll carotenoids (lutein, zeaxanthin, β-cryptoxanthin and β-carotene) were removed, incorporation of 19% of either treated or untreated corn gluten meal (19% in the diet) significantly decreased astaxanthin deposition in the muscle of rainbow trout compared to that of trout fed diet without corn gluten meal, or diet with untreated corn gluten meal (Saez et al., 2015). Generation of highly reactive peroxyl radicals during the carotenoid bleaching process led to rancid feed and eventually the oxidative destruction of astaxanthin. It was suggested that the carotenoid bleaching process should be further optimized, or additional antioxidant should be added to the diet (Saez et al., 2015). White corn has the potential to be used to produce less pigmented corn gluten meal. However, white corn is more expensive to grow, mainly due to the isolation requirements from being contaminated by yellow corn. Anti-nutrients such as fiber, lectins, saponins, trypsin inhibitors and soy antigens in soy protein concentrate have been reduced compared to traditional soybean meal after aqueous ethanol extraction and thermal treatment. However, the high level dietary inclusion of soy protein concentrate (50-60%) can still compromise growth

performance of Atlantic salmon and rainbow trout (Storebakken et al., 2000; Escaffre et al., 2007). Atlantic salmon fed soy protein concentrate suffered no enteritis problems fed soy protein concentrate (Escaffre et al., 2007). However, the enterocyte structure and hepatocyte volume in these fish was modified and the causative agent was not clear. Canola protein concentrate has lower crude fiber content at 2% compares to solvent-extracted canola meal at 11% (NRC, 2011). It is highly digestible by salmonids and low in phytate and glucosinolates. Rainbowt trout tolerated up to 30% canola protein concentrate in their diet without adverse growth performance after 56 days (Collins et al., 2012). However, in a 38-week trial, Atlantic salmon showed a lower tolerance to a commercial canola protein concentrate between 10-20%. Salmon fed a diet containing 20% canola protein concentrate exhibited similar feed efficiency to controls, but both feed intake and weight gain were reduced (Burr et al., 2013). The difference in tolerance to canola protein concentrate in two different trials might be due to difference in quality of canola protein concentrate used (neither were provided in the paper), fish species, size of fish or duration of the trial. CPC contains 50-70% crude protein and has an amino acid profile balanced in a similar way to fishmeal. It has great potential as an alternative protein source to fish meal in salmonid diets singly or in combination with other plant proteins described in Table 2.2.

Table 2.2 Amino acid composition and digestibility of camelina products compared to common plant ingredients and crude protein and amino acid requirements of Atlantic salmon and rainbow trout <20g (dry matter basis unless otherwise specified).

	Solvent-extracted camelina meal[1]	Canola meal (double low)[2]	Solvent extracted soybean meal without hulls[2]	Camelina protein concentrate[1]	Corn gluten meal[2]	Canola protein concentrate[2]	Soy protein concentrate[2]	Fish meal (herring)[2]	Requirement of Atlantic salmon[2]	Requirement of rainbow trout[2]
Crude protein (%, as fed basis)	35.6	38.0	48.5	52.5-68.3	63.7	69.2	63.6	72.0	48.0	48.0
Amino acids (%)										
Arginine	3.6	2.5	4.0	7.0	2.1	4.7	5.0	4.0	1.8	1.9
Histidine	1.2	0.6	1.4	2.2	1.3	1.9	1.7	1.6	0.8	0.8
Isoleucine	1.3	1.2	2.9	2.7	2.5	3.1	3.2	3.9	1.3	1.3
Leucine	2.5	3.1	4.2	5.3	10.3	5.4	5.3	5.1	2.3	2.3
Lysine	1.8	0.8	2.4	3.2	1.2	3.4	4.2	7.9	2.6	2.5
Methionine	0.7	0.5	0.8	1.4	2.1	1.4	0.9	2.4	N/A[4]	N/A[4]
Cystine	0.9	0.5	0.8	1.0	1.2	1.4	1.0	1.7	N/A[4]	N/A[4]
Methionine+Cystine	1.5	1.1	1.6	2.3	3.3	2.9	1.8	4.1	1.3	1.3
Phenylalanine	1.6	1.5	3.0	3.4	4.2	3.1	3.5	2.9	N/A[4]	N/A[4]
Tyrosine	1.1	1.1	1.4	2.1	1.0	1.1	2.5	2.3	N/A[4]	N/A[4]
Phenylalanine+Tyrosine	2.7	2.6	4.4	5.5	5.2	4.2	6.0	5.2	2.7	2.5
Threonine	1.7	1.1	2.2	3.2	2.2	2.8	2.7	2.7	1.6	1.8
Tryptophan	0.4	0.1	0.8	0.9	0.3	0.2	0.9	0.8	0.4	0.4
Valine	1.8	1.6	3.0	3.8	3.0	3.7	3.3	3.6	1.8	1.9
ADC[5] of crude protein for Atlantic salmon (%)	88	79	77-94[2]	70	92	N/A[4]	90	91-95[2]		
ADC[5] of crude protein for rainbow trout (%)	85-87	N/A[4]	90-99[2]	78	92-97[2]	90.3	98-100[2]	95		

[1] Fraser, 2016
[2] NRC, 2011
[3] Thiessen et al., 2004
[4] N/A= data not available
[5] ADC= apparent digestibility coefficient

2.3.2 Antinutritional Factors in Camelina

Like other plant, camelina contains antinutritional factors when considered as animals feed ingredients. Mucilage, glucosinolates, phytic acid, and condensed tannins are the main concerns in camelina protein products (Russo and Reggiani et al., 2012; Fraser et al., 2017). The deleterious effects of antinutritional substances are generally related to their concentration in the diet. They can negatively affect growth, feed consumption, nutrient digestibility and utilization, the function of internal organs or disease resistance (Krogdahl et al., 2010). Fortunately, most can be eliminated either by heat treatment, water soaking, enzyme treatment or fermentation (Fraser et al., 2017).

2.3.2.1 Mucilage

Mucilage is one of the main antinutritional factors in camelina. Most mucilages are tasteless water-soluble, non-starch polysaccharides that exist mostly in hulls of the seed (Kaewmanee et al., 2014). In flaxseed, mucilage is composed of D-galacturonic acid, L-rhamnose, L-galactose, L-fucose, and D-xylose (Anderson and Lowe, 1947; Bhatty and Cherdkiatgumchai, 1990). It has a high water-absorption capacity and forms a gel coating on the seed called a “halo”. The gel consistency of mucilage is favored in feed manufacturing for its binding effect. However, salmonids, like other monogastric animals, cannot digest mucilage. Upon ingestion, mucilage absorbs water in the intestine and increases the viscosity of digesta (Rebolé et al., 2002; Choct et al., 1996). Subsequently, it reduces enzyme accessibility to the nutrients, causing a lower rate of nutrient break down, diffusion, and absorption (Marambe et al. 2013). Lower digestion and absorption rate resulted in the increased amount of undigested nutrients being flushed to the lower

gut. Additionally, it can stimulate the activities of gut microflora in the lower gut and subsequently affect the well-being of the host (Choct et al., 1996). To eliminate mucilage, water extraction and enzymatic treatment were effective in flaxseed (Ziolkovska, 2012; Wanasundara and Shahidi, 1997). Mucilage in camelina has not been well investigated. The yield of mucilage from camelina seed was 6.7% (dry matter basis; Zurb, 2010). Sarv et al. (2017) reported camelina seed mucilage was about 10% of the seed mass, which was similar to that in flaxseed at 10% of seed mass (Ziolkovska, 2012). It should be noted that the yield of mucilage extraction is affected by extraction conditions such as seed to water ratio, water temperature and duration of agitation while mixing with water (Ziolkovska, 2012).

Fraser (2016) reported that treating SECM with Superzyme-OM (units g^{-1}: 2,800 cellulase, 400 mannanase, 50 galactanase, 1,000 xylanase, 600 glucanase, 2,500 amylase, 200 protease; Canadian Bio-Systems Inc., Calgary, Alberta, Canada) lowered the viscosity (wood stick test measuring the amount of product attached to a wooden stick) of SECM when soaked in water and incubated at 35-40 °C for 24 hours. Since mucilage can cause high viscosity, it was concluded that Superzyme-OM was effective for mucilage removal in camelina meal. However, the exact mucilage content in the SECM was not directly measured (Fraser, 2016).

2.3.2.2 Glucosinolates

Glucosinolates are sulfur and nitrogen containing secondary metabolites in plants from the Brassica family (Tripathi and Mishra, 2007). More than 110 different glucosinolates

have been characterized and they all share a β-D-thioglucose group (Chen and Andreasson, 2001). Glucosinolates such as sinigrin, progoitrin, glucobrassicin and neoglucobrassicin are responsible for the bitterness taste in Brassicaceae crops such as cabbage, Brussel sprouts, cauliflower, turnip, and kale. (Wieczorek et al., 2017). In intact seeds, glucosinolates are stored separately from glycosylated thioglucosidases called myrosinase (Bones and Rossiter, 1996). Upon processing or ingestion, physical damage to the plant/seed tissues releases myrosinase for glucosinolate hydrolysis, leading to a range of degradation products. These products include isothiocyanates, thiocyanates, nitriles, epithionitriles, and oxazolidine-2-thione, depending on pH and other factors (Figure 2.3). Glucosinolates are biologically inactive molecules, however, their bioactive breakdown products can be detrimental to animals at specific concentrations. The adverse effects including growth depression, reduced feed intake, iodine deficiency, thyroid disorders, and liver hypertrophy have been observed in a wide range of animals including ruminant animals and monogastric animals including swine, poultry, and fish (reviewed by Tripathi and Mishra, 2007). Isothiocyanates were a greater contributor to bitterness taste than glucosinolates themselves (Wieczorek et al., 2017). Both isothiocyanates and thiocyanates contribute to thyroid disorders, but through different mechanisms. The former prevents iodination of tyrosine (Langer and Greer, 1968; Nugon-Baudon and Rabot, 1994) whereas the latter competes with iodide during active transportation (Tonacchera et al, 2004). Both lead to reductions in the biosynthesis of thyroxine and triiodothyronine. Inclusion at 10% or higher levels of a nitrile-rich rapeseed meal (5.67 mg g^{-1}) in the diet of both rats and chickens contributed to hypertrophy of liver and kidney tissues (Srivastava et al. 1975), and high doses were lethal. A nitrile concentration

of 2.27 mg g^{-1} in the diet resulted in 100% mortality rate of rats within seven days. Chicken fed the same diet had 62% mortality on day 15 (Srivastava et al. 1975). Effective methods of glucosinolate removal in plant meals include Cu treatment, water soaking and extraction, acid treatment, heat treatment and micronization (Tripathi and Mishra, 2007).

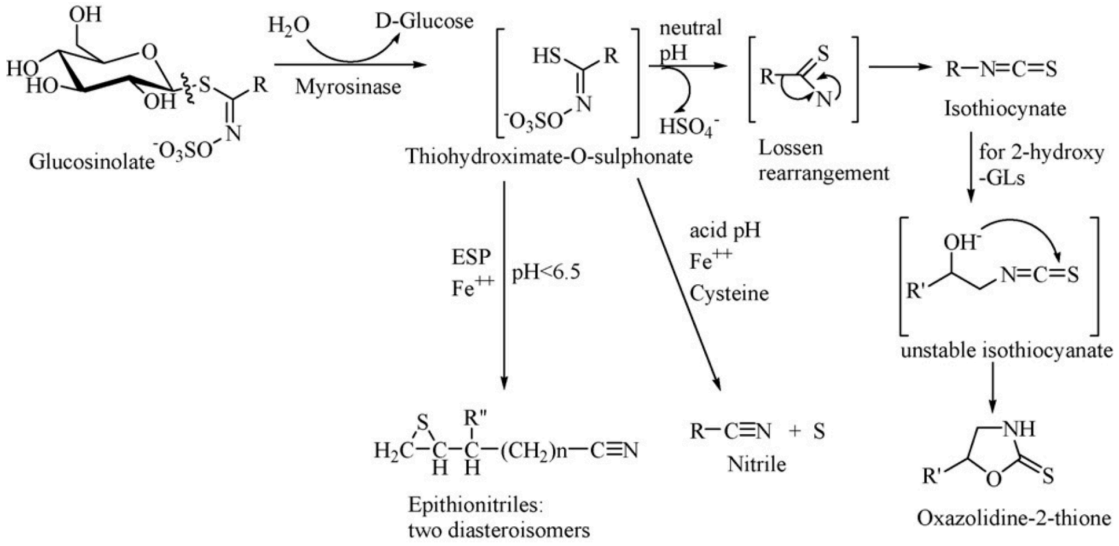

Figure 2.3 Outline of glucosinolate enzymatic hydrolysis. Source: Tripathi and Mishra, 2007.

The typical glucosinolates present in camelina seed are 9-methyl-sulfinyl-nonyl (glucoarabin), 10-methyl-sulfinyl-decyl (glucocamelinin) and a trace of 11-methyl-sulfinyl-undecyl glucosinolates (Schuster and Friedt, 1998). Total glucosinolate content in camelina meal ranges from 15.2 to 24.6 μmol g^{-1} on dry matter basis, in twelve camelina genotypes (Russo and Reggiani et al., 2012). The wide range of glucosinolate content might be associated with the different availability of sulfur nutrient (Schuster and Friedt, 1998). Glucosinolate content in camelina seed is relatively low when compared to other seeds from the Brassica family such as leaf mustard seed at 87.7 μmol g^{-1} (dry matter basis) or broccoli seed at 110.8 μmol g^{-1} (dry matter basis; Bhandari et al., 2015), but high when compared to canola seeds harvested in western Canada 2017-2018 (8.9-

11.4 μmol g^{-1} on dry matter basis; Canadian Grain Commission, 2017). Generally, higher levels of glucosinolates are expected in the meal due to the removal of oil. Glucosinolate content in HORM and SECM was 40.0 and 41.2 μmol g^{-1}, respectively on dry matter basis (Fraser et al., 2017). Water soaking of the meal entirely removed glucosinolates from the meal (Fraser et al., 2017).

The effects of camelina glucosinolates in fish have not been documented in the primary literature. Atlantic salmon can tolerate diets containing 5% SECM with 1.9 μmol g^{-1} (dry matter basis) (Ye et al., 2016). However, glucosinolates were not the only variable in the trial. The antinutritional effects from other substances could not be differentiated and thyroid morphology and function were not examined in Ye et al. (2016). A diet with 30% rapeseed meal that contained 1.4 μmol g^{-1} fed to 20g rainbow trout diet significantly depressed growth over a 64-day trial (Burel et al., 2000). Poor growth was attributed to reductions in feed intake, nutrients utilization and retention and impaired thyroid function. Hyperthyroid condition was detected by significantly decreased plasma concentration of thyroid hormones including triiodothyronine and thyroxine (Burel et al., 2000). However, the severely depressed growth reported by Burel et al. (2000) was likely more than a result of the glucosinolates level but also other anti-nutrients due to high dietary inclusion levels of rapeseed meal (30 and 50%) and the authors failed to take into consideration. Additionally, myrosinase is likely to be activated during the process of crushing oilseed into meals and leads to degradation of glucosinolates. Thus, even if the analyzed glucosinolates content are low in meals or diets, it is necessary to check the levels of their degradation products which can also be toxic to animals.

2.3.2.3 Phytic Acid

Phytic acid is the principal phosphorus storage pool in plant seeds. It is unstable as free acid and mostly exists as the salt phytate (Cheryan and Rackis, 1980). Phosphorus in this form is poorly available to animals due to lack of digestive enzyme for its degradation, and also reduces the utilization of certain nutrients and depresses the somatic growth in animals. Upon ingestion, phytate becomes negatively charged in the stomach due to the low pH. When it enters the small intestine, where the pH rises to 6-7, the phosphate groups act as a chelating agent for cations such as Mg^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+}, or Fe^{3+}. The formation of these insoluble mineral and phytic acid complexes reduces the availability of those minerals (Kratzer and Vohra, 1986). Phytate can also bind with proteins over a wide range of pH both in stomach and small intestine resulting in lower digestibility of the proteins (Dersjant-Li et al., 2015). Additionally, phytate can interact with digestive enzyme such as trypsin to lower nutrient digestibility (Dersjant-Li et al., 2015).

There are several ways to reduce phytate. Phytate locates in the aleurone layer (a layer under seed coat) in most oils seeds and cereals, and mechanical separation of this layer can reduce the phytate content. Other methods such as storing under warm and humid condition, water soaking, germination, fermentation and direct enzyme treatment (phytase) change on enzymatically dephosphorylated phytate into its degraded products with fewer phosphate groups and weaker affinity for binding minerals and proteins (Humer et al., 2015).

The phytate content of camelina seed cake from 30 samples collected from five European countries during 1995-1998 ranged from 21.9 to 30.1 g kg^{-1} phytate (Matthäus and Zubr, 2000). Phytate content in full-fat camelina seed, high-oil residue camelina meal and SECM were 5.4, 6.8, and 7.0 g kg^{-1} (dry matter basis), respectively (Fraser et al., 2016). Phytate in SECM was successfully eliminated when treated with Bio-phytase (5000 phytase FYT units g^{-1} enzyme; Canadian Bio-systems Inc., Calgary, AB, Canada; Fraser et al., 2017).

Atlantic salmon were able to tolerate phytate (sodium phytate) in the range between 4.7 to 10.0 g kg^{-1} diet (Denstadli et al., 2006). When fed phytate exceeding this threshold, fish showed depressed feed consumption and weight gain. Digestibility as well as the whole-body retention of phosphorus and magnesium decreased with increasing levels of dietary phytate. High dose of dietary phytic acid at 20.7g kg^{-1} resulted in significantly lower trypsin activity in intestine chyme and bile acid concentration in the pyloric caeca (Denstadli et al., 2006). Supplement of 0.8 g kg^{-1} phytase (5000 FYT units g^{-1}) in plant-based diet which contained 13 g kg^{-1} of phytate significantly improved the FCR and final weight of rainbow trout (Morales et al., 2016). The added phytase significantly improved the apparent digestibility of P, Ca, Mg, and Zn, and subsequently the body retention of Zn P, and Ca (Morales et al., 2016).

2.3.2.4 Condensed Tannins

Tannins can bind with digestive enzymes or interact with proteins and minerals to form substances that are less digestible (Sandoval and Carmona, 1998). The content of

condensed tannins in camelina seed cake range from 1.0 to 2.4 mg g^{-1} (Matthäus and Zubr, 2000). Camelina meal contains 1.9 to 4.4 mg g^{-1} (dry matter basis) condensed tannins (Russo and Reggiani, 2012). Up to 25 mg g^{-1} (as fed basis) condensed tannin in the diet had no negative effect on growth performance, feed efficiency and body composition of Nile tilapia (*Oreochromis niloticus*) (Buyukcapar et al., 2011). In common carp (*Cyprinus carpis* L.), dietary inclusion of 20 mg g^{-1} (as fed basis) condensed tannins did not affect growth performance or carcass composition of common carp (*Cyprinus carpis* L.) (Becker and Makkar, 1999). The effect of condensed tannins on salmonids is not reported in the primary literature.

2.4 Camelina Products as a Protein Source in Animal Feeds

Camelina meal has been used a protein source in animal feed, with the residual oil in the meal providing n-3 fatty acids to enrich the fatty acid profiles of intended animal products such as milk, meat, and eggs (Hurtaud and Peyraud, 2007; Aziza et al., 2010; Kakani et al., 2012). Camelina meal has been used to replaced protein-rich feed ingredients in diets for ruminants (Moloney et al., 1998), and increased the monounsaturated fatty acids in milk (Hurtaud and Peyraud, 2007). Broiler chicken fed camelina meal replacement for canola meal at inclusion rates from 3 to 15% for 21 days showed lowered nutrient digestibility, weight gain and FCR (Thacker and Widyaratne, 2012). This is consistent with the finding of Pekel et al. (2015) that ileal digestibility of nitrogen and energy were significantly decreased by camelina meal incorporation in broiler diets, and was possibly meditated through high viscosity in jejunal digesta due to mucilage in camelina. By contrast, birds fed up to 10% camelina meal inclusion in the

diet exhibited no growth suppression and the meat n-3 fatty acid content increased after a 42-day feeding trial (Aziza et al. 2010). The γ-tocopherols, which effectively prevent lipid oxidation, significantly increased in the thigh meat but not breast meat (Aziza et al., 2010). Similarly, up to 10% camelina meal in the diet of laying hens had no effects on growth and increased the egg n-3 fatty acid content (Kakani et al., 2012).

The nutritional quality of camelina meal for finfish has been assessed in a few studies (Table 2.3 and 2.4). Feeding camelina meal to Atlantic cod showed contradictory results in two trials (Table 2.3). In the first trial, cod (19.4 g) were able to tolerate the highest test level of 24% SECM in the diet without compromised growth performance in a 9.5-week trial. In the second trial, cod (14.4 g) showed significantly lower weight gain and feed intake at 15% dietary inclusion of the same SECM in a 13-week trial (Hixson et al., 2015b). In the second trial, the visceral somatic index (VSI) was significantly lower in cod fed a diet with 40% SECM inclusion, indicating a lower visceral fat accumulation. In a parallel study to the second trial, cod fed 0, 15 and 30% SECM-containing diets were tested for orexigenic peptide mRNA expression in their brain. The increased transcript expressions of *pmch* and *hcrt* suggested that cod on SECM treatments were still hungry and not actually fed to satiation even though they were hand-fed to apparent satiation, and this was possibly due to reduced palatability and/or antinutritional factors of the SECM (Tuziak et al., 2014). SECM showed relatively high apparent digestibility of protein (88%) and energy (75%) by cod (Fraser et al., 2017), and the amount of crude protein and the amino acid profiles of the diets were similar. However, cod consumed less feeds when high levels of camelina meal was included (15% and up), indicating a palatability issue

Table 2.3 Feeding trials conducted to evaluate camelina products as a protein source in Atlantic cod and Atlantic salmon diet.

Experimental set up							Results				References
Species	Initial weight (g)	Trial duration (week)	Water characteristic	Camelina product tested[1]	Treatments	Camelina protein contribution to total dietary CP (% as fed basis)	Weight gain (g)	Feed consumption (g)	FCR	VSI	
Atlantic cod	14.4	13	10°C seawater	SECM (42.2% CP, 6.0% CL; DM basis)	0, 15, 30, and 40% of SECM inclusion	0, 12.7, 25.2, and 33.7	0%>15%>30%>40%	0% > 15% > 30% = 40%	0%<40%	0% = 15% > 40%	Hixson et al., 2015b; Tuziak et al., 2014
	19.4	9.5	10°C seawater	SECM (42.2% CP, 6.0% CL; DM basis)	0, 12, and 24% of SECM inclusion	0, 9.4, and 18.6	NSD[7]	N/A	N/A	NSD	Hixson et al., 2015b
	8.4	16	12°C freshwater	SECM (42.2% CP, 6.0% CL; DM basis)	0, 5, 10, 15, and 20% of SECM inclusion	0, 4.5, 9.0, 13.6 and 18.2	NSD	NSD	NSD	N/A	Ye et al., 2016
	62	16	12°C freshwater for week 1-5, salinity= 2ppt after week5	HORM (30.6% CP, 10.3% CL; as fed basis)	0, 8, 16, 24% of HORM inclusion	0, 5.4, 10.9 and 16.1	0%>8%>16%> 24%	0%>8%>16% >24%	0%<16% <24%	0%<8%<24 %, 8% =16% = 24%	Ye, 2014
Atlantic salmon	242	16	14°C seawater	SECM (42.2% CP, 6.0% CL; DM basis)	0, 8, 16, 24% of SECM inclusion	0, 7.3, 15.0, and 22.2	0%=8%, 0%>16%>24%	0%>8%=16% =24%	NSD	NSD	Hixson et al., 2015a; Ye, 2014
	242	16	14°C seawater	SECM (42.2% CP, 6.0% CL; DM basis)	Control diet with FO, 100% FO replacement with CO and 10% inclusion of SECM (100CO10CM), 100% FO replacement with CO and 10% inclusion of SECM (100COSEFM10CM)	0, 9.2, and 9.5	FO > 100CO10CM= 100COSEFM10CM	FO > 100CO10CM = 100COSEFM10CM	NSD	FO < 100COSEFM10CM	Hixson et al., 2014b; Xue et al., 2014

SECM= solvent extracted camelina meal; HORM= high oil camelina residue meal; CS= camelina seed; CP= crude protein; CL= crude lipid; FM= fishmeal; FO= fish oil; VSI= visceral-somatic index; NSD = no significant difference; N/A= data not available.

Table 2.4 Feeding trials conducted to evaluate camelina products as a protein source in rainbow trout diet.

Experimental set up							Results				References
Species	Initial weight (g)	Trial duration (week)	Water characteristic	Camelina product tested[1]	Treatments	Camelina protein contribution to total dietary CP (% as fed basis)	Weight gain (g)	Feed consumption (g)	FCR	VSI	
Rainbow trout	1	16	14 ºC freshwater	HORM (39.9% CP[2], 11.0% CL[3]; DM basis)	0, 10, 20 and 30% of HORM inclusion	0, 8.0, 16.1, and 23.7	0% = 10% = 20%	NSD	NSD	N/A	Bullerwell et al., 2016
	1	16	14 ºC freshwater	CS (27.4% CP, 37.7% CL; DM basis)	0, 10, 20 and 30% of CS inclusion	0, 5.6, 11.1 and 16.5	0% = 10% = 20%	NSD	0%=20%=30%, 0%>10%	N/A	Bullerwell et al., 2016
	1	16	14 ºC freshwater	SECM (43.0% CP, 3.1% CL, DM basis)	Control (0%) and a double substitution that contained 10% SECM and full replacement of added FO with CO (10SECM100CO)	0 and 8.6	0% = 10SECM100CO	NSD	NSD	N/A	Bullerwell et al., 2016
	2.4	16	14 ºC freshwater	SECM (43.0% CP, 3.1% CL, DM basis)	0, 5, 10, 15, and 20% of SECM inclusion	0, 4.4, 8.6, 12.9 and 17.6	0% = 5% = 10%= 15%	NSD	NSD	N/A	Bullerwell et al., 2016
	45	12	14 ºC freshwater	SECM (43.0% CP, 3.1% CL, DM basis)	0. 7. 14 and 21% of SECM inclusion	0, 5.9, 12.2 and 18.0	0% = 7% = 14%, 0%> 21%	NSD	0%=7%=14%, 0%>21%	NSD	Hixson et al., 2015a

SECM= solvent-extracted camelina meal; HORM= high oil residue camelina meal; CS= camelina seed; CP= crude protein; CL= crude lipid; FM= fishmeal; FO= fish oil; VSI= visceral-somatic index; NSD = no significant difference; N/A= data not available.

of camelina meal, rather than efficiency of utilization. The differences in growth performance in these two trials might be explained by different amounts of camelina protein ingested based on different trial durations (9.5 vs 13 weeks). It might also be a result of different sizes of the experimental fish; larger cod might have better ability to adapt to new diets. In both trials, inclusion of camelina meal did not alter the amino acid profile of the diets and cod muscle tissues.

Use of camelina products as a protein source for Atlantic salmon has been investigated (Table 2.3). High incorporation levels of camelina products reduced the palatability of the diet and therefore feed intake. Feed efficiency was affected in some trials. Enteritis, muscle fatty acid profile, and the associated gene expression alterations were detected in fish fed camelina meal in some trials (Ye et al., 2016; Ye, 2014; Hixson et al., 2014b and 2015a; Xue et al., 2014). Salmon parr (8.4 g), showed no differences in weight gain, FCR, HSI and carcass composition when fed 0, 5, 15 or 20% SECM in a 16-week trial (Ye et al., 2016). During the last weigh period (weeks 13-16, fish grew from 10-12 to 15-18 g), fish fed control diet gain significantly more weight compared to fish fed 15 and 20% SECM diets. Morphology of the villi in the distal intestine was independent of SECM inclusion rate. However, fish fed both 15 and 20% camelina diets had a thicker lamina propria (a layer lies beneath the mucosal epithelium in intestine) compared to the controls, indicating enteritis. In the second trial, salmon smolts (62 g) was not able to tolerate any test level of HORM inclusion (8, 16 or 24%) based on growth performance in freshwater after 16 weeks (Ye, 2014). Weight gain and feed consumption were negatively correlated with dietary inclusion level of HORM. Fish fed both 16 and 24% HORM diets deposited

significantly less protein and lipid in their carcass than fish fed control diet at week 16. In the same study, enteritis was detected in fish fed 16 and 24% HORM diets based on the morphology of the distal intestine including intestinal wall thickness, villus width, height and area, and goblet cell scoring (Ye, 2014). Post-smolt Atlantic salmon (242 g) had significantly lower weigh gain and feed intake when fed any SECM containing diet (8, 16 or 24%) than fish fed fishmeal and fish oil based diet in seawater after 16 weeks (Hixson et al., 2015a), which is consistent with the finding about HORM in the study of Ye (2014). However, different from the results of Ye (2014), the depression in weight gain and feed intake was not dose dependent in Hixson's case. FCR, VSI and carcass composition were similar among all treatments (Hixson et al., 2015a, Ye, 2014). Fish fed 24% SECM showed a higher score of supranuclear vacuoles and sub-mucosa indicating enteritis (Ye, 2014). In another trial, weight gain and feed intake were significantly decreased by the two double substitution treatments, one with 100% fish oil replaced with CO and 10% inclusion of SECM (100CO10CM); and the other with 100% fish oil replaced with CO, 100% fish meal replaced with solvent extracted fish meal and 10% inclusion of SECM (100COSEFM10CM), while FCR was similar among treatments (Hixson et al., 2014b). The VSI for 100COSEFM10CM was higher than control treatment, suggesting higher lipid accumulation in the gut (Hixson et al., 2014b). The parallel study (same fish) on liver transcriptome using indicated that hepatic gene expressions associated with lipid metabolism, carbohydrate metabolism, cell differentiation and proliferation, and immune function were negatively impacted by feeding 100COSEFM10CM diet, an extreme diet with little or no fish oil (Xue et al., 2014).

Different camalina products have been tested on rainbow trout with different initial sizes (1.0, 2.4 and 45 g; Table 2.4). Trout with initial weight of 1.0 g were able to tolerate dietary inclusion of ground camelina seed and HORM at 20% without depressed growth performance (Bullerwell et al., 2016). However, dietary incorporation of 30% ground camelina seed led to significantly lower weight gain of rainbow trout. This was due to lower feed consumption as a negative linear relationship was observed between inclusion rate of camelina seed and feed consumption. The authors speculated that the 30% HORM diet also led to a significant reduction in final weight gain, however, possibly this effect was meditated through lower nutrient utilization than feed intake, according to the significantly lowered PER. Measurements on villi in both midgut and hindgut, and carcass composition were similar among all treatments (Bullerwell et al., 2016). In the second trial, up to 15% of SECM was successfully included in the diet of rainbow trout (2.4 g) measured by weight gain, feed consumption and FCR (Bullerwell et al., 2016). The 20%SECM diet resulted in a significant reduction in the final weight gain, and is possible related to feed intake according to a regression analysis. In another trial, among rainbow trout starting at 45 g, up to 14% of dietary inclusion of SECM had no negative effects on weight gain, feed intake, FCR and after 12 weeks. Notably, the final mean weight of fish fed 14%SECM diet was significantly lower than the controls. At 21% dietary inclusion rate, fish had significantly lower weight gain, FCR and PER compared to fish fed the control diet, while the feed intake was not affected ($p>0.05$). In the trout muscle tissues, no treatment difference was observed in either the EPA, DHA, the total n-3 fatty acids, or the amino acid profile of trout muscle tissue (Hixson et al., 2015a). It appears from these studies that younger trout (1.0 g) may tolerate higher amounts of

camelina protein than older ones (2.4 and 44.9 g), although the types of camelina meal used was different among trials. The current study will investigate the tolerance level of camelina products including SECM and CPC on young rainbow trout including both first feeding and fry stages.

2.5 Development of the Digestive System of Salmonid Fish

The digestive system of salmonid fish consists of mouth, esophagus, stomach, pyloric caeca, intestine and two secretory glands including liver and pancreas. During the embryo development before hatching, the mouth, esophagus, stomach, intestine and liver had started to form, and bile acids start to appear in the gut (Gorodilov, 1996). At seven-day post hatch (dph), newly hatched alevins exhibited mouth with teeth, tongue, and taste buds (Sahlmann et al., 2015). Taste buds were observed in the esophagus at a later sampling point at 27 dph. Between 7 dph to first feeding (46 dph), the straight stomach developed into a "u" shape and the simple straight intestine was divided into the proximal and distal intestine where the former can be distinguished by a larger diameter than the latter (Sahlmann et al., 2015). Pyloric caeca buds were not observed until a relative late state at 27 dph. They continued to develop and was connected to pancreatic tissues when ready for first-feeding at 46 dph. Functional liver and pancreatic cells were evidenced since 7 dph (Sahlmann et al., 2015). By the time of first feeding, the gastrointestinal track is fully functional.

2.6 Nutritional Requirements of Atlantic Salmon and Rainbow Trout

Both Atlantic salmon and rainbow trout have dietary requirements for amino acids, fatty acids and micronutrients including vitamins and minerals to maintain normal growth and health. Although they do not have dietary requirements of proteins, providing a minimal of 48% crude protein usually provides sufficient levels of amino acids to optimize the growth of both species under 20 g (NRC, 2011). As fish grow, lower levels of proteins are needed. When salmon and trout reaches 1.5 kg, the recommended levels of dietary protein decrease to about 35% (NRC, 2011). Due to high costs of proteins, it is more desirable for dietary proteins being used for metabolic needs and protein retention than as energy sources. Higher protein retention in Atlantic salmon and rainbow trout can be achieved by optimizing ratios of dietary digestible protein (DP) to digestible energy (DE). Protein retention in salmon (25 g) was higher when fed diets with a DP/DE ratio of either 18 or 20 g MJ^{-1} when compared to 22 and 24 g MJ^{-1} (Azevedo et al., 2004). Rainbow trout (47 g) had similar protein retention when fed diets containing DP/DE ratio either at 18, 20, 22 or 24g MJ^{-1} (Azevedo et al., 2004). Neither Atlantic salmon nor rainbow can synthesize some amino acids including arginine, histidine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine and thus have dietary requirements of these essential amino acids (Table 2.2; NCR, 2011). Dietary requirements for essential fatty acids including ALA (18:3n-3), EPA (20:5n-3) and DHA (22:6n-3) for Atlantic salmon and rainbow trout are 1, 0.5, 0.5-1%, and 0.7-1, 1, 0.5% of the diet, respectively (NRC, 2011; Food and Agriculture Organization of United Nations (FAO), 2008a and b). Both of the species have dietary requirement of linoleic acid. The requirement for rainbow trout is 1% of the diet; however, it is not determined for Atlantic salmon (NRC,

2011). Linoleic acid and ALA can be sourced from terrestrial plants, while EPA and DHA are mainly derived from marine organisms. Carbohydrates are not required in Atlantic salmon and rainbow trout diets. In salmonids farming industry, they are often provided in the diets as energy sources or as binders to enhance the integrity of feed pellets. Simple carbohydrates like glucose and complex carbohydrates such as cooked starch can be utilized by both salmon and trout. However, the levels of these carbohydrates should not exceed 10-12% in Atlantic salmon diet and 15-17% in rainbow trout diet (FAO, 2018a and b). Carbohydrates such as raw starch and non-starch polysaccharides cannot be digested by salmon and trout.

2.7 Focus of the Literature and Objectives of the Thesis

Camelina products with high ALA, have the potential to be used as feed ingredients in animal diets, particularly in salmonid diets. CO has been successfully used to fully replace the added fish oil in the diet of Atlantic salmon and rainbow trout at the juvenile and grow-out stages. However, salmon or trout at juvenile and grow-out stages showed limited acceptance to the dietary inclusion of HORM or SECM. The poor growth performance and enteritis found in fish fed high levels of camelina meal were likely to be associated with antinutrients in the meal. Camelina protein concentrate has higher protein content compared to the meal, and is more appealing as a feed ingredient. There is some evidence for introduction of plant feed ingredients at early growth stage to enhance the utilization of the same ingredients later in the life cycle and it has not been investigated for camelina products.

This thesis expanded the knowledge of using camelina products including SECM, CPC and CO in the diet of first feeding rainbow trout, trout fry and Atlantic salmon fry through growth performance, mortality and carcass composition.

Chapter 3

Use of Oil, Solvent-Extracted Meal, and Protein Concentrate from *Camelina sativa* L. Crantz for First Feeding Rainbow Trout *Oncorhynchus mykiss*

3.1 Abstract

Feeding swim-up rainbow trout fry with plant-based diets may be useful to assess the acceptability, negative effects and performance potential of new feed ingredients. Fish at this stage are undergoing high growth rate and are sensitive to dietary changes. No studies were found using *Camelina sativa* products as feed ingredients for first feeding rainbow trout (0.105 g). One hundred fry per tank (20 L tank, with initial water temperature at 10 °C, increasing to 16°C) in four replicate tanks were fed with one of ten experimental diets containing 0, 6, 12, or 18% solvent-extract camelina meal (SECM) or camelina protein concentrate (CPC) or with 50 or 100% fish oil replaced with camelina oil (CO) for 112 days. Up to 100% fish oil can be replaced with CO based on all measured growth parameters ($p>0.05$). Fish fed 100%CO diet had a higher visceral somatic index and hepato-somatic index, indicating higher levels of fat deposition in viscera and liver. On day 112, fish fed 18%SECM diet consumed more feed, gained more weigh, and had a lower mortality rate compared to other treatments ($p<0.05$).

3.2 Introduction

Salmonid feed industry continuously evaluates the potential new feedstuffs to reduce the dependency on fishmeal and fish oil. The oil crop, *Camelina sativa*, has drawn increasing attention as such in recent years. Camelina seed contains a high percentage of oil at 38-43% (Gugel and Falk, 2006). The oil has a unique fatty acid profile, 90% of which is highly unsaturated (Zubr, 1997). Among the total fatty acids, alpha-linolenic acid, an essential n-3 fatty acid for Atlantic salmon and rainbow trout, accounts for 36.2-39.4%. The high content of n-3 fatty acids of camelina oil (CO) makes it promising for fish oil substitution in the salmonid diet. Camelina press cake is a by-product from oil extraction. This by-product can be further processed into high-oil residue camelina meal (HORM), solvent extracted camelina meal (SECM), or camelina protein concentrate (CPC) to be used as protein sources in fish diets (Hixson et al., 2015a; Bullerwell et al., 2016; Ye et al., 2016).

The effect of using CO and camelina meals in fish diet including rainbow trout, Atlantic salmon, and Atlantic cod. CO was used to replace fish oil in rainbow trout diet without impacting growth performance (Bullerwell et al., 2016; Hixson et al., 2013 and 2014a;). Both EPA and DHA content, as well as the n3/n-6 fatty acid ratio in trout muscle tissue decreased significantly due to dietary replacement of fish oil with CO (Hixson et al., 2014a). On the positive side, DHA content in muscle tissue doubled the amount in the diet, suggesting the use of metabolic pathway from ALA to DHA. Incorporating up to 20% of ground camelina seed or HORM in trout diet (1.0 g) did not have negative impacts on growth performance or midgut and hindgut morphology (Bullerwell et al., 2016). However, a negative linear relationship between the inclusion rate of ground camelina seed and feed consumption was observed. Up to 15% of dietary inclusion of SECM was acceptable to rainbow trout (2.4 g), while weight gain was significantly decreased at an inclusion rate at 20% (Bullerwell et al., 2016). Similarly, incorporating 14% SECM in a trout diet had no adverse effects on growth performance of rainbow trout (44.9 g), while incorporating 21% of SECM resulted in a reduction in weight gain (Hixson et al., 2015a). Despite the different forms of camelina meal were used in these trials, smaller trout in the former study seemed to utilize high levels of protein from camelina meal compared to larger fish in the latter study (Bullerwell et al., 2016; Hixson et al., 2015a).

Exposing fish to plant-based diets during the early life stage can induce persistent metabolic adaptations, which can improve their ability to tolerate higher levels of such diets in later life stages (Geurden et al., 2007, 2013; Vagner et al., 2007). In the current study, first feeding rainbow trout fry were used as a model to investigate whether first

feeding rainbow trout can tolerate CO, SECM or CPC in their diet; to find the optimal dietary inclusion rate of these three ingredients; and to compare with results from trials done on rainbow trout at later life state.

3.3 Objectives

The 112-day feeding trial introducing CO, SECM or CPC-containing diets to first feeding rainbow trout investigated the following objectives:

1. To evaluate the acceptability of three camelina products fed to rainbow trout based on their growth performance.
2. To identify the optimal dietary inclusion rate of these ingredients based on growth performance up to 112 days of feeding.

3.4 Null Hypotheses

1. Replacement of 0, 50 or 100% fish oil with CO will result in different growth performance by first feeding rainbow trout. Carcass composition of rainbow trout will be altered by diets containing different levels of CO.

2. Inclusion of 0, 6, 12 or 18% SECM or CPC in the diet of first feeding rainbow trout will result in different growth performance and proximate carcass composition.

3.5 Materials and Methods

3.5.1 Experimental Diets

3.5.1.1 Test Ingredients

Camelina (cultivar-MIDAS) was grown and harvested in Canning and Truro, Nova Scotia, Canada under the supervision of Dalhousie University, Faculty of Agriculture. CO was obtained from seeds pressing using a EGON KELLER KEK 0500 expeller-press at Atlantic Oilseed Processing, Ltd. in Summerside, Prince Edward Island, Canada. CO was stabilized by the addition of ethoxyquin (60% ethoxyquin, 40% silica) at 0.2% of the oil. The meal cake from oil extraction was hammer milled to produce HORM and sent to POS BIO-Sciences in Saskatoon, Canada to make SECM and CPC. The SECM was prepared by the following procedures: 1) HORM was washed with n-hexane three times; 2) hexane was drained and the meal was spread in the fume hood for two days for the remaining hexane to evaporate. HORM was also used to produce CPC using a modified version of the process described by Bilgi and Celik (2004). The process is summarized in Figure 3.1. The yield of CPC from SECM was low and only represented around 10% of the original meal, based on multiple batches made by the author following the procedure of Bilgi and Celik (2004). Both SECM and CPC were shipped back to the Chute Animal Nutrition Centre, Dalhousie University, Faculty of Agriculture until feed manufacture. The chemical compositions of SECM and CPC used in the current study are found in Table 3.1.

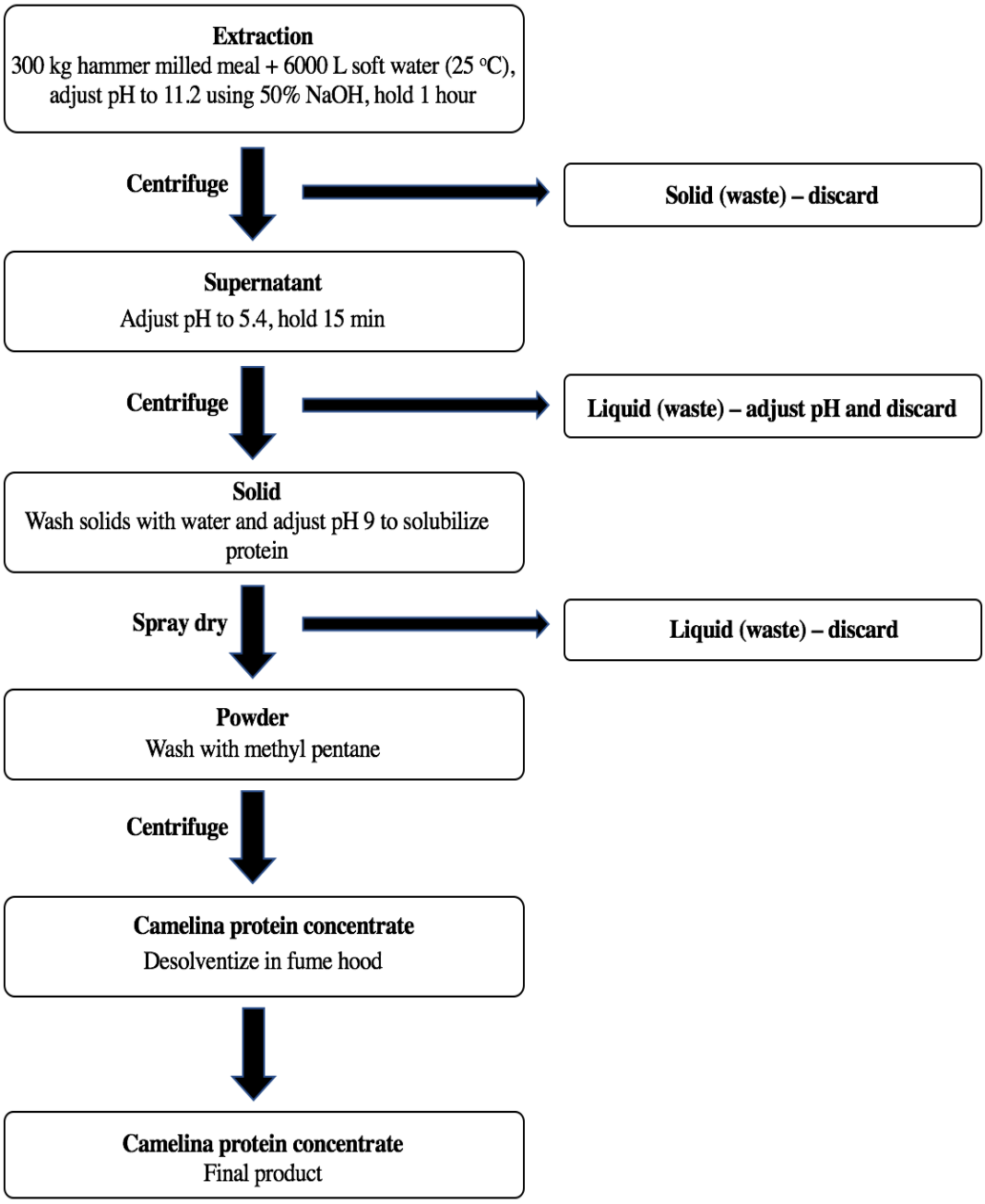

Figure 3.1 Procedures for camelina protein concentrate preparation using high oil residue camelina meal based on the modified method of Bilgi and Celik (2004).

Table 3.1 Nutrient composition of solvent-extracted camelina meal and camelina protein concentrate (as is basis).

	Solvent-extracted camelina meal	Camelina protein concentrate
Proximate analysis[1]		
Dry matter (%)	89.1	92.7
Gross energy (kcal/kg)	4244	4713
Crude protein (%)	38.2	52.5
Crude fat (%)	3.3	7.9
Ash (%)	6.8	16.0
Macro-minerals[2] (%)		
Calcium	0.39	0.10
Potassium	1.3	0.2
Magnesium	0.46	0.05
Phosphorus	1.15	3.55
Sodium	BDL[5]	5.5
Micro-minerals[2] (mg kg^{-1})		
Copper	11	23
Manganese	40	6
Zinc	80	17
Boron	19	11
Molybdenum	1.4	3.7
Barium	0.9	0.5
Cobalt	0.06	0.10
Cadmium	0.05	0.06
Arsenic	BDL[5]	0.10
Chromium	BDL[5]	0.90
Lead	BDL[5]	0.08
Selenium	BDL[5]	BDL[5]
Titanium	BDL[5]	3
Mercury	BDL[5]	0.01
Sinapine[3] (mg kg^{-1})	2576	BDL[5]
Total Glucosinolates[4] (μmol g^{-1})	47.0	2.5
9-methyl-sulfinyl-nonyl	11.8	0.5
10-methyl-sulfinyl-decyl	30.1	1.8
11-methyl-sulfinyl-undecyl	5.1	0.2

[1] Completed in Nutrition Lab of Dalhousie University, Faculty of Agriculture, Truro, NS, Canada.

[2] Completed by Maxxam Analytics International Corporation, Mississauga, Ontario, Canada.

[3] Completed by Agriculture and Agri-Food Canada, London, Ontario, Canada.

[4] Completed by Agriculture and Agri-Food Canada, Saskatoon, SK, Canada.

[5] Below detectable limits.

3.5.1.2 Diet Formulation and Preparation

Ten experimental diets were formulated to be isonitrogenous and isocaloric to provide a calculated crude protein level of 52.5% and estimated digestible energy level of 4475 kcal kg^{-1}. All diets met the nutritional requirements of rainbow trout (NRC, 2011). The ten treatments were: two oil diets with either 50 or 100% fish oil replaced with CO (Table 3.2); four SECM diets with 0, 6, 12 and 18% SECM inclusion (Table 3.2); four CPC diets with 0, 6, 12 and 18% CPC inclusion (Table 3.2). Each diet was fed to four replicate tanks. All diets were prepared at Chute Animal Nutrition Centre (Dalhousie University, Faculty of Agriculture, Truro, Nova Scotia, Canada) within seven days of the starting point of the trial (January 25, 2014). Each diet was mixed in a KitchenAid Classic Series 4.5-Quart Tilt-Head Stand Mixer, with warm water (50-60 °C) added at 300g kg^{-1} diet. The wet mix was blended again and then extruded through a Cuisinart electric meat grinder (Model No. MG-100C) fitted with a 3 mm die. The pelleted feed was dried at about 55°C for 4-5 hours in a forced air oven. Extruded feed was allowed to cool down to room temperature, vacuum packed into individual bags (approximate 500 g per bag) using a food sealer and stored in a -20°C freezer until use. Feed was hand-crumbled and sifted into different particle sizes ranging from 0.5-0.7, 0.7-0.85, 0.85-1.0, 1.0-1.4, 1.4-1.7, and 1.7-2.0 mm, respectively. The excessive feed and fines were discarded. According to feeding behavior of the fish, optimal particle size of feed particles was provided with the smallest range (0.5-0.7 mm) initially then increased as fish grew. All feed was stored in a -20°C freezer and only being exposed to room temperature during feeding. Diets were sent to Nova Scotia Agriculture and Food Operations Analytical Lab in Harlow Institute, Truro, Nova Scotia for nutrient composition analysis (Table 3.2). Dry

matter was determined following method 935.29 (Association of Official Analytical Chemists (AOAC), 2011). Crude protein content was analyzed according to combustion method 990.03 (AOAC, 2011). Crude fat was measured using an Ankom XT10 extraction system following method 920.39 (AOAC, 2011). Ash content was determined by method 942.05 (AOAC, 2011). Total glucosinolates was analyzed by Agriculture and Agri-Food Canada, Saskatoon, SK, Canada by an HPLC method of Lange et al. (1995).

A few diets were selected from the total of eight diets and checked for oxidation status and vitamin C content in consideration of the costs. Frozen feed samples from four diets including 0%SECM/0%CPC, 50%CO, 18%SECM and 18%CPC were vacuum-packed, placed on ice packs, and sent for peroxide and p-anisidine analysis in the Coastal Zones Research Institute Inc. in Shippagan, New Brunswick, Canada, following the method described by Firestone (1998).

Three diets including 0%SECM/0%CPC, 18%SECM and 6%CPC were analyzed for vitamin C content (L-ascorbyl-2-phosphate) six months after diet preparation. Frozen samples from these diets were packed in vacuum sealed bags, placed on ice packs, sent to the Maxxam Analytics International Corporation (Mississauga, Ontario, Canada) and analyzed according to the method of Hitchins et al. (1998).

Table 3.2 Ingredients and analyzed nutrient composition of the experimental diets containing solvent-extracted camelina meal (SECM), camelina protein concentrate (CPC) and camelina oil (CO) of first feeding rainbow trout (as fed basis)

Diet	SECM				CPC				CO	
Ingredient (%)	0%	6%	12%	18%	0%	6%	12%	18%	50%	100%
Fishmeal	47.8	43.9	40.1	36.3	47.8	45.3	42.8	40.3	47.8	47.8
SECM	0	6	12	18	0	0	0	0	0	0
CPC	0	0	0	0	0	6	12	18	0	0
Fish oil	12.1	12	12.2	12.5	12.1	12.3	12.7	13.1	6.05	0
CO	0	0	0	0	0	0	0	0	6.05	12.1
Wheat	11.9	9.9	7.5	5.0	11.9	8.0	4.0	0.1	11.9	11.9
CPSP-G[a]	7	7	7	7	7	7	7	7	7	7
Wheat gluten meal	5	5	5	5	5	5	5	5	5	5
Blood meal	5	5	5	5	5	5	5	5	5	5
Krill meal	3	3	3	3	3	3	3	3	3	3
Pre-gelatinized starch	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Poultry byproduct meal	2	2	2	2	2	2	2	2	2	2
Sodium bicarbonate	1	1	1	1	1	1	1	1	1	1
Soy lecithin	1	1	1	1	1	1	1	1	1	1
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.2	0.2	0.2	0.2	0.2	0.5	0.5	0.5	0.4	0.4
Mineral premix[b]	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.2	0.2
Vitamin premix[c]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Special premix[d]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Threonine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Total	100	100	100	100	100	100	100	100	100	100
Analyzed nutrient[e] composition										
Dry matter (%)	97.3	97.9	97.1	97.6	97.3	97.7	97.3	97.7	97.4	97.9
Crude protein (%)	54.9	54.2	53.7	53.7	54.9	54.5	54.3	54.2	55.2	55.6
Crude fat (%)	20.4	20.7	20.1	20.7	20.4	19.9	20.6	20.8	18.6	19.5
Ash (%)	10.0	9.8	9.6	9.7	10.0	10.2	10.3	10.8	9.9	10.2
Calcium (%)	2.4	2.3	2.2	2.2	2.4	2.3	2.2	2	2.5	2.7
Potassium (%)	0.62	0.64	0.68	0.70	0.62	0.59	0.53	0.58	0.57	0.60
Magnesium (%)	0.19	0.21	0.23	0.25	0.19	0.19	0.18	0.17	0.19	0.2
Phosphorus (%)	1.6	1.6	1.6	1.6	1.6	1.7	1.9	2.0	1.7	1.8
Sodium (%)	0.7	0.7	0.7	0.7	0.7	0.9	1.3	1.6	0.8	0.8
Copper (mg kg^{-1})	43	9	9	11	43	12	12	12	8	8
Manganese (mg kg^{-1})	24	26	25	26	24	26	24	22	25	25
Zinc (mg kg^{-1})	144	148	147	151	144	146	141	135	160	144
Total glucosinolates[f] (µmol g^{-1})	0	0.6	2.0	2.7	0	0.5	0.7	1.1	N/A[g]	N/A[g]

[a] CPSP-G is a soluble fish protein concentrate. Manufacturer: Sopropeche. Wimille, France.

[b] Mineral added to supply the following (per kilogram diet): magnesium (magnesium sulfate, 9.86% Mg), 250 mg; iron (ferrous sulfate, 0.1% Fe), 30 mg; copper (copper sulfate, 25.4% Cu), 5 mg; zinc (zinc sulfate, 22.7% Zn), 75 mg; cobalt (cobalt chloride, 24.8% Co), 2.5 mg; fluorine (sodium fluorine, 42.5% F), 4mg; manganese (manganous sulfate, 32.5% Mn) 12.5 mg; wheat middling, 724 mg.

[c] Vitamin added to supply the following (per kilogram diet): vitamin A (retinal acetate, 1000*10^{6} IU kg^{-1}), 4000 IU; vitamin D3 (premix, 500*10^{6} IU kg^{-1}), 3200 IU; vitamin K3 (meadione sodium bisulfate, 33%), 20 mg; thiamin (thiamin mononitrate, 893199 mg kg^{-1}), 20 mg; riboflavin (spray dried, 80%), 15 mg; pantothenate (dicalcium pantothenate, 920000 mg kg^{-1}), 40 mg; biotin (spray dried, 2%), 0.5 mg; folic acid (spray dried, 3%), 8 mg; vitamin B12 (premix, 1000mg kg^{-1}), 0.03 mg; Niacin (Rivomix niacin, 100%), 100 mg; pyroxidine (Pyridoxine hydrochloride, 821513 mg kg^{-1}), 12mg; inositol (pure, 100%), 250 mg; ethxyquin (60%), 40mg; wheat middlings, 1086 mg.

[d] Special premix (IU or mg kg^{-1} diet): selenium (premix, 2000mg kg^{-1}), 0.3 mg; ascorbic acid (mono/polyphosphate, 975000 mg kg^{-1}), 200 mg; vitamin E (dl-alpha tocopherol acetate, 500000 IU kg^{-1}), 400 IU; wheat middlings, 645 mg.

[e] Analyzed by Nova Scotia Agriculture and Food Operations Analytical Lab in Harlow Institute, Truro, Nova Scotia, Canada

[f] Analyzed by Agriculture and Agri-Food Canada, Saskatoon, SK, Canada.

[g] Not available. Camlina oil does not contain glucosinolates.

A sample of CO was sent to the Experimental Station's Chemical Laboratories, University of Missouri, USA for a 20-hour Active Oxygen Method (AOM) peroxide test (American Oil Chemists' Society Official Method Cd 12-57, 1980).

3.5.2 Fish Stock and Rearing Conditions

Eyed rainbow trout eggs (Kamloops stock) were shipped to the Aquaculture Centre of Dalhousie University, Faculty of Agriculture, Truro, Nova Scotia, Canada from Troutlodge Inc. in Washington State, USA. After arrival, trout eggs were disinfected in 10°C fresh water containing 100 ppm Ovadine® solution for 10 minutes, rinsed with 10°C fresh water, then transported into a MariSource 8-tray vertical incubator until hatching. Four thousand newly-hatched alevins were randomly selected and distributed (with water) into 40 of 2 L enclosures (Figure 3.2) suspended within 20 L white cone-shaped tanks in a flow through system. About ten days after initiation of feeding, the enclosures were removed and the fry was released into the tanks.

A B C

Figure 3.2 Set up of a 2 L enclosure in a 20 L white cone-shaped tank (A). Rainbow trout fry were released into the 20 L white cone-shaped tank (B). Two identical flow-through system with a total of 40 tanks (C).

The system was initially supplied with 10±0.5°C fresh water. Water temperature was gradually increased to 16°C at a rate of 1°C per three days after fry adapted to feed. The initial water flow for each tank was 0.6±0.05 L min^{-1}, increased to 0.8±0.05 L min^{-1} on day 28, and again increased to 1.0±0.05 L min^{-1} on day 56. Water was oxygenated with an onsite oxygen generator to provide 100-120% dissolved oxygen. Water temperature and oxygen level in each tank was checked daily. Photoperiod was fixed at 12-hour light:12-hour dark (0700 to 1900h) from January 25th to May 17th, 2014. The duration of the trial was 112 days. Six meals were provided at the interval of every two hours between 0730 and 1730h. Fish were overfed consistently among tanks until day 14. After day 15, fish were hand fed to apparent satiation. Feeding frequency was reduced to three times per day (0830, 1200, and 1630h) when fish reached 2 g. On day 56, the number of fish in each tank was reduced to 30 to maintain a reasonable stocking density. Tanks were cleaned daily by siphoning and brushing. Mortalities were recorded and dead fish were removed daily. Moribund was recorded, removed and euthanized using an overdose of tricaine methanesulfonate (TMS, 400-500 ppm) if loss of equilibrium and feeding response. Each dead fish and moribund was examined and categorized into normal and abnormal group based on the symptoms by the author. The normal group were fish with no obviously pathological symptoms while the abnormal group were fish with symptoms including skin discoloration, scoliosis, lordosis or hemorrhage. On the last day of the trial, in each tank, all survival fish were assessed and categorized into normal and abnormal groups by the author.

3.5.3 Data Collection and Analysis

3.5.3.1 Sampling

The last meal was withheld on the day before weighing day or sampling day. Fish in each tank were counted and batch weighed at an interval of 14 days. No anesthetic was used. Weight gain, feed consumption, feed conversion ratio (FCR), and protein efficiency ratio (PER) were determined every 14 days. On day 0, one hundred first feeding fry were randomly selected from the original population in the incubator, euthanized with an overdose of TMS (400-500 ppm), and stored at -20°C as an initial carcass sample until future analysis. On day 112, six fish per tank were euthanized using an overdose of TMS to determine viscera-somatic index (VSI) and hepato-somatic index (HSI) determination. VSI is the ratio of viscera weight to the whole-body weight of a fish. Viscera included the whole digestive tract from the esophagus to the end of hindgut, along with all attached organs, except for heart and kidney. HSI is the ratio of liver weight to the whole-body weight of a fish. To measured VSI and HSI, body weight of individual fish was recorded. Fish were then dissected to have viscera removed and weighed. The liver attached to the viscera was carefully removed and weighed.

3.5.3.2 Calculation

$$\text{Weight gain (g fish}^{-1}) = \frac{\text{final batch weight (g)}}{\text{number of fish counted}} - \frac{\text{inital batch weight (g)}}{\text{number of fish counted}}$$

$$\text{Feed consumption (g fish}^{-1}) = \frac{\text{total feed consumed (g)}}{\text{number of fish counted}}$$

$$\text{Feed conversion ratio} = \frac{\text{total feed consumed (g)}}{\text{weight gain (g)}}$$

$$\text{Protein efficiency ratio} = \frac{\text{total weight gain (g)}}{\text{total protein intake (g)}} = \frac{\text{total weight gain (g)}}{\text{total feed intake(g)} * \%\ \text{of the crude protein}}$$

$$\text{Visceral-somatic index} = \frac{\text{viscera weight of an individual fish (g)}}{\text{whole body weight of an individaul fish (g)}} * 100$$

$$\text{Hepato-somatic index} = \frac{\text{liver weight of an individual fish (g)}}{\text{whole body weight of an individaul fish (g)}} * 100$$

3.5.3.3 Statistical Analysis

Data obtained from fish fed camelina protein products including SECM and CPC were analyzed separately from fish fed diets with fish oil replaced with CO. A randomized complete block design was used for the oil replacement treatments (Figure 3.3). Each tank was an experimental unit. The 0%SECM treatment from the camelina protein products treatments was used as the control treatment 0%CO. The statistical model statement of the experiment was listed as follows:

$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$

Y_{ijk} was the response variable

μ was the overall mean of the response variable

α_i was the effect of different replacement levels (0, 50 or 100%)

β_j was the blocking effect (j=1, 2, 3 or 4)

ε_{ij} was the random error

Analysis of variance (ANOVA) was performed for cumulative mortality rate, weight gain, feed consumption, FCR, PER, VSI, and HSI, using the Proc Mixed procedure of Statistical Analysis System (SAS) 9.3 (SAS Institute Inc., Cary, NC) (Littell et al., 2006). Tukey-Kramer test was used for multiple comparisons whenever the ANOVA indicated significant difference (α=0.05; Gbur et al., 2012).

The experiment design for the camelina protein products treatments was a 2×4 factorial arrangement on four randomized complete blocks, with two types of camelina products (SECM or CPC) and four graded levels of inclusion (0, 6, 12 and 18%) (Figure 3.3). Each

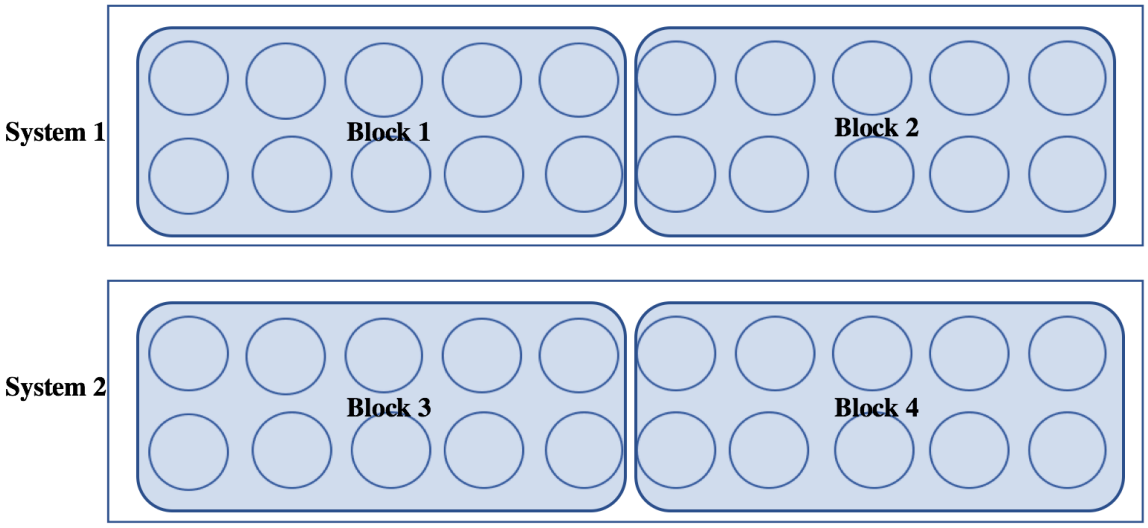

Figure 3.3 Experimental set-up within two identical systems divided into four blocks. Each block contains 10 tanks.

tank was an experimental unit. Cumulative mortality rate, weight gain, feed consumption, FCR, PER, VSI, and HSI were subjected to analysis of variance by the Proc Mixed procedure of SAS 9.3 (SAS Institute Inc., Cary, NC) (Littell et al., 2006) following the model as follow:

$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \varepsilon_{ijk}$

Y_{ijk} was the response variable

μ was the overall mean of the response variable

α_i was the effect of types of camelina protein products (i=SECM or CPC)

β_j was the effect of the inclusion level (j= 0, 6, 12 or 18%)

$(\alpha\beta)_{ij}$ was the two-way interaction effects of i^{th} of camelina protein product and j^{th} inclusion rate

γ_k was the blocking effect (k=1, 2, 3 or 4)

ε_{ijk} was the random error

If the ANOVA indicated significance of the interaction effects or main effects, Tukey-Kramer test was applied for multiple means comparisons (α=0.05; Gbur et al., 2012).

3.6 Results

3.6.1 Composition of the Experimental Diets

The three diets used in oil treatments had 0, 50 or 100% fish oil replaced with CO. The analyzed crude protein (54.9-55.6%), crude fat (18.6-20.4%), ash (9.9-10.2%), and minerals in all three diets were similar except for slight variation in copper content from 9-43mg kg^{-1} (Table 3.2).

Eight diets with 0, 6, 12 and 18% of either SECM or CPC inclusion had similar nutritional profiles crude protein, crude fat, ash ranged from 53.7 to 54.9%, 19.9 to 20.8%, and 9.6 to 10.8%, respectively (Table 3.2). The mineral content was similar among all eight diets (Table 3.2). The total glucosinolates in the SECM and CPC were 47 and 2.5 μmol g^{-1}, respectively. The total glucosinolates in the eight diets increased with the increasing inclusion levels of SECM or CPC, although not in a proportional way. The analyzed total glucosinolates in the eight diets including 0, 6, 12 and 18% SECM and CPC diets were 0, 0.6, 2.0, 2.7 and 0, 0.5, 0.7 and 1.1 μmol g^{-1}, respectively (Table 3.2).

CO had an initial peroxide and 20-hour AOM peroxide of 3.7 and 37.9 mEq kg^{-1}, respectively (Table 3.3). Four diets, including the control diet (0%SECM/CPC),

18%SECM, 18%CPC and 50%CO, had peroxide of 9.1, 14.6, 11.2 and 108 mEq kg^{-1}, respectively (Table 3.3). The p-anisidine in these diets was 43.6, 74, 58.1 and 277, respectively. Three diets including 0%SECM/CPC, 18%SECM and 6%CPC had less than 5 mg of L-ascorbyl-2-phosphate per kg diet (under the reportable detection limit; Table 3.3).

Table 3.3 The stability of camelina oil (CO), oxidation tests and vitamin C contents of selected diets containing CO, solvent-extracted camelina meal (SECM) or camelina protein concentrate (CPC).

Parameter		Ingredient/Diet						Date of analysis
		CO	0%CO/SECM/CPC	50%CO	18%SECM	6%CPC	18%CPC	
Stability of CO[1]	Initial peroxide (mEq kg^{-1})	3.7						Feb-Mar, 2015
	20-Hour AOM peroxide (mEq kg^{-1})	37.9						
Oxidation tests[2]	Peroxide (mEq kg^{-1})		9.1	108.0	14.6		11.2	Feb 12-18, 2014
	p-anisidine		44	277	74		58	
Vitamin C[3]	L-ascorbyl-2-phosphate (mg kg^{-1})		<5		<5	<5		July 28, 2014

[1] Completed by Experimental Station's Chemical Laboratories, University of Missouri, USA.
[2] Completed by Coastal Zones Research Institute Inc. Shippagan, New Brunswick, Canada.
[3] Completed by Maxxam Analytics International Corporation in Mississauga, Ontario, Canada.

3.6.2 Performance of Rainbow Trout Fed Graded Levels of Camelina Oil

3.6.2.1 Mortality Rate

The cumulative mortality rate was calculated during periods day 0-56 and 56-112, separately. During day 0-56, the cumulative mortality rates of rainbow trout fed all three diets, including 0, 50 and 100%CO, were less than 5% (Figure 3.4). Trout fed 0%CO diet had the highest cumulative mortality rate at 2.7%, which was significantly higher than fish fed 50%CO diet at 0.29% (Figure 3.4). On day 112, the cumulative mortality rates of fish fed all three diets were not significantly different at 15.8, 15.0, and 22.5% respectively ($p>0.05$) (Figure 3.5). The rates of normal and abnormal fish survived on day 112 were similar among all three treatments ($p>0.05$) (Table 3.4).

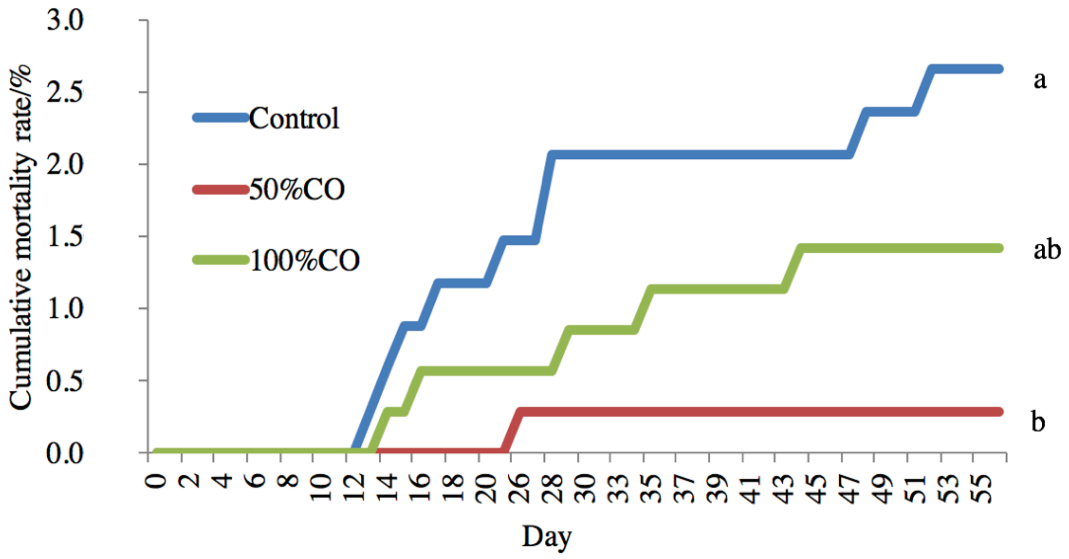

Figure 3.4 The cumulative mortality rate of first feeding rainbow trout fed diets with 0, 50 and 100% of fish oil replaced by camelina oil from day 0 to 56 (n=4). Statistics was based on the cumulative mortality rate on day 56. Standard deviation for the control, 50%CO and 100%CO treatment were 0.52%, 0.57% and 1.08%, respectively.
[ab] Mean with different superscripts are significantly different ($p<0.05$).

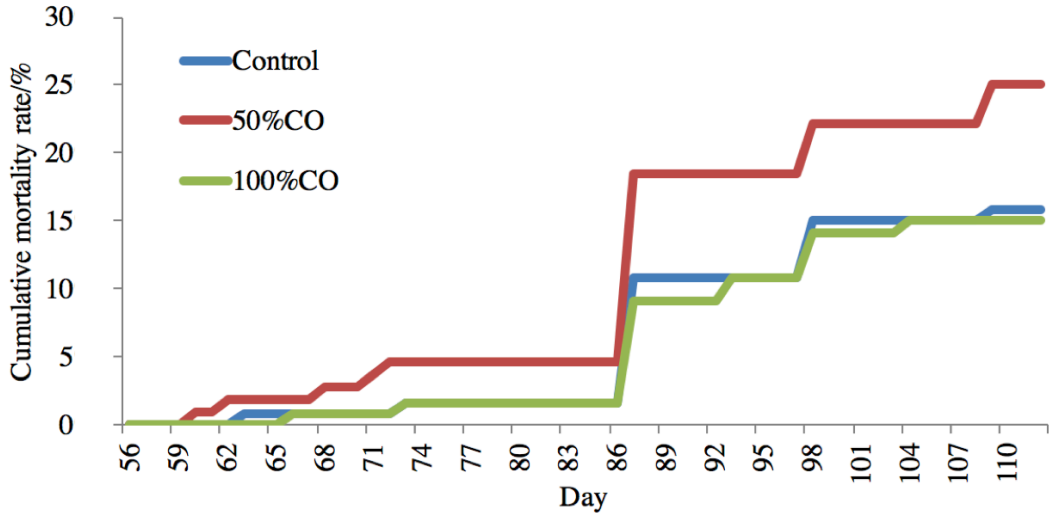

Figure 3.5 The cumulative mortality rate of first feeding rainbow trout fed diets with 0, 50 and 100% of fish oil replaced by camelina oil from day 56 to 112 (n=4).

Table 3.4 Rate of overall mortality, abnormal fish, and normal fish of rainbow trout fed graded levels of camelina oil at day 112 (n=4).

Diet	Camelina oil (%)		
	0	50	100
Mortality[a] (%)	15.8±5.0	22.5±11.3	15.0±3.3
Surviving fish			
Abnormal fish[b] (%)	28.3±5.8	17.5±8.8	23.3±7.2
Normal fish (%)	55.8±8.3	60±16.1	61.7±4.3

Mean±SD

[a] Data based on the period of day 56-112 with 30 fish in each tank.

[b] Abnormal fish refers to fish with symptoms typical of vitamin C deficiency including skin discoloration, scoliosis, lordosis or hemorrhage.

During the 112 days feeding trial, fish grew from 0.105 to 19.1-22.1 g (Table 3.5). Weight gain of fish fed all three diets were similar in all weigh periods except for first and last weigh period 0-14 and 98-112 (Table 3.5). During day 0-14, fish fed 50%CO diet gained significantly more weight and demonstrated better utilization of nutrients with significantly lower FCR and higher PER than fish fed 100%CO diet (Table 3.5 and 3.6). During day 98-112, weight gain of fish fed 50%CO diet was significantly lower than fish

Table 3.5 Weight gain, feed consumption, and feed conversion ratio of rainbow trout fed diet with graded levels of camelina oil (n=4).

Diet	Camelina oil (%)		
	0	50	100
Initial weight (g fish^{-1}) (n=100)		0.105g	
Weight gain (g fish^{-1})			
Day 0-14	0.075±0.005^{ab}	0.078±0.005^{a}	0.067±0.004^{b}
Day 14-28	0.247±0.011	0.235±0.010	0.242±0.016
Day 28-42	1.14±0.04	1.14±0.06	1.09±0.04
Day 42-56	2.34±0.06	2.27±0.30	2.10±0.17
Day 0-56	3.79±0.10	3.72±0.34	3.51±0.10
Day 56-70	3.9±0.3	4.0±0.3	3.9±0.3
Day 70-84	3.8±0.4	4.2±1.2	4.5±0.6
Day 84-98	5.7±0.7	4.2±2.0	5.9±0.4
Day 98-112	4.8±1.1^{a}	2.8±0.9^{b}	4.4±0.3^{a}
Day 0-112	22.0±2.2^{a}	19.1±1.5^{b}	22.1±0.8^{a}
Feed consumption (g fish^{-1})			
Day 0-14	0.032±0.001	0.030±0.003	0.033±0.002
Day 14-28	0.123±0.007	0.134±0.028	0.134±0.005
Day 28-42	0.59±0.02	0.59±0.06	0.63±0.02
Day 42-56	1.62±0.07^{ab}	1.72±0.07^{a}	1.53±0.08^{b}
Day 0-56	2.36±0.08	2.47±0.10	2.33±0.09
Day 56-70	2.5±0.1	2.6±0.3	2.5±0.1
Day 70-84	2.9±0.2^{b}	3.3±0.3^{ab}	3.8±0.3^{a}
Day 84-98	3.9±0.5^{b}	3.8±0.3^{b}	5.0±0.2^{a}
Day 98-112	5.9±0.8	5.2±0.3	5.9±0.4
Day 0-112	18.2±1.2	17.1±0.5	19.6±0.5
Feed conversion ratio			
Day 0-14	0.43±0.03^{ab}	0.39±0.008^{b}	0.49±0.03^{a}
Day 14-28	0.50±0.01^{b}	0.51±0.02^{b}	0.56±0.14^{a}
Day 28-42	0.52±0.00^{b}	0.51±0.03^{b}	0.58±0.01^{a}
Day 42-56	0.69±0.03	0.73±0.03	0.76±0.08
Day 0-56	0.63±0.02	0.67±0.04	0.67±0.02
Day 56-70	0.63±0.03	0.64±0.03	0.65±0.02
Day 70-84	0.78±0.05	0.80±0.14	0.86±0.06
Day 84-98	0.68±0.02^{b}	0.74±0.12^{ab}	0.85±0.04^{a}
Day 98-112	1.24±0.15	1.72±0.47	1.35±0.11
Day 0-112	0.80±0.02	0.85±0.01	0.88±0.03

^{ab} Mean±SD within rows with different superscripts are significant different (P<0.05).

fed control and diets containing 100%CO (Table 3.5). The PER followed a similar pattern; fish receiving 50%CO diet had a significantly lower PER than fish fed control diet (Table 3.6). The feed consumption and FCR were not different among all three treatments ($p>0.05$).

During periods when the weight gain was similar among all three treatments, fish fed control diet showed better nutrient utilization than the CO replacement diets; fish fed 100%CO diet had to consume significantly more feed to gain similar weight to the control group during periods of day 70-84 and 84-98 (Table 3.4); during day 14-28, 28-42, and 84-98; when fed 100%CO diet, fish had significantly poorer FCR than fish fed control and 50%CO diets (Table 3.4); during day 28-42 and 84-98, fish fed control diet had significantly higher PER than fish fed 100%CO (Table 3.6).

The total weight gain during days 0-112 among the fish fed the 50%CO diet was significantly lower than fish fed the control and 100%CO diets (Table 3.5). The PER of fish receiving control diet was significantly higher than fish fed any of the CO diets (Table 3.6). The overall feed consumption and FCR were similar among all three treatments ($p>0.05$) (Table 3.5). On day 112, both VSI and HSI were significantly lower in fish fed 100%CO diet than any other diets (Table 3.6).

Table 3.6 Protein efficiency ratio, visceral-somatic index (VSI), and hepato-somatic index (HSI) of rainbow trout fed graded levels of camelina oil (n=4).

Diet	Camelina oil (%)		
	0	50	100
Protein efficiency ratio			
Day 0-14	4.23±0.33[ab]	4.67±0.22[a]	3.65±0.38[b]
Day 14-28	3.65±0.08	3.27±0.64	3.24±0.14
Day 28-42	3.50±0.03[a]	3.53±0.19[a]	3.17±0.06[b]
Day 42-56	2.64±0.11	2.39±0.24	2.47±0.09
Day 0-56	2.93±0.09	2.73±0.17	2.70±0.07
Day 56-70	2.88±0.12	2.82±0.15	2.76±0.08
Day 70-84	2.33±0.14	2.33±0.46	2.10±0.15
Day 84-98	2.67±0.09[a]	2.48±0.39[ab]	2.12±0.09[b]
Day 98-112	1.48±0.17[a]	1.00±0.37[b]	1.33±0.11[ab]
Day 0-112	2.28±0.05[a]	2.09±0.01[b]	2.03±0.06[b]
VSI (Day 112)	16.66±0.84[b]	16.94±1.07[b]	19.41±0.43[a]
HSI (Day 112)	1.56±0.07[b]	1.62±0.11[b]	2.14±0.08[a]

[ab] Mean±SD within rows with different superscripts are significant different (P<0.05).

3.6.3 Performance of Rainbow Trout Fed Graded Levels of Solvent-Extracted Camelina Meal and Camelina Protein Concentrate

3.6.3.1 Mortality Rate

Throughout the first 56 days, no obvious pathological symptoms were observed in all mortalities during this period. Therefore, the difference in cumulative mortality rate was more likely treatment-related. The cumulative mortality rate of fish fed all diets except for the 12 and 18%CPC diets were less than 5% (p>0.05) (Figure 3.5). The cumulative mortality rate of fish receiving 12 and 18%CPC diets was under 5% until day 30, and then gradually increased to 12.6 and 52.8% on day 56, which was significantly higher than the rest of the treatments (Figure 3.5). The 18%CPC treatment was terminated on day 56 due to high mortality.

From day 56 to 70, the cumulative mortality rate was relatively low across all remaining treatments (<5%; Figure 3.5). From day 70 onwards, fish with symptoms such as skin discoloration, scoliosis, lordosis or hemorrhage, started to occur and the mortality rate started to increase. On day 112, fish fed 18%SECM diet had the highest survival rate of normal fish at 92.5%, which was significantly higher than the rest of the treatments ranged from 55.8 to 65.8% (Table 3.6). Correlatively, the 18%SECM treatment had the lowest rate of mortality (6.7%) and abnormal fish (0.8%) on day 112. Except for the 18%SECM, all treatments had similar rates of mortality (15.8 to 27.5%), abnormal fish (10.0 to 28.3%) and normal fish (55.8 to 65.8%) on day 112 ($p>0.05$) (Table 3.7). The abnormal fish showed symptoms were photographed and are reported in Appendix A.

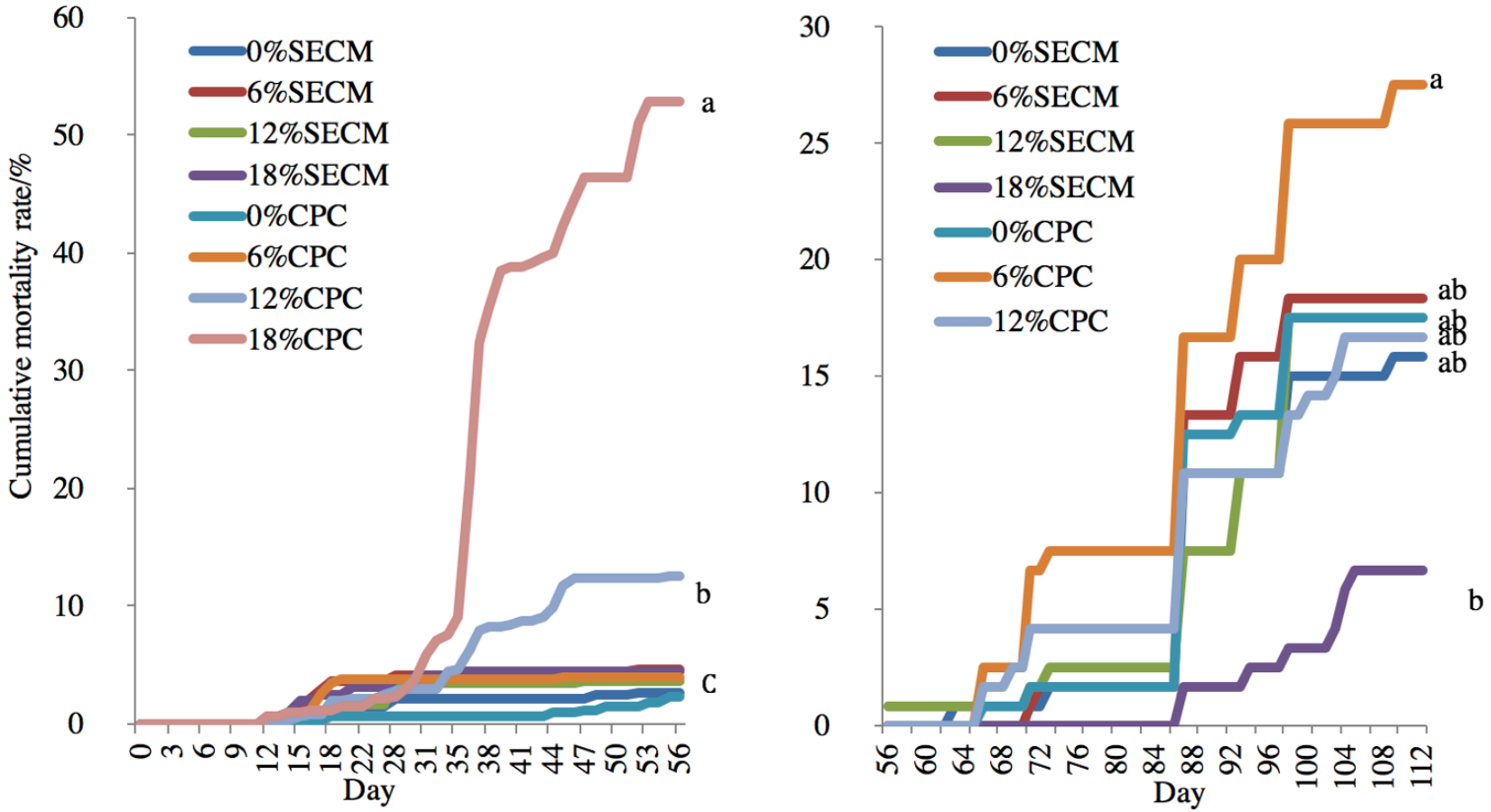

Figure 3.6 The cumulative mortality rate of first feeding rainbow trout fed diets with 0, 6, 12 and 18% of solvent extracted camelina meal or camelina protein concentrate inclusion from day 0-56 and 56-112 (n=4). On day 56, standard deviation for 0, 6, 12 and 18% SECM treatments were 0.5%, 4.1%, 1.9% and 1.5%, respectively; standard deviation for 0, 6, 12 and 18% CPC treatments were 1.6%, 4.7%, 4.3% and 5.0%, respectively.

[a-c] Mean with different superscripts are significantly different ($p<0.05$).

Table 3.7 Rate of overall mortality, abnormal fish, and normal fish of rainbow trout fed graded levels of solvent extracted camelina meal or camelina protein concentrate on day 112 (n=4).

Diet	Solvent extracted camelina meal (%)				Camelina protein concentrate (%)			
	0	6	12	18	0	6	12	18
Mortality (%)	15.8±5.0^{ab}	18.3±8.4^{ab}	17.5±8.8^{ab}	6.7±2.7^{b}	17.5±6.9^{ab}	27.5±6.9^{a}	16.7±4.7^{ab}	N/A
Fish survived								
Abnormal fish (%)	28.3±5.8^{a}	16.7±7.2^{ab}	16.7±11.9^{ab}	0.8±1.7^{b}	23.3±14.7^{a}	10.0±7.7^{ab}	27.5±9.6^{a}	N/A
Normal fish (%)	55.9±8.3^{b}	65.0±8.8^{b}	65.8±9.6^{b}	92.5±3.2^{a}	59.2±13.4^{b}	62.5±9.6^{b}	55.8±8.3^{b}	N/A

^{ab} Mean±SD within rows with different superscripts are significant different ($P<0.05$).

N/A= Data not available as the 18%CPC treatment was terminated on day 56 due to high mortality rate.

Data was based on the period of day 56-112 with 30 fish in each tank.

Abnormal fish refers to fish with symptoms typical of vitamin C deficiency including skin discoloration, scoliosis, lordosis or hemorrhage.

3.6.3.2 Growth Performance

Lower inclusion level of either SECM or CPC at 6% had no negative effects on the growth performance of rainbow trout. Fish fed 6%SECM and control diets had similar growth performance during days 0-14, 14-28, 28-42, 42-56 or the overall performance during day 0-56, based on weight gain, feed consumption, FCR and PER ($p>0.05$) (Table 3.8 and 3.9). Fish receiving 6%CPC diet had similar weight gain, feed consumption, FCR and PER to control diet-fed fish during days 0-14, 14-28, 28-42 ($p>0.05$) (Table 3.7 and 3.8). During day 42 to 56, the 6%CPC diet encouraged feed consumption and subsequently weight gain of rainbow trout during day 42-56 and 0-56 ($p<0.05$), although the FCR and PER were similar among these two treatments ($p>0.05$) (Table 3.8 and 3.9). However, beyond the threshold of the 6%, the growth performance of rainbow trout decreased significantly with increased dietary inclusion levels of either SECM or CPC. Fish offered 12 and 18%SECM diets exhibited significantly lower weight gain and feed consumption compared to the control group during day 14-28, 28-42 and 42-56 and over the entire period up to day 56 (Table 3.8). The 12%CPC diet fed fish also consumed less feed and gained less weight compared to fish fed the control diet, but the difference being significant from day 28-42 and 42-56 ($p<0.05$). The FCR and PER of fish fed 18%SECM diet were significantly higher than the control, 6 and 12%SECM or 12%CPC during day 14-28, however, was not significant during days 28-42, 42-56 or 0-56 (Table 3.9). Among all treatments, the 18%CPC treatment resulted in the poorest growth performance with lowest weight gain, feed consumption, and highest FCR and PER during day 14-28, 28-42, 42-56 and the entire period of day 0-56 ($p<0.05$) (Table 3.8 and 3.9).

Table 3.8 Weight gain and feed consumption of rainbow trout fed graded levels of solvent extracted camelina meal or camelina protein concentrate (n=4).

Diet	Solvent extracted camelina meal (%)				Camelina protein concentrate (%)			
	0	6	12	18	0	6	12	18
Initial body weight (g fish^{-1}) (n=100)	0.105							
Weight gain (g fish^{-1})								
Day 0-14	0.075±0.005^{ab}	0.072±0.011^{ab}	0.075±0.000^{ab}	0.068±0.008^{ab}	0.077±0.008^{a}	0.072±0.004^{ab}	0.071±0.012^{ab}	0.058±0.003^{b}
Day 14-28	0.247±0.011^{ab}	0.227±0.028^{abc}	0.195±0.005^{c}	0.119±0.010^{d}	0.258±0.014^{a}	0.258±0.009^{a}	0.212±0.018^{bc}	0.150±0.013^{d}
Day 28-42	1.14±0.04^{ab}	1.08±0.08^{ab}	0.85±0.08^{c}	0.65±0.02^{d}	1.22±0.06^{ab}	1.25±0.03^{a}	1.04±0.05^{bc}	0.57±0.14^{d}
Day 42-56	2.34±0.06^{b}	2.39±0.03^{ab}	2.00±0.13^{c}	1.52±0.05^{d}	2.32±0.07^{b}	2.59±0.06^{a}	1.99±0.13^{c}	0.72±0.19^{e}
Day 0-56	3.79±0.10^{b}	3.78±0.12^{b}	3.13±0.10^{c}	2.36±0.08^{d}	3.87±0.03^{b}	4.17±0.06^{a}	3.31±0.11^{c}	1.50±0.15^{e}
Day 56-70	3.9±0.3^{b}	4.4±0.3^{ab}	3.9±0.5^{b}	3.6±0.3^{b}	3.5±0.4^{b}	5.3±0.4^{a}	3.9±0.5^{b}	*
Day 70-84	3.8±0.4	4.0±0.4	3.8±0.9	5.0±0.4	3.7±1.4	4.9±0.9	3.0±0.8	*
Day 84-98	5.7±0.7^{b}	5.5±0.7^{b}	6.6±0.8^{b}	10.6±1.5^{a}	5.2±1.4^{b}	5.5±0.7^{b}	4.7±0.8^{b}	*
Day 98-112	4.8±1.1^{bc}	4.9±1.4^{bc}	6.3±0.5^{b}	11.5±1.2^{a}	4.7±1.0^{bc}	5.3±0.7^{bc}	3.7±1.4^{c}	*
Day 0-112	22.0±2.2^{bcd}	22.7±1.4^{bcd}	23.7±1.1^{bc}	33.1±2.9^{a}	21.0±1.2^{cd}	25.2±1.0^{b}	18.6±1.0^{d}	*
Feed consumption (g fish^{-1})								
Day 0-14	0.032±0.001	0.030±0.003	0.031±0.002	0.034±0.003	0.034±0.002	0.032±0.002	0.034±0.001	0.030±0.002
Day 14-28	0.123±0.007^{ab}	0.123±0.004^{ab}	0.115±0.003^{d}	0.116±0.007^{c}	0.132±0.010^{ab}	0.129±0.005^{a}	0.123±0.010^{b}	0.110±0.003^{cd}
Day 28-42	0.59±0.02^{ab}	0.59±0.03^{ab}	0.47±0.04^{c}	0.34±0.01^{d}	0.63±0.01^{ab}	0.67±0.03^{a}	0.57±0.04^{b}	0.42±0.07^{cd}
Day 42-56	1.62±0.07^{b}	1.64±0.11^{b}	1.37±0.03^{c}	1.05±0.00^{d}	1.64±0.04^{b}	1.89±0.09^{a}	1.39±0.14^{c}	0.71±0.07^{e}
Day 0-56	2.36±0.08^{b}	2.38±0.13^{b}	1.98±0.04^{c}	1.54±0.01^{d}	2.44±0.04^{b}	2.72±0.11^{a}	2.11±0.17^{c}	1.27±0.11^{e}
Day 56-70	2.5±0.1^{bc}	2.9±0.3^{b}	2.4±0.1^{c}	2.2±0.1^{c}	2.3±0.2^{c}	3.4±0.1^{a}	2.3±0.3^{c}	*
Day 70-84	2.9±0.2^{c}	3.4±0.2^{abc}	3.1±0.3^{bc}	3.9±0.3^{ab}	2.8±0.1^{c}	4.3±0.8^{a}	2.6±0.3^{c}	*
Day 84-98	3.9±0.5^{bc}	4.6±0.5^{b}	4.6±0.4^{b}	7.7±0.9^{a}	3.8±0.1^{bc}	4.7±1.0^{b}	3.1±0.3^{c}	*
Day 98-112	5.9±0.8^{bc}	6.0±1.1^{bc}	6.6±0.3^{bc}	11.6±1.4^{a}	5.5±0.4b^{c}	7.1±0.9^{b}	4.5±0.6^{c}	*
Day 0-112	18.2±1.2^{c}	19.3±1.3^{bc}	18.7±0.4^{c}	27.0±2.5^{a}	16.7±0.2^{cd}	22.2±1.2^{b}	14.6±0.6^{d}	*

$^{a-e}$ Mean±SD within rows with different superscripts are significant different (P<0.05).

* Treatment of 18% camelina protein concentrate inclusion was terminated at day 56 due to high mortality rate.

Table 3.9 Feed conversion ratio, protein efficiency ratio, visceral-somatic index (VSI) and hepato-somatic index (HSI) of rainbow trout fed graded levels solvent extracted camelina meal or camelina protein concentrate (n=4).

Diet	Solvent extracted camelina meal (%)				Camelina protein concentrate (%)			
	0	6	12	18	0	6	12	18
Feed conversion ratio								
Day 0-14	0.43±0.03	0.43±0.09	0.42±0.02	0.50±0.10	0.45±0.02	0.44±0.03	0.49±0.07	0.53±0.04
Day 14-28	0.50±0.01[c]	0.55±0.05[c]	0.59±0.02[c]	0.98±0.09[a]	0.51±0.06[c]	0.50±0.03[c]	0.58±0.01[c]	0.73±0.07[b]
Day 28-42	0.52±0.00[b]	0.54±0.02[b]	0.55±0.01[b]	0.53±0.02[b]	0.52±0.02[b]	0.54±0.01[b]	0.55±0.02[b]	0.72±0.05[a]
Day 42-56	0.69±0.03[b]	0.69±0.04[b]	0.68±0.04[b]	0.69±0.02[b]	0.71±0.01[b]	0.73±0.04[b]	0.70±0.05[b]	1.14±0.17[a]
Day 0-56	0.63±0.02[b]	0.63±0.02[b]	0.63±0.02[b]	0.65±0.02[b]	0.63±0.01[b]	0.65±0.02[b]	0.064±0.03[b]	0.85±0.08[a]
Day 56-70	0.63±0.03	0.61±0.02	0.57±0.03	0.60±0.04	0.67±0.09	0.64±0.04	0.59±0.07	*
Day 70-84	0.78±0.05	0.85±0.04	0.76±0.02	0.78±0.02	0.66±0.12	0.88±0.08	0.80±0.05	*
Day 84-98	0.68±0.02	0.85±0.13	0.70±0.04	0.73±0.04	0.82±0.15	0.74±0.14	0.66±0.08	*
Day 98-112	1.24±0.15	1.04±0.19	1.04±0.08	1.01±0.08	1.20±0.20	1.27±0.06	1.17±0.22	*
Day 0-112	0.80±0.02	0.85±0.08	0.79±0.02	0.81±0.03	0.80±0.04	0.88±0.07	0.78±0.02	*
Protein efficiency ratio								
Day 0-14	4.23±0.33	4.42±0.92	4.41±0.22	3.76±0.68	4.10±0.20	4.16±0.31	3.87±0.66	3.53±0.25
Day 14-28	3.65±0.08[a]	3.40±0.31[ab]	3.13±0.11[b]	1.90±0.17[d]	3.58±0.38[ab]	3.67±0.19[a]	3.17±0.08[ab]	2.53±0.26[c]
Day 28-42	3.50±0.03[a]	3.39±0.12[a]	3.34±0.08[a]	3.47±0.10[a]	3.52±0.14[a]	3.43±0.09[a]	3.38±0.11[a]	2.47±0.28[b]
Day 42-56	2.64±0.11[a]	2.70±0.15[a]	2.70±0.15[a]	2.68±0.08[a]	2.57±0.04[a]	2.52±0.13[ab]	2.65±0.18[a]	1.85±0.47[b]
Day 0-56	2.93±0.09[a]	2.93±0.07[a]	2.90±0.09[a]	2.82±0.09[a]	2.89±0.04[a]	2.82±0.10[a]	2.90±0.15[a]	2.17±0.19[b]
Day 56-70	2.88±0.12	2.84±0.37	2.99±0.48	3.10±0.22	2.75±0.33	2.86±0.18	3.13±0.39	*
Day 70-84	2.33±0.14[ab]	2.18±0.11[b]	2.43±0.07[ab]	2.37±0.06[ab]	2.82±0.47[a]	2.14±0.19[b]	2.14±0.35[b]	*
Day 84-98	2.67±0.09	2.21±0.30	2.63±0.14	2.52±0.15	2.58±0.73	2.29±0.72	2.81±0.37	*
Day 98-112	1.48±0.17	1.57±0.57	1.77±0.13	1.84±0.16	1.56±0.27	1.37±0.15	1.49±0.36	*
Day 0-112	2.28±0.05	2.17±0.15	2.34±0.10	2.27±0.09	2.29±0.12	2.09±0.16	2.36±0.07	*
VSI (Day 112)	16.66±0.8	16.74±1.74	16.44±0.93	15.42±1.81	15.84±1.48	15.80±0.17	18.29±1.65	*
HSI (Day 112)	1.56±0.07	1.85±0.30	1.63±0.18	1.72±0.12	1.57±0.09	1.50±0.06	1.81±0.09	*

[a-d] Mean±SD within rows with different superscripts refer to significant differences (P<0.05).

* Treatment with 18% camelina protein concentrate inclusion was terminated at day 56 due to high mortality rate.

Between day 56 and 70, all previous differences in growth performance across all treatments disappeared except for fish fed 6%CPC diet. The 6%CPC treatment showed better growth with significantly higher weight gain, feed consumption compared to the rest of the treatments (Table 3.8 and 3.9). During day 70-84, even the 6%CPC treatment lost the advantage in growth and no difference was evident in weight gain across all treatments ($p>0.05$) (Table 3.8 and 3.9). During day 84-98 and 98-112, fish fed 18%SECM diet consumed more feed and gain more weight compared to any other treatments ($p<0.05$) (Table 3.8). All other treatments had similar weight gain and feed consumption ($p>0.05$) (Table 3.8). The FCR and PER were similar among all treatments during 84-98 and 98-112 ($p>0.05$) (Table 3.9).

Over the 112 days, weight gain and feed consumption were significantly higher in 18%SECM diet fed fish, followed by the 6%CPC diet fed fish ($p<0.05$) (Table 3.8). The rest of the treatments had similar growth performance in terms of weight gain and feed consumption when compared to each other. The overall FCR and PER were not affected by treatments ($p>0.05$) (Table 3.9). On day 112, the VSI and HSI were not different in all treatments ($p>0.05$) (Table 3.9).

3.7 Discussion

3.7.1 Diets

The current trial was the first to explore the use of CO, SECM, and CPC, in the diets of first feeding rainbow trout. This is also the first trial using CPC in animal feed. However, the trial was possibly compromised by oxidation of diets and vitamin C deficiency, even

though all diets were carefully stored in vacuum-sealed bags, frozen at -20 ºC and kept in the freezer until use. It should be noted that the analysis of oxidation tests and vitamin C content of the diets were done about a month and six months, respectively, after preparation and does not represent the time frame when the fish were fed. The exact onset point of oxidation or vitamin C loss of the diets cannot be determined.

Four diets including the control (0%CO/SECM/CPC), 50%CO, 18%SECM, and 18%CPC were sent for precautionary oxidation test, even though no diet smelled rancid when sent for analysis. Peroxides are the primary oxidation products. When subject to heat or in the presence of metals, peroxides decompose to alkoxy radicals and then produce aldehydes which cause a rancid odor. Aldehydes can be measured by p-anisidine test (Choe and Min, 2006). The p-anisidine test is usually paired with the peroxide test to describe the oxidation status of a product. No established industry standard was found for the peroxide and p-anisidine in fish feed. A salmonid diet with peroxide value of 11 mEq kg^{-1} is considered un-oxidized and diets with peroxide range from 43-62 are termed moderately oxidized (Hamre et al., 2001). The p-anisidine value was not determined in these diets. Herring oil with 20.4 mEq kg^{-1} peroxide and 12.6 p-anisidine, respectively, was considered fresh and these values in an oxidized herring oil were 33.2-183 mEq kg^{-1} and 28.5-51.1, respectively (Kubiriza et al., 2017). In another trial, a fresh fish oil had 11.5 mEq kg^{-1} peroxide and 4.3 p-anisidine. An oxidized fish oil had peroxide of 132 mEq kg^{-1} and p-anisidine of 15.1 (Chen et al., 2012). Samples from four of our diets including control (0%CO/SECM/CPC), 50%CO, 18%SECM, and18%CPC diet analyzed about a month after being made contained peroxide of 9.1, 14.6, 11.2 and 108 mEq kg^{-1},

and p-anisidine of 43.6, 74, 58.1 and 277, respectively. Diets including control, 18%SECM and 18%CPC might be termed as slightly oxidized and the 50%CO diet was moderately to highly oxidized. Both the 50 and 100%CO diets were re-made immediately after the analyses were received.

The oxidation of the 50 and 100% CO diets was not due to CO. A 20-hour AOM peroxide test indicates the oxidative stability of an oil. The industry standard for edible vegetable oils in terms of initial peroxide value is 10-15 mEq kg^{-1} (Joint Food and Agriculture Organization of the United Nations and World Health Organization CODEX Alimentarius Commission, 2001). A commercial food-grade soybean oil has an initial peroxide and 20-hour AOM peroxide of 3.8 and 340 mEq kg^{-1} (Pesti et al., 2002). In our case, CO had an initial peroxide of 3.7 mEq kg^{-1} and 20-hour AOM peroxide of 37.9 mEq kg^{-1}, which was comparable with the industry standard and superior to the commercial soybean oil. As the stability test of CO was conducted about twelve months after the feed was prepared, it was safe to assume CO was not oxidized at the time when used for diet preparation. The oxidation of the 50 and 100%CO diets was probably during diet preparation or during storage.

Because of high mortality rate and symptoms including skin discoloration (darkened skin), scoliosis, lordosis or hemorrhage of fish on almost all treatments started around day 56, fish were examined by a veterinarian. These symptoms were consistent with rainbow trout fed vitamin C deficient diets (Teskeredžić et al. 1989; Tacon, 1992; Frischknecht et al., 1994; Falahatkar et al., 2011). Sample from three diets (0%CO/SECM/CPC,

18%SECM and 6%CPC, vacuum-sealed and frozen) were sent for vitamin C analysis for further confirmation. The deficiency symptoms were observed in older rainbow trout (7 g) during week 18-23, and trout fry (1 g) during week 16-20 (Frischknecht et al., 1994). These symptoms were discerned even earlier in first feeding trout (0.105 g) since week 10 (day 56) in the current study. Spinal deformation was not observed in the older trout (7 g) fed vitamin C deficient diet. It seems that the effects of vitamin C deficiency were more pronounced on younger fish and took less time to manifest itself.

Vitamin C is critical for fish to maintain normal health and growth. It acts as an antioxidant against lipid oxidation, enhances the immune system, disease and stress resistance (Oliva-Teles, 2012). Evidence also suggested that vitamin C can regenerate vitamin E to its functional form (Oliva-Teles, 2012). Rainbow trout is one of many teleost species that does not possess the ability to synthesize ascorbic acid endogenously. This is due to the absence of the L-gulono-lactone oxidase gene that codes the enzyme to finish the biosynthesis cycle of vitamin C (Drouin et al., 2011). Hence, rainbow trout have a dietary requirement for this indispensable micronutrient at a level of >40 mg kg^{-1} diet (NRC, 2011). In the current study, vitamin C (mono/polyphosphate) was added to each diet at a concentration of 200 mg kg^{-1}, but the vitamin C content in all three samples analyzed was under the reportable limit (L-ascorbyl-2-phosphate >5 mg kg^{-1}) six months after the feed was made. Apparently, most of this water-soluble vitamin was oxidized and lost during either feed preparation or storage.

Vitamin C is water soluble and unstable during the processing of diets or storage of feed. Environmental factors such as pH, temperature, moisture, light, and presence of enzymes, oxygen, and metallic catalyzers can lead to vitamin C degradation or leaching (Santos and Silva, 2008; Oliva-Teles, 2012). Vitamin C in ascorbyl monophosphate or ascorbyl polyphosphate form are more stable when subject to heat or during storage. During feed manufacture process, commercial extrusion (80-82 ºC in preconditioner and 167-206 ºC of calculated product temperature behind die) generates heat which can denature vitamin C resulting in retention as low as 5% in other forms of vitamin C while the retention of L-ascorbyl-2-monophosphate could be over 90% (Riaz et al., 2009; Anderson and Sunderland, 2002). Storing fish feed at -20 ºC resulted in 87% of retention of vitamin C in the form of L-ascorbyl-2-monophosphate after 6 months, while vitamin C in its sodium salt form had a retention rate at 7% (Soliman et al., 1987).

In the current study, vitamin C in the form of mono-polyphosphate, was added to each diet at 200 mg/kg. Since mono-polyphosphate is a more stable form of vitamin C, loss of the added vitamin C was unlikely due to heat or storage condition. Temperature of the warm water added to the mixture of the raw ingredient before hand-pelleting was 50-60 ºC. The temperature range did not exceed the temperature used in the commercial extrusion process. All feed were vacuum sealed into small packages and stored in -20 ºC until being used. Feed, in feed cups, was only exposed to room temperature during feeding, and otherwise remained in a -20 ºC freezer. Therefore, vitamin C losses in these diets were less likely to be attributed to heating or storage conditions. It should be noted that a substantial amount of warm water (30%) was added to the mixture of raw

ingredients before hand-pelleting. However, whether this was a factor that contributed to vitamin C loss and subsequently oxidation of the diets was unknown.

3.7.2 Mortality

Rainbow trout seemed to be less impacted during the period of day 0-56 than day 56-112 based on the mortality rate. On day 56, except for fish fed 18%CPC diet, the cumulative mortality rates of trout fed all diets including CO, SECM and CPC were under 13%. It is comparable to the result of Ramzanzadeh et al. (2016), where the cumulative mortality rate of rainbow trout was under 18% after 44 days since first feeding. Fish fed 18%CPC diet had an outstanding high mortality rate at 52.8% on day 56. No pathological symptoms were observed in these fish. The cause of high mortality was unclear; however, it could be associated with oxidative diet or the high inclusion rate of CPC. During day 56-112, rainbow trout grew from 2.37 g to at least 18.6 g and the cumulative mortality ranged from 15.0 to 27.5% for trout fed all diets except 18%SECM. The cumulative mortality rates were much higher compared to a previous study in which rainbow trout with initial body weight of 1.2 g had mortality rate under 5% (Haghbayan and Mehrgan, 2015).

3.7.3 Growth Performance

By day 84, up to 100% fish oil can be replaced by CO in the diet of rainbow trout. This is in agreement with previous studies (Ye et al., 2016; Hixson et al., 2014c). However, during the 14 days of the trial, fish fed 50%CO diet showed significantly lower growth compare to those fed control and 100%CO diets. Since fish appeared to be severely

affected by vitamin C deficiency in the last few weeks of the trial, whether the cause of the poor growth was a result of treatment effects or the oxidized diets/deficiency was not clear.

Growth of fish fed both SECM and CPC diets during day 0-56 followed the typical pattern of plant protein inclusion in salmonid feeds; low or moderate dietary inclusion level did not have negative impact on their performance while incorporating high levels of plant protein in salmonid diets compromised their growth (Hua and Bureau, 2012). In our case, both SECM and CPC at a low inclusion rate of 6% did not compromise the growth performance of the fish. In fact, the 6%CPC diet even encouraged feed consumption and subsequently weight gain during day 42-56 ($p<0.05$). However, both the 12 and 18% dietary inclusion of SECM and CPC depressed the weight gain of rainbow trout. The possible causes seem to differ. The significant reduction in weight gain of fish fed 12 and 18%SECM was simply correlated to the decreased feed consumption, suggesting palatability issue of SECM. Fish feed 12%CPC diet had significantly lower feed intake while those fed 18%CPC diet showed significantly lower feed intake, FCR and PER, revealing both palatability issue and lower nutrient utilization of CPC.

The same palatability issue of SECM in rainbow trout diets has been reported by Bullerwell et al. (2016). Rainbow trout fed increased levels of SECM demonstrated decreased feed intake, although this negative linear relationship was not reflected in inclusion levels of SECM and weight gain (Bullerwell et al. 2016). It is not surprising that SECM and CPC caused lower feed intake in our study, as camelina is a member of

brassica family that is characterized by their bitter taste. The bitter taste is a result of glucosinolates and their degraded products isothiocyanates. The three glucosinolates present in camelina are 9-methyl-sulfinyl-nonyl (glucoarabin), 10-methyl-sulfinyl-decyl (glucocamelinin) and a trace of 11-methyl-sulfinyl-undecyl glucosinolates (Schuster and Friedt, 1998). The bitterness of these three glucosinolates have not yet been defined. They are not on the established list of glucosinolates known for bitterness including sinigrin, progoitrin, glucobrassicin and neoglucobrassicin (Wieczorek et al., 2017). It is possible that glucosinolates in these camelina products also have the bitter taste and led to reduced feed intake and subsequently decreased weight gain of trout when fed diets containing high levels of either SECM or CPC in our study. The other possible explanation for the less palatable diets could be the degradation of glucosinolates and formation of isothiocyanates during either preparation of the ingredients (both SECM and CPC) or feeds, as isothiocyanates were reported to contribute more to the bitterness than glucosinolates themselves (Wieczorek et al., 2017). We analyzed the total glucosinolates contents in 6, 12, and 18% of both SECM and CPC diets, respectively (0.6, 2.0, 2.7, 0.5, 0.7 and 1.1 μmol g^{-1}, respectively). However, the isothiocyanates were not determined in these diets. It is unclear whether the depressed feed intake when fed high levels of both SECM and CPC of trout was a result of glucosinolates, isothiocyanates or both. Profiling of both glucosinolates and their degradation products in the diets is recommended in future studies. Dietary inclusion of both 12 and 18% of CPC resulted in the significantly higher FCR and lower PER, indicating difficulties in nutrient utilization in these diets. Although all diets were formulated to be isonitrogenous and isocaloric, it is possible that

certain amino acids in CPC were less digestible and resulted in poorer feed efficiency and lower PER. No literature has been reported to support or refute this hypothesis.

From day 56 to 112, typical pattern where growth performance was negatively affected by the dietary inclusion of either SECM or CPC at high levels disappeared. Weight gain of fish fed 12%SECM, 18%SECM and 12%CPC diets started to catch up with those fed control diets during day 56-70. Eventually, during day 70-84, growth performance became similar among all treatments ($p>0.05$), and remained similar throughout the trial except for fish fed 18%SECM diet. Rainbow trout fed 18%SECM showed best growth performance among all treatment during the last 56 days, which was unexpected. This might have been a result of the interference from vitamin C deficiency rather than treatment effects. Certain components in 18%SECM diet canceled off the negative effect of vitamin C deficiency/oxidized diets, and these components were missing or not sufficient in low-SECM inclusion diets, CPC diets or the CO diets. Although glucosinolates can be anti-nutritional factors in animal feed as discussed, evidence has showed that some glucosinolates including gluconapin, epiprogoitrin, glucoraphanin, sinalbin, and glucobrassicin possess an antioxidant capacity (Natella et al., 2014). Their breakdown products isothiocyanates can also act as antioxidants (Valgimigli and Lori, 2009). It is possible that the 18%SECM diet might contain higher levels of glucosinolates which acted as antioxidants and resulted in less oxidized diets and better performance of the fish. Future studies should be devoted to investigating the potential antioxidant property of SECM.

3.8 Conclusions

Up to 100% fish oil can be replaced with CO with no adverse effects on the growth of first feeding rainbow trout. In the first 56 days, up to 6% of either SECM or CPC can be included in the diet of first feeding rainbow trout without affecting their growth performance. Higher inclusion levels of both SECM and CPC at either 12 or 18% resulted in lower weight gain and feed intake during day 0-56, possibly related to the anti-nutritional factors of SECM and CPC such as glucosinolates and their breakdown products. Further investigation is recommended in future studies. The 18%CPC diet led to poor growth and nutrient utilization and over 50% of mortality rate on day 56. On day 112, among all meal treatments, fish fed 18%SECM diet consumed more feed, gained more weight, and had a lower mortality rate ($p<0.05$). This is the first study using CPC in animal feed, the process of preparing CPC should be optimized in the future to increase its protein content and yield.

Chapter 4

Use of Oil and Solvent Extracted Meal from *Camelina sativa* L. Crantz for Atlantic Salmon *Salmo salar* Fry

4.1 Abstract

Camelina sativa seed products, including solvent-extracted camelina meal (SECM), and camelina oil (CO), are potentially sustainable feed ingredients for farmed salmonids. The meal has a well-balanced amino acid profile and the oil has a unique fatty acid profile with 36-39% α-linolenic acid. Atlantic salmon fry (1.1 g), were fed one of the six diets with either 50 or 100% replacement of fish oil with CO, or 0, 6, 12, or 18% dietary inclusion of SECM, for 112 days to evaluate the suitability of these novel ingredients. For oil replacement treatments, fish fed 100% CO diet had significantly higher overall weight gain than fish fed the control and 50% CO diets. Overall feed consumption exhibited a similar trend that fish fed 100% CO diet consumed more feed than fish fed 50% CO diet ($p<0.05$). Feed conversion ratio (FCR), protein efficiency ratio (PER), visceral somatic index (VSI), hepato-somatic index (HSI) and carcass composition were similar among the dietary treatments after 112 days ($p<0.05$). For meal inclusion, salmon fed 18% SECM diet gained significantly lower weight than fish fed 6 and 12% SECM diets. Overall feed consumption, FCR, PER, VSI, HSI and carcass composition were similar among treatments after 112 days ($p>0.05$).

4.2 Introduction

Success has been achieved in searching and implementing alternative feed ingredients that are more available, sustainable and cost-effective to relieve the dependency on fish oil and fishmeal in salmonid diets. Currently, terrestrial plant ingredients such as canola oil, palm oil, soybean protein concentrate, wheat gluten, pea protein, corn protein concentrate, canola meal and protein concentrate are widely used in salmonid diets (Ytrestøyl et al., 2015). Fish oil and fishmeal are mostly being used more strategically at minimal levels to ensure the dietary requirements of salmonid fish for essential nutrients such as n-3 HUFAs are met. Although the growth of the fish was not affected in most cases, the quality of salmon fillets based on fatty acid profile has consequently declined. The n-3/n-6 ratio of farmed Atlantic salmon fillet reduced by half, dropped from 6:1 to

2.9:1 during 1987-2012 (van Vliet and Katan, 1990; Strobel et al., 2012). Similarly, the EPA+DHA content has dropped dramatically from 2.75 to 1.36 g per 100 g fillet over 2006-2015 and is predicted to continue to decline to decline (Sprague et al., 2016). Most plant ingredients are poor sources of n-3 fatty acids, and entirely devoid of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (NRC, 2011). The changes in fillet composition is a reflection of the dietary composition changes due to use of these plant ingredients without compensatory adjustment from other sources. Thus, the challenge of aquaculture feed manufacturers has transitioned from using alternative feed ingredients without compromising the growth performance of the fish to maintaining the nutritional value and subsequently commercial value of the fish.

Unlike most plants oil, oil extracted from camelina seeds is rich in alpha-linolenic acid (ALA; 36.2-39.4% of the total fatty acids). The ALA is an essential n-3 fatty acid required in salmonid diets. The biosynthesis *de novo* of EPA and DHA from the dietary supplement of ALA was observed in Atlantic salmon and rainbow trout (Collins et al., 2011; Lazzarotto et al., 2015; Hixson et al., 2014a and b). Therefore, camelina oil (CO) is an appealing lipid source in salmonid diets to provide the n-3/n-6 fatty acid ratio and EPA and DHA levels in fish fillets. CO has been successfully used in the diets of salmonid fish such as Atlantic salmon, rainbow trout and Atlantic cod in replacement of fish oil (Hixson et al., 2013, 2014a and 2014b; Bullerwell et al., 2016; Morais et al., 2012). Up to 100% replacement of fish oil with CO did not result in reduction in growth of salmon at parr and smolt stages (Ye et al., 2016; Hixson et al., 2014b). However, fish fed 100%CO diet had to consume significantly more feed to gain similar weight to fish fed control diet

(Hixson et al., 2014b). Although the n-3/n-6 fatty acid ratio in the white muscle was halved due to the replacement of fish oil with CO, the EPA+DHA level in the white muscle was not altered by the different treatments (Hixson et al., 2014b). The current study will employ salmon fry as the target to extend the understanding of the effects of using CO in the diet of salmon during the full life cycle.

The residual meal resulting from oil extraction are often used as the protein source in feed to maximize the value of the oilseed. The current study will simulate the common industry practice and assess the potential of using solvent-extracted camelina meal (SECM) in salmon fry diet. SECM has a crude protein content of 35.6% (as fed basis) and its protein is highly digestible at 88% by salmon (Fraser et al., 2017). This is comparable to some common plant meals used in salmonid diets such as canola meal (38.0% crude protein as fed basis) with an apparent digestibility coefficient of 79% for protein (NRC, 2011). The amino acids profile of SECM was balanced and in a proportion similar to the fishmeal or the requirements of salmon (Fraser et al., 2017; NRC, 2011). Previous studies have demonstrated the possibility of including camelina protein such as SECM or the high oil residue meal in the diet of salmon at later life stage including parr and smolt. However, only up to 8-10% of the inclusion of either the SECM or the high oil residue meal (HORM) was recommended. When included above these limits, growth reduction and enteritis was observed in salmon (Ye et al., 2016; Hixson et al., 2015b; Ye, 2014).

4.3 Objectives

Feeding Atlantic salmon fry with solvent extracted camelina meal or camelina oil-containing diets investigated the following objectives:

1. To evaluate the acceptability of CO and SECM for salmon fry based on their growth performance.

2. To identify the optimal dietary inclusion rate of these ingredients based on growth performance, and carcass composition of salmon fry.

4.4 Null Hypotheses

1. Replacing 0 (control), 50 or 100% fish oil with CO will result in different growth performance of salmon fry. The proximate body composition will be altered by the level of CO in the diets.

2. Incorporating 0 (control), 6, 12 or 18% SECM in the diet of salmon fry will result in different growth performance, and proximate body compositions.

4.5 Materials and Methods

4.5.1. Experimental Ingredients and Diets

Both CO and SECM used in this study were from the same batches as described in Chapter 3, Section 3.5.1.1. All diets were formulated to be isonitrogenous (50% crude protein) and isocaloric (4600 kcal digestible energy kg^{-1} diet) to meet the nutritional requirements of Atlantic salmon (NRC, 2011) (Table 4.1). All diets were prepared at the Chute Animal Nutrition Centre, Dalhousie University, Faculty of Agriculture, Truro, Nova Scotia, Canada. For each diet, all ingredients were homogenized in a Hobart mixer

(Hobart Corporation Model L-800; USA) and steam-pelleted through a pellet mill (California Pellet Mill Co., USA) fitted with a 3 mm die. Pelleted feed was then dried at about 55ºC for 4-5 hours in a forced air oven. Once cooled down to room temperature, feed was vacuum packed into individual plastic bags (approximate 500 g per bag), sealed using a food sealer and stored in -20 ºC freezer until use. Feed was hand-crumbled and sifted into different sizes including 0.7-0.85, 0.85-1.0, 1.0-1.4, 1.4-1.7, and 1.7-2.0 mm using standard test sieves (Model SL-GT-093, Sun Labtek Equipments PVT. LTD). According to the feeding behavior of the fish, optimal size of feed particles was provided. Representative samples of the diets were stored in sealed bags and froze until subsequent nutrient analysis. Samples of the diets were sent to Nova Scotia Agriculture & Food Operations Analytical Lab in Harlow Institute, Truro, Nova Scotia for major nutrients analysis (Table 4.2). The methodology used were the same as described in Chapter 3. The amino acid profiles of the diets were analyzed by the Experimental Station's Chemical Laboratories, University of Missouri, USA, following AOAC Official Method 982.30E (a, b, c, 2006; Table 4.2).

Table 4.1 Ingredients and analyzed nutrient composition of the six experimental diets fed to Atlantic salmon fry (as fed basis).

Diets	Control	Camelina oil		Solvent extracted camelina meal		
		50%	100%	6%	12%	18%
Ingredients (%)						
Fishmeal	47.7	47.7	47.7	44.9	42.1	39.2
Fish oil	12.3	6.15	0	12.8	13.2	13.8
Solvent-extracted camelina meal	0	0	0	6	12	18
Camelina oil	0	6.15	12.3	0	0	0
Wheat	11.5	11.5	11.5	7.8	4.2	0.5
CPSP-G[a]	7	7	7	7	7	7
Wheat gluten meal	5	5	5	5	5	5
Poultry byproduct meal	5	5	5	5	5	5
Blood meal	5	5	5	5	5	5
Pre-gelatinized starch	3	3	3	3	3	3
Soy lecithin	2	2	2	2	2	2
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mineral premix[b]	0.25	0.25	0.25	0.25	0.25	0.25
Special premix[c]	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100
Analyzed nutrient composition[d]						
Dry matter (%)	91.9	95.3	95.0	93.4	93.1	94.5
Crude protein (%)	50.8	50.8	50.1	51.5	50.8	49.3
Crude fat (%)	18.5	19.5	22.0	18.8	19.6	19.2
Ash (%)	10.0	10.3	10.0	9.9	9.6	9.1
Calcium (%)	2.7	2.7	2.8	2.7	2.5	2.4
Potassium (%)	0.62	0.64	0.62	0.69	0.79	0.86
Magnesium (%)	0.15	0.16	0.16	0.18	0.19	0.22
Phosphorus (%)	1.8	1.8	1.8	1.8	1.8	1.8
Sodium (%)	0.54	0.55	0.55	0.54	0.52	0.51
Copper (mg kg^{-1})	6.0	8.2	6.9	6.6	7.5	8.2
Manganese (mg kg^{-1})	164	161	168	169	159	175
Zinc (mg kg^{-1})	175	183	176	175	181	182

[a] CPSP-G is soluble fish protein concentrate. Manufacturer, Sopropeche. Wimille, France.

[b] Vitamin mineral premix (kg^{-1} diet): zinc, 96.9 mg; manganese, 156 mg; iron, 105 mg; copper, 3.1 mg; iodine, 9.4 mg; vitamin A, 6250 IU; vitamin D, 5000 IU; vitamin K, 2.5mg; vitamin B_{12}, 0.005mg; thiamin, 10mg; riboflavin, 22.5 mg; pantothenic acid, 50mg; niacin, 125mg; folic acid, 5 mg; biotin, 0.75 mg; pyridoxine, 18.8 mg; ethoxyquin, 52.5 mg; wheat shorts, 1715 mg.

[c] Special premix (kg^{-1} diet): selenium, 0.22 mg; vitamin E, 250 mg; ascorbic acid, 200 mg; astaxanthin, 60 mg; wheat shorts, 1988 mg.

[d] Nutrient composition of the diets was completed by Nova Scotia Agriculture & Food Operations Analytical Lab in Harlow Institute, Truro, Nova Scotia

Table 4.2 Analyzed amino acid composition of the experimental diets fed to Atlantic salmon fry (as fed basis).

Diet	Control	CO		SECM		
		50%	100%	6%	12%	18%
Indispensable amino acids (%)						
Arginine	2.9	2.8	2.8	2.9	2.9	2.9
Histidine	1.4	1.4	1.3	1.4	1.4	1.3
Isoleucine	1.9	2.0	1.9	1.9	1.9	1.8
Leucine	4.0	4.1	3.9	4.0	4.0	3.8
Lysine	3.6	3.6	3.5	3.6	3.5	3.3
Methionine	1.2	1.2	1.2	1.2	1.2	1.1
Phenylalanine	2.2	2.4	2.3	2.3	2.3	2.3
Threonine	2.1	2.0	2.0	2.0	2.0	2.0
Tryptophan	0.6	0.64	0.52	0.59	0.65	0.63
Valine	2.8	2.8	2.6	2.7	2.7	2.7
Total indispensable amino acids	22.7	22.9	22.0	22.7	22.6	21.9
Dispensable amino acids (%)						
Alanine	3.0	3.0	2.9	3.0	2.9	2.8
Aspartic acid	4.4	4.4	4.3	4.4	4.4	4.2
Cysteine	0.48	0.47	0.46	0.50	0.51	0.55
Glutamic acid	7.2	7.1	7.0	7.2	7.1	7.0
Glycine	3.2	3.2	3.2	3.2	3.2	3.2
Proline	2.7	2.7	2.6	2.6	2.6	2.5
Serine	2.0	1.9	1.9	1.9	2.0	1.9
Tyrosine	1.6	1.7	1.6	1.6	1.6	1.5
Taurine	0.29	0.29	0.28	0.29	0.27	0.25
Hydroxyproline	0.41	0.41	0.46	0.43	0.43	0.45
Lanthionine	0	0	0	0	0	0
Hydroxylysine	0.09	0.14	0.15	0.14	0.13	0.13
Ornithine	0.05	0.04	0.05	0.05	0.05	0.04
Total dispensable amino acids	25.4	25.3	25.0	25.3	25.1	24.5

Analyses were conducted by the Experimental Station's Chemical Laboratories, University of Missouri, USA.

4.5.2 Fish Stock and Rearing Conditions

Five thousand eyed Atlantic salmon (St. John River stock) eggs were obtained from Big Falls Fish Grower Ltd., Wolfville, Nova Scotia, Canada, and shipped to the Aquaculture

Centre, Dalhousie University, Faculty of Agriculture, Truro, Nova Scotia, Canada. Once received, eggs were disinfected in 6 ºC freshwater containing 100 ppm Ovadine® solution for 10 minutes, rinsed with 6±0.5 ºC fresh water, then placed into a MariSource 8-tray vertical incubator supplied with 6±0.5 ºC fresh water until the fish hatched out. Dead eggs were removed daily. Water temperature was gradually increased to 10±0.5 ºC by 1ºC every three days after the alevins hatched out. When ready for first feeding, salmon fry were transported into a green fiberglass tank (~790 L) supplied with 10±0.5 ºC fresh water. A vibratory automatic feeder was provided to distribute feed every 20 minutes. Hand-feeding was provided at 0830, 1030, 1300 and 1630h. Fry were fed with Skretting® Nutra Starter 0.05 and 0.07 mm until they reached 1 g. Once the fry adapted to the feed, water temperature was increased to 13±0.5 ºC by 1ºC every three days.

Salmon fry (1200 fish in total, 50 fish per tank), with initial body weight of 1.1±0.1 g, were randomly distributed into 24 dark green tanks (40 L) in a flow through system. Each treatment was randomly assigned to four tanks. The tanks were supplied with 13±0.5ºC water with salinity ranging from 0.5 to 1 ppt. The initial water flow in each tank was 0.8±0.05 L/min, and increased to 1.0±0.05 L/min on day 56. The water was oxygenated from an onsite oxygen generation system to provide 100-120% dissolved oxygen. Photoperiod was simulated as natural day length based on 45 °N latitude from May to September 2014. Fish were hand-fed to apparent satiation four times per day (0900, 1100, 1400, and 1630h). Feeding frequency was reduced to three times per day (0830, 1200 and 1630h) when the fish reach 2 g. To minimize the stress of relocation and handling, all of experimental fish were fed with Skretting® Nutra Starter 0.7 mm during the first seven

days as an acclimation, and test diets were offered after the adaptation. Dead fish were monitored daily, removed and weighed if present. The duration of the trial was 112 days. On day 56, the number of fish in each tank was reduced to 30. Fish were cared for under the guidelines of the Canadian Council of Animal Care (2005).

4.5.3 Data Collection and Analysis

4.5.3.1 Growth Response and Sampling

Weight gain, feed consumption, feed conversion ratio and protein efficiency ratio were determined every 14 days following the same protocol as described in Chapter 3, Section 3.5.3. Condition factor, viscera-somatic index (VSI) and hepato-somatic index (HSI) were measured at day 112 based on six randomly selected fish from each tank using same methodology as described in Chapter 3, Section 3.5.3. Condition factor was calculated following the equations as fellow:

$$\text{Condition factor} = \frac{100 * \text{weight of an individual fish(g)}}{\text{fork length of an individual fish (cm)\^3}}$$

Carcass samples were obtained at day 0 and day 112. At day 0, one hundred fish were randomly sampled from the original population, euthanized by an overdose of TMS (400-500 ppm) and stored at -20 ºC as the initial carcass sample. Due to the small size of the fish, viscera were not removed. At day 112, 12 fish per tank was euthanized with an overdose of TMS (400-500 ppm) and stored at -20 ºC after removal of viscera for future proximate body composition analysis.

4.5.3.2 Carcass Analysis

Twelve fish from the same tank, were pooled as one carcass sample for each individual tank. Each carcass sample was chopped and homogenized using a food chopper (Proctor Silex 72500RY, USA), weighed and stored in -20 ºC freezer until completely frozen. Frozen samples were dried in a freeze-dryer (MODULYOD-115, Thermo Fisher Scientific, USA) for about 24 hours, and weighed immediately once removed from the freeze-dryer. Samples were then homogenized in a coffee grinder (Smartgrind® CBG100SC, Black & Decker, China) for crude protein, crude fat and ash analysis. Each sample was analyzed in duplicate. Samples were dried to constant weight in a drying oven (Isotemp® 300 series, Fisher Scientific, Canada) at 100 ºC for at least three hours following AOAC Official Method 934.01(2011). Moisture content was calculated. Nitrogen content was determined with a LECO nitrogen/protein analyzer (LECO FP-528, USA). Crude protein (N×6.25) was calculated (AOAC Official Method 992.15, 2011). Crude fat was determined using an ANKOM XT15 extractor and an ANKOMRD dryer (Ankom Technology, USA), following AOAC Official Method 920.39 (AOAC, 2011). Samples were ashed at 550 ºC for at least two hours in an Isotemp® Muffle Furnace (Model 650, Fisher Scientific, USA) following AOAC Official Method 942.05 (2011).

4.5.3.3 Statistical Analysis

The trial was conducted in a 24-tank system with a completely randomized experimental design. Each tank was an experimental unit. Diet was the primary effect. The two sets of data, fish oil replacement with CO and SECM inclusions, were analyzed separately. For both sets of data, repeated measures including weight gain, feed consumption, FCR, and

PER were subjected to analysis of variance using Proc Mix in SAS 9.3 (SAS Institute Inc., Cary, NC) (Littell et al., 2006) following the model below:

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

Where:

Y was the response variables (weight gain, feed consumption, FCR, or PER)

μ was the overall mean of the response variables

α_i was the effect of the treatment (i=0, 6, 12 or 18%SECM inclusion) or (i=0, 50 or 100%CO replacement)

β_j was the effect of time (j= day 0-14, 14-28, 28-42, 42-56, 56-70, 70-84, 84-98, or 98-112)

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

ε_{ijk} was the random error

The non-repeated measures such as condition factor, VSI, HSI and proximate carcass composition were subjected to analysis of variance using Proc Mix procedure in SAS 9.3 (SAS Institute Inc., Cary, NC) (Littell et al., 2006) following the model below:

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

Where:

Y_{ij} was the response variables (condition factor, VSI, HSI or proximate body composition)

μ was the overall mean of the response variables

α_i was the effect of the treatment (i=0, 6, 12 or 18%SECM inclusion) or (i=0, 50 or 100%CO replacement)

ε_{ij} was the random error

Whenever the analysis of variance indicated significant difference ($p<0.05$), Tukey-Kramer test was conducted for multiple comparisons (Gbur et al. 2012).

4.6 Results

4.6.1 Growth Performance of Atlantic Salmon Fed Graded Levels of Camelina Oil

Weight gain, feed consumption, FCR and PER for day 0-14 were not included in the statistical analysis due to weight loss of salmon fry. In each weighing period, no difference was evident in weight gain for salmon fed treatments containing different levels of CO ($p>0.05$; Table 4.3). However, the overall weight gain (day 0-112) of fish fed 100% CO diet was significantly higher than fish fed control and 50% CO diets (Table 4.3). Salmon fry fed different CO-containing diets consumed similar amount of feed during each weighing period. However, fish fed 100% CO diet had a significantly higher total feed consumption (day 0-112) compared to fish fed 50% CO diet (Table 4.3). FCR and PER remained similar among all treatments throughout the trial (Table 4.3). The VSI and HSI did not differ by treatments on day 112 (Table 4.4). After 112 days, fish fed different diets showed similar carcass composition in regard to moisture, crude protein, crude lipid and ash content (Table 4.4).

Table 4.3 Weight gain, feed consumption, feed conversion ratio and protein efficiency ratio of Atlantic salmon fry fed graded levels of camelina oil (CO) (n=4).

Diet	Control	CO	
Level of CO (%)	0	50	100
Parameters			
Initial body weight (g fish^{-1})	1.10±0.01	1.11±0.02	1.12±0.10
Weight gain (g fish^{-1})			
Day 0-14[1]	-0.01±0.04	0.00±0.02	0.00±0.02
Day 15-28	0.59±0.08	0.59±0.06	0.69±0.04
Day 29-42	1.09±0.21	1.05±0.08	1.21±0.11
Day 43-56	1.19±0.10	1.31±0.10	1.36±0.13
Day 57-70	1.7±0.3	1.6±0.1	2.2±0.4
Day 71-84	2.8±0.4	2.8±0.2	3.4±0.5
Day 85-98	3.4±0.5	3.3±0.5	4.1±0.4
Day 99-112	4.6±0.5	3.8±0.5	5.2±1.0
Day 0-112	15.5±1.7^{b}	14.5±0.4^{b}	18.0±0.9^{a}
Feed consumption (g fish^{-1})			
Day 0-14[1]	0.09±0.02	0.08±0.01	0.08±0.02
Day 15-28	0.56±0.07	0.52±0.04	0.56±0.06
Day 29-42	1.01±0.08	1.01±0.10	1.22±0.11
Day 43-56	1.33±0.13	1.38±0.10	1.53±0.24
Day 57-70	1.9±0.2	2.0±0.1	2.3±0.3
Day 71-84	3.0±0.2	3.0±0.2	3.4±0.4
Day 85-98	3.4±0.3	3.2±0.2	3.7±0.4
Day 99-112	3.8±0.3	3.5±0.3	4.5±0.3
Day 0-112	15.1±1.1^{ab}	14.8±0.3^{b}	17.3±1.7^{a}
Feed conversion ratio			
Day 0-14[1]	-	-	-
Day 15-28	0.97±0.20	0.89±0.05	0.81±0.07
Day 29-42	0.95±0.17	0.97±0.05	1.01±0.06
Day 43-56	1.11±0.08	1.06±0.10	1.12±0.10
Day 57-70	1.09±0.05	1.22±0.07	1.07±0.10
Day 71-84	1.07±0.14	1.09±0.13	1.03±0.09
Day 85-98	0.98±0.09	0.99±0.09	0.90±0.09
Day 99-112	0.84±0.04	0.92±0.04	0.90±0.26
Day 0-112	0.98±0.06	1.02±0.05	0.96±0.08
Protein efficiency ratio			
Day 0-14[1]	-	-	-
Day 15-28	2.1±0.4	2.2±0.1	2.5±0.2
Day 29-42	2.1±0.3	2.0±0.1	2.0±0.1
Day 43-56	1.8±0.1	1.9±0.1	1.8±0.1
Day 57-70	1.8±0.1	1.6±0.1	1.9±0.2
Day 71-84	1.9±0.2	1.8±0.2	2.0±0.2
Day 85-98	2.0±0.2	2.0±0.2	2.2±0.2
Day 99-112	2.4±0.1	2.1±0.1	2.4±0.6
Day 0-112	2.0±0.1	1.9±0.1	2.1±0.2

[1]Data of weight gain, feed consumption, feed conversion ratio, and protein efficiency ratio over the period of day 0 to 14 were excluded from the statistics analysis.

[ab]Mean±SD within rows with different superscripts are significantly different ($P<0.05$).

Table 4.4 The condition factor, visceral-somatic index (VSI), hepato-somatic index (HSI), and proximate body composition (wet weight basis) of Atlantic salmon fry fed graded levels of camelina oil (CO) on day 112 (n=4).

	Day 0	Day 98		
		Control	50%CO	100%CO
Condition factor	N/A	1.33±0.04	1.33±0.06	1.38±0.04
VSI	N/A	12.19±1.42	11.81±0.59	12.67±0.77
HSI	N/A	1.33±0.18	1.20±0.10	1.40±0.24
Carcass composition (%)				
Moisture	80	73.6±0.7	73.1±0.5	73.2±0.1
Crude protein	14.3	16.5±0.4	16.9±0.2	16.7±0.2
Crude lipid	3.1	6.4±0.6	6.5±0.2	6.2±0.3
Ash	1.9	2.2±0.1	2.2±0.2	2.2±0.1

N/A data not measured due to the size of the fish.
Mean±SD

4.6.2 Performance of Atlantic Salmon Fed Graded Levels of Solvent-Extracted Camelina Meal

In the 112-day feeding trial, salmon fry grew from about 1 g to at least 13.8±1.3 g (Table 4.5). Fish fed all diets had lost weight during day 0-14. The weight loss was 0.01 to 0.02g depending on the treatment (Table 4.5). Weight gain, feed consumption, FCR, and PER of day 0-14 was excluded from statistical analysis due to loss of weight. Weight gain during day 14-28 period was not different among fish fed control diet or any SECM containing-diets (Table 4.5). However, salmon fed 18%SECM diet did consume less feed when compared to fish fed control diet during this period, but the FCR and PER were not affected by different treatments ($p>0.05$) (Table 4.5). For the period of day 28-42 and subsequent weighing periods, no statistical differences were observed in weight gain, feed consumption, FCR or PER among all treatments (Table 4.5). The total weight gain over day 0-112 of fish fed control diet and the SECM-containing diets were not different ($p>0.05$; Table 4.3). However, the total weight gain of fish fed 18%SECM was significantly lower than those fed 6% and 12%SECM diets (Table 4.5). The total feed

Table 4.5 Weight gain, feed consumption and feed conversion ratio of Atlantic salmon fry fed graded levels of solvent-extracted camelina meal (SECM) in a 112-day feeding trial (n=4).

Diet	Control	SECM		
Level of SECM (%)	0	6	12	18
Parameters				
Initial body weight (g fish^{-1})	1.10±0.01	1.11±0.02	1.12±0.02	1.12±0.02
Weight gain (g fish^{-1})				
Day 0-14	-0.01±0.04	-0.01±0.02	-0.02±0.03	-0.02±0.01
Day 14-28	0.59±0.08	0.60±0.01	0.53±0.05	0.45±0.09
Day 28-42	1.09±0.21	1.04±0.05	0.98±0.05	0.92±0.09
Day 42-56	1.19±0.10	1.37±0.14	1.35±0.07	1.19±0.12
Day 56-70	1.7±0.3	1.8±0.1	1.9±0.1	1.6±0.2
Day 70-84	2.8±0.4	3.1±0.2	3.2±0.3	2.6±0.2
Day 84-98	3.4±0.5	3.7±0.4	3.7±0.3	3.1±0.2
Day 98-112	4.6±0.5	4.8±0.5	5.0±0.2	4.0±0.7
Day 0-112	15.5±1.7^{ab}	16.4±0.5^{a}	16.5±0.3^{a}	13.8±1.3^{b}
Feed consumption (g fish^{-1})				
Day 0-14	0.09±0.02	0.08±0.01	0.08±0.00	0.07±0.00
Day 14-28	0.56±0.07^{a}	0.46±0.03^{ab}	0.45±0.02^{ab}	0.40±0.04^{b}
Day 28-42	1.01±0.08	1.01±0.08	1.02±0.04	0.82±0.05
Day 42-56	1.33±0.13	1.35±0.15	1.32±0.12	1.28±0.14
Day 56-70	1.9±0.2	1.9±0.4	2.1±0.2	1.8±0.4
Day 70-84	3.0±0.2	3.1±0.3	3.0±0.2	2.8±0.2
Day 84-98	3.4±0.3	3.4±0.4	3.5±0.2	3.2±0.4
Day 98-112	3.8±0.3	4.1±0.1	3.9±0.1	3.4±0.5
Day 0-112	15.1±1.1	15.4±1.1	15.4±0.5	13.7±1.4
Feed conversion ratio				
Day 0-14[1]	-	-	-	-
Day 14-28	0.97±0.20	0.76±0.05	0.86±0.10	0.91±0.13
Day 28-42	0.95±0.17	0.96±0.06	1.04±0.08	0.89±0.05
Day 42-56	1.11±0.08	0.99±0.06	0.98±0.05	1.08±0.05
Day 56-70	1.09±0.05	1.06±0.20	1.11±0.10	1.11±0.12
Day 70-84	1.07±0.14	0.98±0.09	0.95±0.03	1.08±0.07
Day 84-98	0.98±0.09	0.94±0.07	0.97±0.13	1.02±0.07
Day 98-112	0.84±0.04	0.85±0.08	0.78±0.06	0.85±0.06
Day 0-112	0.98±0.06	0.94±0.05	0.93±0.03	0.99±0.01
Protein efficiency ratio				
Day 0-14[1]	-	-	-	-
Day 14-28	2.1±0.4	2.6±0.2	2.3±0.3	2.3±0.3
Day 28-42	2.1±0.3	2.0±0.1	1.9±0.1	2.3±0.1
Day 42-56	1.8±0.1	2.0±0.1	2.0±0.1	1.9±0.1
Day 56-70	1.8±0.1	1.9±0.4	1.8±0.2	1.8±0.2
Day 70-84	1.9±0.2	2.0±0.2	2.1±0.1	1.9±0.1
Day 84-98	2.0±0.2	2.1±0.2	2.1±0.2	2.0±0.1
Day 98-112	2.4±0.1	2.3±0.2	2.5±0.2	2.4±0.2
Day 0-112	2.0±0.1	2.1±0.1	2.1±0.1	2.1±0.0

[1]Feed conversion ratio and protein efficiency ratio was not calculated for day 0-14 due to the loss of weight during this period. Data of weight gain, feed consumption, feed conversion ratio, and protein efficiency ratio over the period of day 0 to 14 were excluded from the statistics analysis.

[ab]Mean±SD within rows with different superscripts are significantly different ($P<0.05$).

consumption, FCR and PER over day 0-112 was not different for fish fed control and any SECM diets ($p>0.05$; Table 4.5).

After the 112-day feeding trial, the condition factor was significantly lower in salmon fed control diet than the 12%SECM diet (Table 4.5). The VSI, HSI and proximate body composition on day 112 were similar among control diet-fed fish and any SECM diets-fed fish ($P>0.05$; Table 4.6).

Table 4.6 The condition factor, visceral-somatic index (VSI), hepato-somatic index (HSI), and proximate body composition (wet weight basis) of Atlantic salmon fry fed graded levels of solvent-extracted camelina meal on day 112 (n=4).

	Day 0	Day 112			
		Control	Solvent extracted camelina meal (%)		
			6	12	18
Condition factor	N/A	1.33±0.04[a]	1.39±0.05[ab]	1.47±0.05[b]	1.42±0.04[ab]
VSI	N/A	12.19±1.42	10.98±0.58	11.45±0.84	11.23±0.90
HSI	N/A	1.33±0.18	1.35±0.17	1.35±0.19	1.24±0.06
Carcass composition (%)					
Moisture	80.0	73.6±0.7	73.6±0.2	73.6±0.5	73.6±0.5
Crude protein	14.3	16.5±0.4	16.9±0.2	16.7±0.3	17.0±0.2
Crude lipid	3.1	6.4±0.6	6.3±0.1	6.5±0.4	6.2±0.5
Ash	1.9	2.2±0.1	2.3±0.1	2.3±0.1	2.2±0.1

N/A data not available.

[ab] Mean±SD within rows with different superscripts are significantly different ($P<0.05$).

4.7 Discussion

Although the salmonid feed industry has been able to reduce marine-sourced ingredients including fish oil and fishmeal to a minimal level by employing a wild range of terrestrial plant ingredients, an ideal alternative that allows fish to maintain their growth while retaining n-3 fatty acid content such as EPA and DHA in fish fillets are yet to be found. The current study investigated the possibility of using products from an n-3 fatty acids rich oil crop *camelina sativa* as feed ingredients for Atlantic salmon. The present study is

the first to test CO and SECM in salmon fry diet (1.1 g). The 100%CO diet encouraged feed intake and resulted in a significantly higher weight gain compared to fish fed either the control or 50%CO diets after the 112-day feeding trial. Dietary incorporation of up to 18% of SECM in Atlantic salmon fry diet had no negative impacts on their growth performance, feed efficiency or proximate carcass composition.

Replacing up to 100% of fish oil with plant oils often have no negative effects on the growth performance of fish such as Atlantic salmon and rainbow trout (Turchini et al., 2009; Nasopoulou and Zabetakis, 2012), and current results concur with these findings. In the present study, replacing 50% of fish oil with CO in salmon fry diet did not affect any of the measured growth parameters. This was in agreement with results of Ye et al. (2016). Salmon fed 100%CO diet performed similar to fish fed control and 50%CO diets in each 14-day weighing period. Unexpectedly, the overall feed consumption and weigh gain over the 112-day feeding trial was significantly promoted by 100%CO diet. The feed intake promoting effect of the 100%CO diet was not evident in previous studies (Ye et al., 2014; Hixson et al., 2014b and 2017) where older salmon fed either 100% fish oil or CO had similar growth. In other previous studies feeding CO to rainbow trout or Atlantic cod, no advantage was reported for 100%CO (Morais et al., 2012; Hixson et al., 2013, 2014a; Hixson and Parish, 2014). Future studies on fatty acid profiles of fish tissues and expression of fatty acid related genes when CO is fed is recommended.

In the current study, Atlantic salmon fry tolerated up to 18%SECM in their diet. Those fish performed better on SECM diets than the salmon reported in previous camelina

studies (Ye et al., 2016; Ye, 2014; Hixson et al., 2015a). Although Atlantic salmon (8.4 g) fed 20%SECM diet performed similarly to fish fed control diet after 16 weeks, salmon fed both 15 and 20%SECM diets had significantly lower feed intake and weight gain compared to fish fed control diet during the last growth period week 13-16 (Ye et al., 2016). Enteritis was detected in the 15 and 20%SECM fed fish. In other cases, larger salmon, with body weight of either 61.8 or 242 g, consumed less feed and gained less weight when fed the lowest test level of either SECM or and high-oil residue camelina meal at 8%. Enteritis was observed in higher inclusion rate including 16 and 24% (Ye, 2014; Hixson et al., 2015a). The difference in capacity to tolerate camelina meals in diets may be related to the different life stages when they were first introduced. In the current study, SECM was tested in salmon with about 1.1 g initial weight while in the other studies, fish had an initial weight of 8.4, 61.8 and 242 g respectively. It is possible that fish with smaller initial weight had a better ability to adapt to new ingredients. Although taste preference of fish is mostly dependent on the genetics of a species, and sometimes influenced by factors such as taste feeding motivation (hunger), the perception in larvae or fry is not as well defined as in adult fish (Kasumyan and Doving, 2003). Evidence showed that smaller brown trout in the wild consume more carbohydrates and might digest and utilize those carbohydrates better than the large trout (Marandel et al., 2018). Therefore, it is possible that fish with smaller initial weight had a better ability to adapt to new ingredients.

Although salmon fed 18%SECM diet had similar performance to those fed control diet for those performance parameters measured, the 18%SECM fed fish had significantly lower weight gain compared to fish fed 6 and 12%SECM diets ($p<0.05$). The difference

in overall weight gain of fish fed 18% SECM diet, compared to 6 and 12% SECM diets was not associated with FCR or PER. The lower weight gain of salmon fry fed 18% SECM diet was probably related to the slightly lower feed consumption ($p>0.05$). The difference was likely not large enough to be detected by Tukey-Kramer test due to the high standard deviation of overall feed consumption.

Reduction in feed intake and subsequently weight gain possibly related to glucosinolates has been discussed in previous camelina studies using older Atlantic salmon (Ye et al., 2016; Hixson et al., 2015a). Both glucosinolates and its breakdown products isothiocyanates can contribute to bitter taste, and the latter contributes more to the bitter taste than glucosinolates themselves (Wieczorek et al., 2017). In our case, the depressed feed intake could possibly be due to the bitterness from glucosinolates themselves. More than 110 glucosinolates have been characterized. Certain glucosinolates, including sinigrin, progoitrin, glucobrassicin and neoglucobrassicin, are bitter while others are not (Wieczorek et al., 2017). Three typical glucosinolates presented in camelina are 9-methyl-sulfinyl-nonyl (glucoarabin), 10-methyl-sulfinyl-decyl (glucocamelinin) and a trace of 11-methyl-sulfinyl-undecyl glucosinolates (Schuster and Friedt, 1998). These glucosinolates in camelina have not been well studied and the bitterness of them is unknown. Glucosinolates metabolized to compounds such as isothiocyanates that already existed in SECM, or isothiocyanates formed during feed preparation could be a factor in reduced feed intake. Glucosinolates can be hydrolyzed into isothiocyanates and other derivatives in the presence of myrosinase (Tripathi and Mishra, 2007). The hydrolyzing process is not likely to happen in intact plant tissues since glucosinolates are stored

separately from myrosinase (Bones and Rossiter, 1996). When plant cells are ruptured during processing such as seed crushing, grinding, solvent extraction, preparation for feed, glucosinolates could potentially be in contact with myrosinase and breakdown to isothiocyanates and other derivatives. Wetting a rapeseed meal that contained 150.3 μmol g^{-1} of total glucosinolates led to their hydroxylation and resulted in 6.5, 3.6 and 4.6 μmol g^{-1} of isothiocyanate, nitrile and oxazolidine-2-thione, respectively (Smolinska et al., 1997). Both production of camelina seed to SECM and SECM to the SECM diets could potentially lead to degradation of glucosinolates and result in production of isothiocyanates and causing a less palatable diet (18%SECM). Combinations of either of these factors could also be involved. The calculated level of glucosinolates in 6, 12 and 18%SECM diets were 3.2, 6.3 and 9.5 μmol g^{-1} (dry matter basis), respectively, based on the analyzed total glucosinolates in SECM used in the current trial. The degradation products such as isothiocyanate, nitrile and oxazolidine-2-thione in SECM were unfortunately not determined. The total glucosinolates and its degradation products of each diet were not analyzed. Therefore, all of the three scenarios discussed are possible in our case, and the threshold of sensitivity cannot be established using current data. There is currently no literature explicitly evaluating the effects of camelina glucosinolates and their degradation products in fish. Most studies on the effect of these glucosinolates on animal often neglect the aspect of its degradation products. Atlantic salmon tolerated a diet containing 5% SECM with a total glucosinolate content of 1.9 μmol g^{-1} (dry matter basis) after a 112-day trial (Ye et al., 2016). The degradation products of glucosinolates in their diets were not determined. In another trial, growth performance including weight gain, feed intake and FCR of rainbow trout was compromised when fed a diet containing

30% dietary inclusion of rapeseed meal (Burel et al., 2000). The rapeseed meal had 5 μmol of glucosinolates per gram meal (dry matter basis). Glucosinolates and their degradation products in the diet were not analyzed. Additionally, the depressed growth performance of rainbow trout could be attributed to other antinutrients in rapeseed meal. It is important for future studies to have profiles of both glucosinolates and their degradation products in the diet to establish safe levels in animal feed. Berhow et al. (2013) was able to separate and purify glucosinolates from camelina. Future studies assessing the biological effects of camelina glucosinolates should employ the purified ones instead of using camelina meal as their source to eliminate the interference from other antinutrients that potentially exist in the meal.

4.8 Conclusions

Both SECM and CO can be used as feed ingredients in salmon fry diet. Inclusion of up to 18%SECM in the diet of Atlantic salmon fry did not affect their growth performance, feed efficiency and proximate carcass composition when compared to the control treatment after 112 days feeding trial. The 100%CO diet encouraged the feed intake and resulted in a significantly higher weight gain compared to those receiving either the control or 50%CO diets.

Chapter 5

Use of Solvent Extracted Meal, Protein Concentrate and Oil from *Camelina sativa* for Rainbow Trout *Oncorhynchus mykiss*

5.1 Abstract

A 98-day feeding trial was conducted in a 24-tank flow through system with 14 ºC fresh water to evaluate the effects of using camelina oil (CO), solvent extracted camelina meal (SECM), and camelina protein concentrate (CPC) in rainbow trout fry diet (1.0 g, 50 fish/tank). Each tank was randomly fed one of the eight diets including a control diet, two diets with either 50 or 100% fish oil replaced with CO, three diets with SECM incorporated at 6, 12 or 18%, two diets with CPC included at 6 or 12%. Up to 100% fish oil can be replaced with CO diets without negatively affecting all the measured biometric parameters including growth performance, nutrient utilization and proximate carcass composition of rainbow trout fry after 98 days feeding trial. Fish tolerated up to 18% of SECM or 12% of CPC in their diet without reductions in any measured growth parameters, feed efficiency or changes in approximate body composition. However, higher inclusion level of SECM, 18% in particular, did require an acclimation period to the feed and had less weight gain possible due to a poorer protein utilization efficiency at the beginning of the trial.

5.2 Introduction

Seeking environment and budget-friendly alternative feed ingredients to the finite supplies of fishmeal and fish oil has been the focus of salmonid industry in support of its rapid expansion during past decades. The usage of fishmeal and fish oil in salmon diets has been successfully reduced from 66 to 18% and 24 to 11%, respectively, and replaced with terrestrial plant ingredients (Ytrestøyl et al., 2015). Studies have proven the possibility of either partial or entire replacement of fish oil with plant oils without deleterious effect on fish growth performance, nutrients utilization, or even body proximate composition (Turchini et al., 2009; Nasopoulou and Zabetakis, 2012). However, plant oils are low in n-3 fatty acids and completely devoid of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Hence, the replacement often contributes

to the alteration of the fatty acid profiles in fish muscle tissues, reductions in EPA, DHA, and n-3/n-6 fatty acid ratio to be precise. To preserve the nutritional value and thus commercial value of salmonid flesh, the search for fish oil alternatives continue. In the current study, camelina oil (CO) was chosen due to its high content of alpha-linolenic acid (ALA; 36.2-39.4%) (Gugel and Falk, 2006). ALA is an indispensable n-3 fatty acid in salmonid diets. Evidence showed that both rainbow trout and Atlantic salmon were able to use dietary ALA as the precursor for EPA and DHA bioconversion (Collins et al., 2011; Lazzarotto et al., 2015; Hixson et al., 2014a and b). High ALA content in CO could potentially improve the n-3/n-6 ratio in diet and subsequently in fish tissue. CO extraction results in a by-product called pressed cake. This pressed cake can be further processed into camelina meal or protein concentrate. Utilization of by-products resulting from oil extraction is a common practice for feed industry to optimize the value of an oil crop. Hence, the current study demonstrates the possibility of using solvent-extracted camelina meal (SECM) and camelina protein concentrate (CPC) in trout diets.

Further reduction of fishmeal in the diets of carnivorous species like rainbow trout and Atlantic salmon is unlikely due to the detrimental effects in growth, feed efficiency or even pathological damage when high levels of plant proteins were incorporated (Hua and Bureau, 2012; Collins et al., 2013). This is likely associated with the high content of carbohydrates or presentation of anti-nutritional factors such as antigen proteins, glucosinolates, phytic acid, or tannins, leading to poor palatability or difficulty in nutrient digestion and utilization. Different approaches have been examined to achieve higher inclusion levels of plant proteins. Young fish like fry are delicate and consume relatively

small amount of feed in comparison to those at the grow-out stage. Hence, fish oil and fishmeal are often generously provided in fry diet. However, research has shown that rainbow trout that was previously exposed to plant ingredients at the fry stage had an improved acceptance and utilization of the same components as existed at the later life stages (Geurden et al., 2013). This is perhaps due to a better capacity to digest certain nutrients such as carbohydrates by small trout than by large trout (Marandel et al., 2018). Thus, young rainbow trout at fry stage were used as the experimental target to explore the potential of dietary incorporation of camelina products including CO, SECM, and CPC. The plan was to compare the results from previous work conducted on larger trout (Hixson et al., 2014a, 2015a; Bullerwell et al., 2016).

5.3 Objectives

Feeding rainbow trout with CO, SECM or CPC-containing diets in order to investigate the following objectives:

1. To evaluate the acceptability of CO, SECM and CPC to trout fry based on their growth performance.

2. To identify the optimal dietary inclusion rate of these ingredients based on growth performance, and carcass composition of fish after 98 days.

5.4 Null Hypotheses

1. Replacing 0 (control), 50 or 100% fish oil with CO will result in different growth performance of trout fry. The carcass compositions will be altered by changes in the source of dietary oil.

2. Including 0 (control), 6, 12 or 18% SECM, or 6 and 12% CPC in the diet of trout fry will result in the different growth performance, and carcass composition.

5.5 Materials and Methods

5.5.1. Experimental ingredients and diets

All three test ingredients including CO, SECM and CPC used in this study were from the same batches described in Chapter 3. Throughout the 98-day trial, two difference phases of diets were used during day 0-56 and day 56-98, respectively. Each phase contained eight diets including one control diet, two diets that had either 50 or 100% fish oil replaced with CO, three diets that contained 6, 12, or 18% of SECM, two diets that contained either 6 or 12% of CPC. Within each phase, all the diets were formulated to be isocaloric and isonitrogenous. First phase of the diets (Table 5.1) was formulated to contain 50% crude protein and 4600 kcal estimated digestible energy kg^{-1} diet (as fed basis) to meet the nutrient requirement of rainbow trout (NRC, 2011). Second phase of the diets (Table 5.2) were formulated to contain 45% crude protein and 4550 kcal estimated digestible energy kg^{-1} diet (as fed basis) to meet the requirement of rainbow trout (NRC, 2011).

Table 5.1 Formulation and analyzed composition of control and experimental diets fed to rainbow trout fry until day 56 (as fed basis).

Diets	Control	CO		SECM			CPC	
		50%	100%	6%	12%	18%	6%	12%
Fishmeal	47.7	47.7	47.7	44.9	42.1	39.2	43.6	39.5
Fish oil	12.3	6.15	0	12.8	13.2	13.8	12.6	12.9
SECM	0	0	0	6	12	18	0	0
CPC	0	0	0	0	0	0	6	12
CO	0	6.15	12.3	0	0	0	0	0
Wheat	11.5	11.5	11.5	7.8	4.2	0.5	9.3	7.1
CPSP-G	7	7	7	7	7	7	7	7
Wheat gluten meal	5	5	5	5	5	5	5	5
Poultry byproduct meal	5	5	5	5	5	5	5	5
Blood meal	5	5	5	5	5	5	5	5
Pre-gelatinized starch	3	3	3	3	3	3	3	3
Soy lecithin	2	2	2	2	2	2	2	2
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mineral premix[b]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Special premix[c]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100
Analyzed nutrient composition[a]								
Dry matter (%)	92.8	95.5	94.5	93.1	95.4	95.3	94.7	95.4
Crude protein (%)	49.0	52.9	50.4	50.2	52	52.8	51.3	51.8
Crude fat (%)	22.3	21.2	21.7	22.7	22.3	22.3	22.5	21.9
Ash (%)	7.9	9	7.9	8.0	8.3	7.9	8.8	9.9
Calcium (%)	2.1	1.8	1.9	1.7	1.7	1.7	1.9	1.6
Potassium (%)	0.69	0.65	0.62	0.65	0.72	0.75	0.66	0.64
Magnesium (%)	0.14	0.15	0.13	0.15	0.17	0.18	0.13	0.14
Phosphorus (%)	1.5	1.4	1.4	1.3	1.3	1.3	1.6	1.6
Sodium (%)	0.56	0.62	0.5	0.49	0.48	0.46	0.8	1.15
Copper (mg kg^{-1})	10.5	13.6	9.7	10.2	11.9	11.4	21.8	11.5
Manganese (mg kg^{-1})	185	180	214	177	168	165	192	175
Zinc (mg kg^{-1})	161	159	160	157	167	167	155	153

[a] CPSP-G is soluble fish protein concentrate. Source of product and origin of ingredients: France. Manufacturer: Sopropeche. Wimille, France.

[b] Vitamin mineral premix (kg^{-1} diet): zinc, 96.9 mg; manganese, 156 mg; iron, 105 mg; copper, 3.1 mg; iodine, 9.4 mg; vitamin A, 6250 IU; vitamin D, 5000 IU; vitamin K, 2.5mg; vitamin B_{12}, 0.005mg; thiamin, 10mg; riboflavin, 22.5 mg; pantothenic acid, 50mg; niacin, 125mg; folic acid, 5 mg; biotin, 0.75 mg; pyridoxine, 18.8 mg; ethoxyquin, 52.5 mg; wheat shorts, 1715 mg.

[c] Special premix (kg^{-1} diet): selenium, 0.22 mg; vitamin E, 250 mg; ascorbic acid, 200 mg; astaxanthin, 60 mg; wheat shorts, 1988 mg.

[d] Performed by Nova Scotia Agriculture Quality Evaluation Division Laboratory Services, Truro, NS, Canada.

Table 5.2 Formulation and analyzed composition of control diet, camelina oil (CO), solvent extracted camelina meal (SECM) and camelina protein concentrate (CPC) containing diets fed to rainbow trout fry during day 57-98 (as fed basis).

Diets	Control	CO		SECM			CPC	
		50%	100%	6%	12%	18%	6%	12%
Fishmeal	31.10	31.10	31.10	28.27	25.45	22.62	27.00	22.84
Fish oil	14.37	7.185	0	14.86	15.36	15.86	14.70	15.07
SECM	0	0	0	6	12	18	0	0
CPC	0	0	0	0	0	0	6	12
CO	0	7.185	14.37	0	0	0	0	0
Wheat	16.03	16.03	16.03	12.37	8.69	5.02	13.8	11.59
Poultry byproduct meal	10	10	10	10	10	10	10	10
Empyreal 75®[a]	10	10	10	10	10	10	10	10
Wheat gluten meal	5	5	5	5	5	5	5	5
Blood meal	5	5	5	5	5	5	5	5
Pre-gelatinized starch	5	5	5	5	5	5	5	5
Soy lecithin	2	2	2	2	2	2	2	2
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mineral premix[b]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Special premix[c]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100
Analyzed nutrient composition[d]								
Dry matter (%)	94.6	94.6	94.6	94.1	93.8	94.3	94.3	94.2
Crude protein (%)	48.1	49.5	47.8	46.9	46.8	48	48.1	48.2
Crude fat (%)	20.3	19	21.2	20.5	20.9	21.7	20.8	21.4
Ash (%)	7.2	7.2	7.0	7.3	6.9	6.8	7.3	7.7
Calcium (%)	1.5	1.6	1.5	1.4	1.4	1.4	1.3	1.2
Potassium (%)	0.58	0.54	0.57	0.59	0.65	0.70	0.54	0.48
Magnesium (%)	0.14	0.14	0.14	0.15	0.18	0.18	0.13	0.12
Phosphorus (%)	1.2	1.2	1.2	1.2	1.2	1.2	1.3	1.4
Sodium (%)	0.6	0.6	0.58	0.59	0.53	0.46	0.80	1.04
Copper (mg kg^{-1})	10.1	8.9	9.0	12.4	10.2	10.9	11.4	8.9
Manganese (mg kg^{-1})	184	195	189	191	179	180	176	164
Zinc (mg kg^{-1})	151	145	142	144	146	144	147	135

[a] Empyreal 75® is a corn protein concentrate. Manufacturer, Cargill Corn Milling, Nebraska, USA.

[b] Vitamin mineral premix (kg^{-1} diet): zinc, 96.9 mg; manganese, 156 mg; iron, 105 mg; copper, 3.1 mg; iodine, 9.4 mg; vitamin A, 6250 IU; vitamin D, 5000 IU; vitamin K, 2.5mg; vitamin B_{12}, 0.005mg; thiamin, 10mg; riboflavin, 22.5 mg; pantothenic acid, 50mg; niacin, 125mg; folic acid, 5 mg; biotin, 0.75 mg; pyridoxine, 18.8 mg; ethoxyquin, 52.5 mg; wheat shorts, 1715 mg.

[c] Special premix (kg^{-1} diet): selenium, 0.22 mg; vitamin E, 250 mg; ascorbic acid, 200 mg; astaxanthin, 60 mg; wheat shorts, 1988 mg.

[d] Performed by Nova Scotia Agriculture Quality Evaluation Division Laboratory Services, Truro, NS, Canada.

All diets were prepared, packed and stored following the same procedure as described in Chapter 4, Section 4.5.1. Two different sizes of feed pellets including 2.5 mm (phase 1) and 3mm (phase 2) were produced. Feed in size 0.7-0.85, 0.85-1.0, 1.0-1.4, 1.4-1.7, and 1.7-2.0, 2.5 and 3mm were provided to fish. Optimal particle size was determined by whether the smallest fish in the tank can swallow the pellet. Feed was hand-crumbled and sifted using standard test sieves (Model SL-GT-093, Sun Labtek Equipments PVT. LTD) whenever needed. All fines were discard. Analysis of major nutrients (Table 5.1 and 5.2) and amino acids were conducted (Table 5.3 and 5.4). More details including methodology for analysis can be found in Chapter 3, Section 3.5.1.

5.5.2 Fish Stock and Rearing Conditions

The feeding trial was conducted at the Aquaculture Centre, Dalhousie University, Faculty of Agriculture, Truro, Nova Scotia, Canada. Two thousand eyed rainbow trout egg (Silver bullet) were provided by Troutlodge Inc. in Washington State, USA. After arrival at the Aquaculture Centre, trout eggs were immersed in 6 °C freshwater containing 100 ppm Ovadine® solution for 10 minutes, rinsed with 6±0.5 °C fresh water for disinfection. The eggs were the placed into a MariSource 8-tray vertical incubator which was supplied with fresh water temperature (6±0.5 °C). Once over 95% of the alevins hatched, the water temperature was gradually increased to 10±0.5 °C by 1°C every three days. When over 95% of the alevins showed active movement, they were relocated to a green shallow tank (~790 L) supplied with 10±0.5 °C fresh water until they reached 1 g. Commercial salmonid feed Skretting® Nutra ST 0.3 mm, Nutra ST 0.5 mm and Nutra ST 0.7 mm were fed during this period. Feed was delivered to the fish by an automatic vibratory feeder

Table 5.3 Amino acid profile of control and experimental diets containing camelina oil (CO), solvent-extracted camelina meal (SECM) or camelina protein concentrate (CPC) fed to rainbow trout fry until day 56 (as fed basis).

Diet	Control	CO		SECM			CPC	
		50%	100%	6%	12%	18%	6%	12%
Indispensable amino acids (%)								
Arginine	2.9	2.9	2.9	2.9	3	3.1	3.0	3.0
Histidine	1.3	1.5	1.4	1.3	1.4	1.4	1.4	1.6
Isoleucine	1.7	1.9	1.8	1.7	1.8	1.8	1.8	1.9
Leucine	3.7	3.9	3.7	3.7	3.7	3.8	3.8	4.0
Lysine	3.0	3.3	3.0	3.0	3.1	3.1	3.1	3.3
Methionine	0.9	1.1	0.9	0.9	0.9	1.0	0.9	1.1
Phenylalanine	2.2	2.3	2.2	2.2	2.3	2.3	2.2	2.4
Threonine	1.8	1.9	1.8	1.8	1.9	1.9	1.9	2.0
Tryptophan	0.49	0.68	0.58	0.59	0.58	0.61	0.66	0.72
Valine	2.4	2.6	2.5	2.4	2.5	2.6	2.5	2.6
Total	20.7	22.0	20.8	20.6	21.2	21.6	21.2	22.5
Dispensable amino acids (%)								
Alanine	3.0	3.1	3.0	2.9	3.0	3.0	3.0	3.0
Aspartic acid	3.8	4.1	3.9	3.8	4.0	4.1	4.0	4.2
Cysteine	0.52	0.55	0.55	0.55	0.57	0.58	0.57	0.58
Glutamic acid	6.9	7.3	7.0	6.9	7.1	7.1	7.2	7.4
Glycine	3.9	3.6	3.8	3.7	3.7	3.8	3.7	3.4
Proline	3.0	2.9	3.0	2.9	2.9	2.9	3.0	2.8
Serine	1.9	1.9	1.9	1.9	1.9	2.0	1.9	2.0
Tyrosine	1.5	1.6	1.6	1.5	1.5	1.6	1.5	1.6
Taurine	0.32	0.36	0.32	0.30	0.30	0.28	0.30	0.34
Hydroxyproline	0.89	0.68	0.81	0.80	0.83	0.88	0.81	0.49
Lanthionine	0	0	0	0	0	0	0	0
Hydroxylysine	0.18	0.07	0.09	0.17	0.17	0.08	0.13	0.06
Ornithine	0.07	0.05	0.06	0.05	0.05	0.05	0.06	0.06
Total	26.0	26.1	25.9	25.5	26.0	26.4	26.2	25.8

Performed by Experimental Station's Chemical Laboratories, University of Missouri, USA.

Table 5.4 Amino acid profile of the experimental diets containing camelina oil (CO), solvent-extracted camelina meal (SECM) or camelina protein concentrate (CPC) fed to rainbow trout fry during day 57-98 (as fed basis).

Diet	Control	CO		SECM			CPC	
		50%	100%	6%	12%	18%	6%	12%
Indispensable amino acids (%)								
Arginine	2.3	2.4	2.3	2.3	2.5	2.5	2.5	2.4
Histidine	1.4	1.5	1.4	1.4	1.4	1.4	1.4	1.3
Isoleucine	1.7	1.8	1.7	1.7	1.7	1.7	1.7	1.6
Leucine	4.4	4.7	4.5	4.3	4.4	4.3	4.6	4.3
Lysine	2.7	2.8	2.8	2.6	2.7	2.7	2.7	2.3
Methionine	1.0	1.1	1.0	0.9	1.0	1.0	1.0	0.9
Phenylalanine	2.3	2.4	2.3	2.2	2.2	2.2	2.3	2.2
Threonine	1.7	1.8	1.8	1.7	1.8	1.8	1.8	1.7
Tryptophan	0.54	0.58	0.59	0.62	0.61	0.52	0.57	0.59
Valine	2.4	2.5	2.5	2.3	2.4	2.4	2.4	2.3
Total	20.5	21.5	20.9	20.0	20.6	20.5	21.0	19.5
Dispensable amino acids (%)								
Alanine	3.0	3.1	3.1	2.8	2.9	3.0	3.0	2.8
Aspartic acid	3.6	3.8	3.7	3.5	3.6	3.7	3.7	3.5
Cysteine	0.55	0.60	0.53	0.57	0.60	0.62	0.61	0.58
Glutamic acid	7.6	8.0	8.2	7.4	7.7	8.1	7.9	7.7
Glycine	2.6	2.7	2.6	2.4	2.5	2.6	2.6	2.4
Proline	2.9	3.0	2.8	2.8	2.7	2.7	3.0	2.7
Serine	1.8	1.9	2.1	1.8	1.8	2.1	1.9	2.0
Tyrosine	1.6	1.7	1.6	1.5	1.5	1.5	1.6	1.5
Taurine	0.29	0.3	0.29	0.26	0.26	0.24	0.26	0.22
Hydroxyproline	0.30	0.26	0.36	0.29	0.28	0.41	0.27	0.33
Lanthionine	0	0	0	0	0	0	0	0
Hydroxylysine	0.11	0.11	0.07	0.06	0.06	0.03	0.04	0.06
Ornithine	0.04	0.05	0.03	0.03	0.03	0.02	0.04	0.03
Total	24.4	25.4	25.2	23.4	24.0	24.9	24.8	23.7

Performed by Experimental Station's Chemical Laboratories, University of Missouri, USA.

every 20 minutes during the day. Hand-feeding was provided at 0830, 1030, 1300 and 1630h. Once the fry adapted to the feed, water temperature was increased to 13±0.5 °C at a rate of 1°C every three days.

One thousand four hundred and forty rainbow trout fry of initial mean weight 1.0±0.1 g were randomly distributed among 24 dark green fiberglass tanks with 50 fish per tank in a flow through system. On day 56, number of fish in each tank was reduced to 30. The tanks were supplied with 13±0.5 °C oxygenated fresh water using an onsite oxygen generation system (100-120% dissolved oxygen). Water temperature and oxygen levels in each tank was monitored daily. The initial water flow in each tank was 0.8±0.05 L min^{-1}, and increased to 1.0±0.05 L min^{-1} on day 56. Photoperiod was simulated as natural day length based on 45°N latitude and 63°N longitude from December 2014 to March 2015. During the day, trout were offered four meals (0900, 1100, 1400, and 1630h) and hand-fed to apparent satiation. Once the fish reached 2 g, three meals was provided at 0830, 1200 and 1630h. All experimental fish were fed Skretting® Nutra ST 0.7 mm during day 1-7 as an acclimation, and test diets were offered afterwards. Dead fish were weighed and recorded throughout the trial.

5.5.3 Data Collection and Analysis

5.5.3.1 Growth Response and Sampling

Fish from each tank were batched weighed and counted on days 0, 14, 28, 42, 56, 70, 84 and 98. Weight gain and feeding intake, weight gain, feed consumption, feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated every 14 days based on

the same methods described in Chapter 3 and 4. On day 98, four fish were randomly selected from each tank and euthanized by an overdose of buffered tricaine methanesulfonate (TMS; 400-500 ppm) for condition factor, visceral-somatic index (VSI) and hepato-somatic index (HSI) determination, following the same protocol as described in Chapter 3 and 4.

Initial carcass sample was obtained on day 0. Apart from the 1440 trout fry selected for the experiment, one hundred fish from the original population was randomly captured, euthanized by an overdose of TMS (400-500 ppm) and stored at -20 °C in an air-tight zip bag as the initial body carcass sample. Viscera was not removed from these fry due to their small body size. On day 98, eight fish per tank were randomly chosen and euthanized with an overdose of buffered TMS.

5.5.3.2 Proximate Carcass Composition

Eight fish collected from the same tank was pooled as one carcass sample. The methodology for proximate carcass composition analysis was described in Chapter 4, Section 4.5.3.2.

5.5.3.3 Statistical Analysis

The experiment was conducted in a completely randomized design. Tank was the experimental unit, and diet was the main effect. Treatments involved camelina protein inclusion, including SECM and CPC treatments, were analyzed separately from the oil replacement treatments.

For oil treatments, the statistic statement for the repeated measures including weight gain, feed consumption, FCR and PER was as follow:

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

Where:

Y was the response of the variables

μ was the overall mean of the variables

α_i was the effect of treatment (i= 0, 50 or 100% fish oil replaced with CO)

β_j was the effect of time (j= day 0-14, 14-28, 28-42, 42-56, 56-70, 70-84, and 84-96)

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

ε_{ijk} was the random error

Data were subjected to analysis of variance by the Proc Mixed procedure in SAS 9.3 (SAS Institute Inc., Cary, NC) (Littell et al., 2006).

Non-repeated measures including condition factor, VSI, HSI and proximate carcass composition analysis were subjected to analysis of variance (ANOVA) by the Proc Mixed in SAS 9.3 (SAS Institute Inc., Cary, NC) (Littell et al., 2006) following the model as follow:

$Y_i = \mu + \alpha_i + \varepsilon_i$

Where:

Y was the response of the variables

μ was the overall mean of the variables

α_i was the effect of treatment (i= 0, 50 or 100% fish oil replaced with CO)

ε_i was the random error

Tukey-Kramer test was used for multiple means comparisons if ANOVA indicated $P<0.05$.

For meal inclusion treatments, data that were repeatedly measured over several time periods, including weight gain, feed consumption, FCR and PER, were subjected to ANOVA by the Proc Mixed procedure in SAS 9.3 (SAS Institute Inc., Cary, NC) (Littell et al., 2006) following the following model:

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

Where:

Y was the response of the variables

μ was the overall mean of the variables

α_i was the effect of treatment (i= 0, 6,12 and 18%SECM, 6 and 12%CPC)

β_j was the effect of time (j= day 0-14, 14-28, 28-42, 42-56, 56-70, 70-84, and 84-96)

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

ε_{ijk} was the random error

Non-repeated measures including condition factor, VSI, HSI and proximate body composition analysis were subjected to ANOVA by the Proc Mixed in SAS 9.3 (SAS Institute Inc., Cary, NC) (Littell et al., 2006) following the model as follow:

$Y_i = \mu + \alpha_i + \varepsilon_i$

Where:

Y was the response of the variables

μ was the overall mean of the variables

α_i was the effect of treatment (i= 0, 6,12 and 18%SECM, 6 and 12%CPC)

ε_i was the random error

In both repeated measures and the non-repeated measures, Tukey-Kramer test was applied for multiple means comparisons if ANOVA indicated significant difference (α= 0.05).

5.6 Results

5.6.1 Composition of the Experimental Diets

Within each phase, all diets had slight variation in levels of crude protein (49.0-52.8% in Table 5.1; 46.9-49.5% in Table 5.2), crude fat (21.2-22.7% in Table 5.1; 19.0-21.7% Table 5.2) and ash (7.9-9.9% in Table 5.1; 6.8-7.7% in Table 5.2). Minerals including calcium, magnesium, phosphorus, sodium, copper, manganese and zinc were similar (Table 5.1 and 5.2), and the amino acid profile of all eight diets were similar (Table 5.3 and 5.4).

5.6.2 Growth Performance of Rainbow Trout Fed Graded Levels of Camelina Oil

Fish gained 64.0 to 74.6 g of body weight during the 98-day trial when fed diets with 0, 50 or 100% fish oil replaced with CO (Table 5.5). During day 0-14, fish fed control diet had significantly higher PER than fish fed 50 or 100%CO diets (Table 5.5). Trout fed control diet retained significantly less water in their carcass compared to those fed either 50 or 100%CO diet at day 98 (Table 5.6). Other growth parameters measured during each weighing period or over the entire trial were similar among all treatments ($p>0.05$) (Table 5.5 and 5.6).

Table 5.5 Growth performance of rainbow trout fed diet with 0, 50 and 100% fish oil replaced with camelina oil after the 98-day feeding trial (n=3).

Diet	Control	Camelina oil (CO)	
Level of CO (%)	0	50	100
Parameters			
Initial body weight (g fish^{-1})	1.00±0.01	1.00±0.00	1.00±0.01
Weight gain (g fish^{-1})			
Day 0-14	1.65±0.04	1.56±0.04	1.50±0.06
Day 14-28	2.87±0.09	2.92±0.16	2.84±0.03
Day 28-42	4.65±0.07	4.38±0.45	4.24±0.14
Day 42-56	6.8±1.1	8.0±0.04	6.7±0.3
Day 56-70	11.7±3.3	11.6±0.2	9.1±0.9
Day 70-84	18.0±1.9	18.9±1.6	16.3±0.7
Day 84-98	26.5±3.4	27.7±1.4	23.4±1.7
Day 0-98	72.2±7.9	74.6±3.2	64.0±2.1
Feed consumption (g fish^{-1})			
Day 0-14	0.99±0.03	1.03±0.04	0.98±0.05
Day 14-28	2.26±0.04	2.41±0.15	2.21±0.03
Day 28-42	3.82±0.15	3.75±0.07	3.65±0.19
Day 42-56	6.2±0.01	6.3±0.4	5.7±0.3
Day 56-70	8.9±1.1	9.4±0.4	8.8±0.3
Day 70-84	18.8±0.7	18.8±0.6	18.4±0.1
Day 84-98	31.5±4.8	31.2±1.7	24.9±2.1
Day 0-98	73.4±7.1	73.0±2.3	64.5±2.0
Feed conversion ratio			
Day 0-14	0.62±0.003	0.61±0.01	0.60±0.01
Day 14-28	0.79±0.02	0.82±0.01	0.78±0.02
Day 28-42	0.82±0.03	0.86±0.08	0.86±0.02
Day 42-56	0.85±0.08	0.78±0.05	0.85±0.08
Day 56-70	0.9±0.01	0.85±0.02	0.97±0.10
Day 70-84	1.05±0.10	1.00±0.05	1.13±0.05
Day 84-98	1.19±0.04	1.13±0.05	1.07±0.15
Day 0-98	1.02±0.01	0.98±0.01	1.01±0.06
Protein efficiency ratio			
Day 0-14	3.40±0.06^{a}	2.87±0.13^{b}	3.04±0.16^{b}
Day 14-28	2.60±0.06	2.29±0.02	2.55±0.05
Day 28-42	2.49±0.08	2.21±0.19	2.31±0.06
Day 42-56	2.40±0.23	2.42±0.15	2.34±0.24
Day 56-70	2.31±0.03	2.38±0.06	2.17±0.22
Day 70-84	2.00±0.18	2.03±0.11	1.86±0.09
Day 84-98	1.76±0.05	1.79±0.08	1.98±0.25
Day 0-98	2.08±0.05	2.04±0.03	2.06±0.12

^{ab}Mean±SD within rows with different superscripts are significantly different ($P<0.05$).

Table 5.6 Condition factor, visceral-somatic index, hepato-somatic index and carcass composition of rainbow trout fed diets with 0, 50 and 100% fish oil replaced with camelina oil (CO) at day 0 and after 98 days feeding trial (n=3).

	Day 0	Day 98		
		Control	50%CO	100%CO
Condition factor	N/A	1.58±0.02	1.55±0.03	1.54±0.03
Visceral-somatic index	N/A	14.1±0.1	13.6 ±0.04	13.6±0.4
Hepato-somatic index	N/A	1.51±0.12	1.26±0.07	1.27±0.12
Carcass composition				
Moisture (%)	80.1	69.6±0.2[a]	70.3 ±0.1[b]	70.4±0.2[b]
Crude protein (%)	14.2	16.4±0.1	16.0±0.3	16.0±0.1
Crude lipid (%)	3.2	10.8±0.3	10.2±0.1	10.4±0.3
Ash (%)	1.6	2.4±0.03	2.3±0.3	2.2±0.2

[ab] Mean±SD within rows with different superscripts are significantly different (P<0.05).
N/A data not available.

5.6.3 Growth Performance of Rainbow Trout Fed Solvent-Extracted Camelina Meal or Camelina Protein Concentrate

In the 98-day feeding trial, rainbow trout fry grew from about 1 g to at least 63.0±1.9 g. The inclusion of SECM significantly affected weight gain in the first three weighing periods including day 0-14, 14-28 and 28-42 (Table 5.7). During these periods, fish offered 18%SECM diet gained significantly less weight compared to fish fed control and 6%SECM diets. However, the depression in weight gain was not related to feed consumption (Table 5.7) or FCR (Table 5.8) as these parameters were similar among treatments during these periods ($p>0.05$). The PER during day 0-14 reflected the pattern of weight gain, where fish offered 18%SECM diet had significantly lower PER compared to fish fed control and 6%SECM diets (Table 5.8). But the difference did not exist in the subsequent periods. After 42 days, SECM-containing diets did not affect weight gain, feed consumption, FCR and PER of fish during any period of time (Table 5.8).

Table 5.7 Growth performance of rainbow trout fed either solvent-extracted camelina meal or protein concentrate after 98-day feeding trial (n=3).

Diet	Control	Solvent-extracted camelina meal (%)			Camelina protein concentrate (%)	
		6	12	18	6	12
Parameters						
Initial body weight (g fish^{-1})	1.00±0.01	0.99±0.01	1.00±0.00	1.00±0.01	1.01±0.00	1.00±0.01
Weight gain (g fish^{-1})						
Day 0-14	1.65±0.04^{a}	1.59±0.02^{a}	1.52±0.07^{ab}	1.42±0.06^{b}	1.64±0.03^{a}	1.59±0.08^{a}
Day 14-28	2.87±0.09^{a}	2.80±0.18^{a}	2.45±0.17^{bc}	2.39±0.06^{c}	2.87±0.08^{a}	2.78±0.06^{ab}
Day 28-42	4.65±0.07^{ab}	4.07±0.11^{bc}	4.13±0.23^{bc}	3.85±0.08^{c}	4.93±0.16^{a}	4.27±0.29^{abc}
Day 42-56	7.36±0.72	7.17±0.86	6.43±0.09	5.93±0.21	6.69±0.49	6.87±0.07
Day 56-70	9.9±1.3	9.3±0.5	8.9±0.4	8.5±0.4	9.4±1.1	9.9±1.4
Day 70-84	18.0±1.9	17.8±0.6	16.2±0.3	16.3±1.0	18.8±0.9	17.3±2.0
Day 84-98	26.5±3.4	26.0±0.8	24.4±1.4	24.6±0.8	23.2±2.0	24.3±1.4
Day 0-98	72.2+7.9	68.7+2.5	64.1+1.9	63.0+1.9	67.6+2.7	67.1+5.0
Feed consumption (g fish^{-1})						
Day 0-14	0.99±0.03	0.97±0.02	0.99±0.06	1.02±0.07	1.03±0.09	1.01±0.07
Day 14-28	2.26±0.04	2.12±0.05	1.88±0.07	2.05±0.17	2.25±0.04	2.19±0.08
Day 28-42	3.82±0.15	3.55±0.11	3.53±0.26	3.49±0.20	4.10±0.19	3.71±0.09
Day 42-56	6.2±0.01	6.2±0.3	5.6±0.2	5.6±0.5	6.2±0.4	5.9±0.4
Day 56-70	8.9±1.1	8.9±0.4	8.9±0.4	9.0±0.3	9.4±0.5	9.8±0.7
Day 70-84	18.8±0.7	17.0±1.2	18.2±0.2	17.8±0.9	17.7±0.9	18.7±0.7
Day 84-98	31.5±4.8	28.5±1.0	26.7±1.1	28.6±1.6	30.0±1.3	27.7±1.1
Day 0-98	73.4±7.1	67.2±1.2	65.8±0.6	67.6±3.5	70.6±1.4	69.0±2.2

$^{a-c}$ Mean±SD within rows with different superscripts are significantly different ($P<0.05$).

Table 5.8 Growth performance of rainbow trout fed either solvent-extracted camelina meal or protein concentrate after 98-day feeding trial (n=3).

Diet	Control	Solvent extracted camelina meal (%)			Camelina protein concentrate (%)	
		6	12	18	6	12
Parameters						
FCR						
Day 0-14	0.62±0.00	0.62±0.00	0.60±0.01	0.59±0.01	0.62±0.01	0.61±0.01
Day 14-28	0.79±0.02	0.76±0.07	0.77±0.03	0.86±0.09	0.79±0.02	0.79±0.04
Day 28-42	0.82±0.03	0.87±0.01	0.85±0.01	0.91±0.04	0.83±0.02	0.87±0.08
Day 42-56	0.85±0.08	0.87±0.08	0.87±0.02	0.94±0.06	0.93±0.07	0.85±0.05
Day 56-70	0.9±0.01	0.95±0.01	1.01±0.02	1.06±0.09	1.00±0.08	1.00±0.07
Day 70-84	1.05±0.10	0.96±0.10	1.12±0.03	1.10±0.11	0.94±0.08	1.09±0.09
Day 84-98	1.19±0.04	1.10±0.01	1.10±0.05	1.16±0.04	1.29±0.08	1.14±0.11
Day 0-98	1.02±0.01	0.98±0.03	1.03±0.02	1.07±0.07	1.05±0.03	1.03±0.06
PER						
Day 0-14	3.40±0.06^{a}	3.28±0.09^{a}	2.96±0.08^{ab}	2.65±0.08^{b}	3.12±0.31^{ab}	3.05±0.07^{ab}
Day 14-28	2.60±0.06	2.63±0.24	2.50±0.10	2.22±0.21	2.48±0.05	2.46±0.11
Day 28-42	2.49±0.08	2.29±0.02	2.25±0.04	2.09±0.09	2.35±0.06	2.22±0.20
Day 42-56	2.40±0.23	2.31±0.19	2.22±0.05	2.01±0.12	2.10±0.17	2.27±0.13
Day 56-70	2.31±0.03	2.24±0.02	2.13±0.04	1.97±0.16	2.10±0.16	2.09±0.15
Day 70-84	2.00±0.18	2.24±0.24	1.90±0.05	1.91±0.18	2.21±0.19	1.91±0.16
Day 84-98	1.76±0.05	1.95±0.01	1.95±0.08	1.80±0.06	1.61±0.11	1.83±0.16
Day 0-98	2.08±0.05^{ab}	2.15±0.07^{a}	2.04±0.04^{ab}	1.91±0.11^{b}	1.96±0.06^{ab}	1.99±0.12^{ab}

^{ab}Mean±SD within rows with different superscripts refer to significant differences (P<0.05).

Over the period of 98 days, fish fed SECM-containing diets showed similar weight gain, feed consumption, FCR and PER to fish fed control diet ($p>0.05$). However, PER of fish fed 6%SECM diet was higher than fish fed 18%SECM diet ($p<0.05$). Trout fry fed any SECM-containing diets had similar condition factor, VSI, HSI and carcass composition after the 98-day trial (Table 5.9).

The inclusion of either 6 or 12% CPC in trout diets did not affect weight gain, feed consumption, FCR or PER during any weighing period ($p>0.05$; Table 5.7 and 5.8). Similarly, the overall weight gain, feed consumption, FCR or PER over the entire experiment were not different among control and CPC-containing diets ($p>0.05$). No differences were found in condition factor, VSI and carcass composition at day 98 (Table 5.9). The HSI was lower in trout fed 6%CPC diet than the control ($P<0.05$; Table 5.9).

During day 28-42, trout fed 6%CPC diet gained more weight than fish fed 6%SECM ($P<0.05$; Table 5.7). On day 98, trout offered 6%CPC diet had lower VSI and HSI than the 6%SECM (Table 5.9). Other than that, dietary inclusion of either 6% SECM or CPC had similar effects on all growth parameters. Trout offered 12% of either SECM or CPC diets performed the same during the 98-day trial ($p>0.05$).

Table 5.9 Condition factor, visceral-somatic index (VSI), hepato-somatic index (HSI) and carcass composition analysis of rainbow trout fed either solvent-extracted camelina meal or protein concentrate at day 0 and after 98 days feeding trial (n=3).

	Day 0	Day 98					
		Control	Solvent extracted camelina meal (%)			Camelina protein concentrate (%)	
			6	12	18	6	12
Condition factor	N/A	1.58±0.02	1.59±0.07	1.55±0.01	1.61±0.05	1.55±0.03	1.52±0.03
VSI	N/A	14.1±0.1^{bc}	15.4±0.5^{ab}	15.2±0.4^{abc}	16.2±0.8^{a}	14.1±0.2^{c}	14.9±0.6^{bc}
HSI	N/A	1.51±0.12^{a}	1.51±0.09^{a}	1.37±0.03^{ab}	1.45±0.07^{ab}	1.26±0.09^{b}	1.40±0.07^{ab}
Carcass composition							
Moisture (%)	80.1	69.6±0.2	70.6±1.0	70.4±0.2	70.9±0.3	69.7±0.8	70.6±0.5
Crude protein (%)	14.2	16.4±0.1	15.0±0.4	16.0±0.3	16.0±0.2	16.5±0.5	15.9±0.2
Crude lipid (%)	3.2	10.8±0.3	10.1±0.3	10.1±0.4	9.7±0.3	10.5±0.7	9.8±0.4
Ash (%)	1.6	2.4±0.03	2.4±0.2	2.4±0.2	2.2±0.01	2.3±0.1	2.4±0.2

$^{a-c}$Mean±SD within rows with different superscripts are significantly different ($P<0.05$).
N/A data not available.

5.7 Discussion

Most of the previous research was conducted on salmonids during their grow-out stage when they consume the most significant amount of feed during the entire lifecycle. A recent study shows that early nutritional intervention on the young fish, with fast growth rate and little flavor experience, allows them to reprogram their metabolism and better adapt to plant ingredients in later life (Geurden et al., 2013). Young rainbow trout (1.0 g) were used as the experimental fish in the current study to evaluate inclusion of plant ingredients, CO, SECM, and CPC in their diets.

In the present study, up to 100% fish oil was replaced with CO diets without detrimental effects on all the measured biometric parameters including growth performance, nutrient utilization and proximate carcass composition of rainbow trout fry after 98 day feeding trial. The results were in agreement with the implication of using CO, or other vegetable oils in trout diets (Bullerwell et al., 2016; Hixson et al., 2014a; Drew et al., 2007; Richard et al., 2006).

Hixson et al. (2014a) indicated that incorporation of CO increased the total n-3 fatty acid in the diet, due to its high content of ALA. However, the n-3/n-6 fatty acid ratio of the 100%CO diet decreased by half from 2.3 to 1.2 due to the high content of linoleic acid (18:2n-6) in CO. The EPA (11.8 to 2.2% ww^{-1}) and DHA (6.8 to 2.8% ww^{-1}) levels were also reduced. The fatty acid profiles of trout muscle were closely related to the diets, where the n-3/n-6 ratio decreased from 3.9 to 1.6, EPA and DHA decreased significantly from 9.5 to 2.3% ww^{-1} and 13.4 to 6.6% ww^{-1} respectively. In other cases, full

replacement of fish oil with terrestrial plant oil leads to a reduction in plasma cholesterol and lipoprotein lipase (Richard et al., 2006). In trout liver, gene expression of lipoprotein lipase receptor was down-regulated (Richard et al., 2006). The current study has proven effective replacement of fish oil with CO in the diet of young trout. It would be beneficial to enhance results by investigating the fatty acid profiles of fish tissues, potential metabolic alteration or immune response when CO is added to the diet.

Trout fry tolerated up to 18% of SECM or 12% of CPC in their diet without reductions in any overall growth parameters or changes in approximate body composition. However, higher inclusion levels of SECM, 18% in particular, did require an acclimation period to the diet and resulting in significantly lower weight gain possibly due to poor protein utilization efficiency at the beginning of the trial. Our results agree with Bullerwell et al. (2016) that up to 20% inclusion of the high-oil residue camelina meal (HORM) had no negative impacts on the growth performance of rainbow trout with a similar initial weight (1.0 g) over a 112-day trial. In another trial, rainbow trout with an initial weight of 2 g were reported to tolerate only up to 15% of dietary inclusion of SECM in a 112-day trial (Bullerwell et al., 2016). Hixson et al. (2015a) reported that trout with the initial weight of 43.4-45.9 g could tolerate 14% SECM in their diet without depressed growth performance in a 84-day trial. In the present trial, both 6 and 12% of CPC were successfully incorporated in trout fry diets without negative effects on their growth, feed utilization, and proximate body composition after 112 days. The current study was the first to test CPC in rainbow trout fry diets, therefore there are no studies with which to compared these results. Due to the technical difficulty of producing CPC on a large-scale,

a limited amount of CPC was available, and the inclusion levels were limited to 6 and 12%. Higher dietary inclusion levels of CPC are recommended in the future studies to identify its optimal incorporation level into rainbow trout diet.

Different camelina products have different levels of crude protein. Ground camelina seed, HORM and SECM contain 25.5, 35.7, and 39.0% crude protein, respectively (as fed basis) (Bullerwell et al., 2016; Hixson et al., 2015a). The SECM and CPC used in the current study have 38.7 and 52.5% of crude protein respectively (as fed basis). When formulated into diets, the protein contribution of different camelina product at different dietary inclusion levels varies. Table 5.10 illustrated the contribution of crude protein from camelina products to the total crude protein in the diet (% as fed basis) using diets from two previous camelina studies and the current study. When camelina protein accounted for 16.5% of the total crude protein in the diet, rainbow trout showed depressed growth (Bullerwell et al., 2016; Hixson et al., 2015). In our study, the highest camelina protein contribution to the total crude protein was 13.2 and 14.5%, which was under the threshold of 16.5% and did not cause adverse growth performance on rainbow trout. Based on this comparison, it unfortunately cannot be concluded that smaller fish have a higher tolerance to camelina protein but they appear to be able to tolerate high levels of SECM itself.

Table 5.10 Comparison of camelina protein contribution to total crude protein in different trials.

Dietary inclusion rate (%)	Camelina protein contribution to the total crude protein												Initial weight of fish
	0	5	6	7	10	12	14	15	18	20	21	30	
Groud Camelina Seed[1]	0				5.6					11.1		16.5*	1.0
HORM[1]	0				8.0					16.1		23.7*	1.0
SECM[1]	0	4.4			8.6			12.9		17.6*			2.4
SECM[2]	0			5.9			12.2				18.0*		44.9
SECM (day 0-56)[3]	0		4.6			8.9			13.2				1.0
SECM (day 56-98)[3]	0		5.0			9.9			14.5				1.0
CPC (day 0-56)[3]	0		5.7			11.3							1.0
CPC (day 56-98)[3]	0		6.1			12.1							1.0

HORM= high oil residue meal; SECM= solvent-extracted meal; CPC= camelina protein concentrate.
[1]Bullerwell et al., 2016
[2] Hixson et al., 2015a
[3]Current study
*Inclusion levels that resulted in growth reduction

Fish fed the 18%SECM diet gained significantly less weight during the first three weighing periods and had a lower PER during day 0-14. The same pattern was found in studies by Bullerwell et al. (2016) and Hixson et al. (2015a) where high inclusion rates, both 20 and 21% of SECM diets led to significant reductions in weight gain and PER. The reduced weight gain may have been a result of difficulties in protein utilization. The apparent digestibility coefficient for crude protein of SECM was 85-87% for rainbow trout (Fraser et al., 2017). This value was lower than some commonly used salmonid feed ingredients such as solvent extracted soybean meal (90-99%), soy protein concentrate (98-100%), canola protein concentrate (90%), or fishmeal (95%, NRC, 2011). The apparent ileal digestibility of crude protein and amino acid in high oil residue camelina meals were mostly similar compared to canola meal when fed to growing pigs (Almeida et al., 2013). Amino acid digestibility of camelina meal products by salmonid fish has not been reported, which make it difficult to know the bioavailability of amino acid from these plant protein sources.

High fiber content and antinutrients such as glucosinolates in SECM might have affected the nutrients utilization by rainbow trout. The SECM used in the current study contained a 38% total fiber and 25% non-starch polysaccharides. The primary source of fiber is the hull. Kracht et al. (2004) indicated that the dehulling procedure drastically decreased the total fiber content of rapeseed cake by 40%. Crude protein content of the rapeseed cake increased by 13%, and the digestibility of crude protein by piglet significantly increase from 65±3.1 to 79±3.9% (Kracht et al., 2004). At present, due to the small size of

camelina seed, dehulling process has not been established. Future work on processing would be beneficial to improve the nutritional value of camelina meal.

Both 6 and 12% CPC were successfully incorporated into the trout fry diets without negative effects on their growth, feed utilization, and proximate body composition in the 112-day trial. The current study was the first to test CPC in rainbow trout fry diets. The CPC used in the current trial had a crude protein content of 52%. The crude protein content was considerably low when compared to the corn protein concentrate (75%) and soy protein concentrate (70%). Another small batch of CPC made from SECM (lower level of residual oil) by the author had higher protein content (69%) demonstrating the potential of the current processing procedure and making it more reasonable as an alternative protein concentrate.

The apparent digestibility of crude protein from CPC was 78% by rainbow trout (Fraser et al., 2018). It was lower than that of canola protein concentrate at 90%, soy protein concentrate at 98-100% or fishmeal at 95% (Fraser, 2017; NRC 2011; Thiessen et al., 2004). Unlike camelina, canola and soybean products have been commercialized as feed ingredients, and the procedure to produce canola protein concentrate or soy protein concentrate has been optimized. Research is needed to produce better quality CPC on a large scale to achieve the goal of commercialization of this product.

5.8 Conclusions

Up to 100% fish oil can be replaced with CO diets without affecting any of the measured biometric parameters including growth performance, nutrient utilization and proximate carcass composition of rainbow trout fry after a 98-day feeding trial. Up to 18% SECM or 12% CPC can be included in the diet of rainbow trout fry without negatively affecting growth performance, or proximate body composition. However, higher inclusion levels of SECM, 18% in particular required an acclimation period for fish to adjust to this experimental diet.

Chapter 6

Conclusions and Future Directions

6.1 Conclusions

Three feeding trials were conducted to evaluate the effects of using camelina products including camelina oil (CO), solvent-extracted camelina meal (SECM) and camelina protein concentrate (CPC) as feed ingredients for salmonid fish at early life stages.

Fish in all three trials showed acceptance of diets with 100% fish oil replaced with CO, based on growth performance and proximate carcass composition. The diet with highest SECM inclusion rate tested, 18%, significantly encouraged feed consumption and subsequently weight gain of first feeding trout after 112 days. Both salmon and trout fry performed well on the 18%SECM diet when compared to the controls ($p>0.05$). The 18%CPC diet resulted in over 50% of mortality rate of first feeding trout and significantly poorer growth after 56 days and was terminated at that time. The highest level of CPC tested, 12%, in trout fry diet did not lead to any difference in growth when compared to the rest of the treatments.

Based on the results of current study, it is recommended that up to 100% of added fish oil can be replaced with CO in diets of first feeding rainbow trout, rainbow trout fry and Atlantic salmon fry. Up to 18% of SECM can be incorporated in both rainbow trout fry and Atlantic salmon fry diets. Up to 12% dietary inclusion of CPC in rainbow trout fry diet is recommended.

6.2 Future Directions

Future studies involving fatty acid compositions of fish tissues would be beneficial to elucidate the impact of the changes in dietary fatty acids when replacing dietary fish oil with CO.

Higher levels of SECM than 18% should be tested in both Atlantic salmon and rainbow trout fry diets in future studies. Higher dietary inclusion levels of CPC should be tested in rainbow trout diet in future studies. Morphology of the intestine should be examined when camelina protein products are incorporated in salmonid diets. The profile of glucosinolates and their degradation products in both camelina protein products and salmonid diets should be studied to understand the feeding response and growth performance of the fish.

Future study is recommended on developing a procedure to dehull camelina seeds. It could potentially remove a large portion of fibers and other antinutritional factors of camelina protein products and may improve their digestibility as animal feed ingredients. Enhancing the procedure for CPC production with higher crude protein content on larger production scale would be helpful for CPC commercialization. This higher crude protein content CPC may compete with other popular commercial protein concentrates such as soy protein concentrate, canola protein concentrate, and corn protein concentrate.

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Appendix A: Rainbow Trout with Symptoms Including Skin Discoloration, Scoliosis, Lordosis or Hemorrhage.