PROCESSING OF CAMELINA SATIVA OILSEED TO EVALUATE DIGESTIBILITY FOR POTENTIAL INCLUSION IN DIETS FOR COLDWATER CARNIVOROUS FISH.

by

Jamie May Fraser

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DEDICATION

I would like to dedicate this thesis to my father, James Edward Fraser, who died on November 13th, 2014.

He taught me that beauty can be seen in everything, including crows. He always felt that they were the most beautiful bird.

He also said that celery and honey will kill you; it's what the ancient Romans ate. These are two foods which never entered his diet.

JELLO is good at any time.

I will always remember his compassion for the little guy or the underdog.

He taught me to love animals big and small.

I miss your mischievous smile every day. Love you Dad.

I also want to dedicate this thesis to my maternal grandmother, Helen Langille, who I have always looked up to. Love her stories about being in the Royal Canadian Air Force during World War 2. She is my hero.

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ABSTRACT

Camelina sativa is an oilseed with potential to be processed into several novel plant-based protein or lipid rich by-products for use in fish feeds. Digestibility values are needed to formulate balanced diets using these feedstuffs. Camelina full fat seed, oil, expelled meal (HORM), and solvent extracted meal (SEM) were developed. Apparent digestibility values were determined for Atlantic cod (AC), Atlantic salmon (AS), and rainbow trout (RT). Digestible protein (DP; %) content was established for the seed, HORM, and SEM for the AC (23.0, 32.6, 34.1, respectively), AS (23.9, 33.4, 35.6, respectively) and RT (21.7, 33.4, 35.2, respectively). Digestible energy (DE; kcal/kg) content was established for the oil (AC, 6596; AS, 8524; RT, 8063). There was no effect from toasting SEM for RT. Water and phytase enzyme pre-treatment of SEM for 24 hours removed the glucosinolates and phytate completely (RT; DP, 35.44%; DE, 3185 kcal/kg). Based on digestibility values, these products show good potential for use in aquaculture feeds.

LIST OF ABBREVIATIONS USED

AA	Amino Acid
AC	Atlantic cod (*Gadus morhua*)
ADC	Apparent Digestibility Coefficient
ADF	Acid Detergent Fibre
AIA	Acid-Insoluble Ash
ALA	Alpha-linolenic acid
AOAC	Association of Official Analytical Chemists
AS	Atlantic salmon (*Salmo salar*)
CFIA	Canadian Food Inspection Agency
CP	Crude Protein
CS	*Camelina sativa*
DE	Digestible Energy
DFO	Department of Fisheries and Oceans Canada
DHA	Docosahexaenoic Acid
DM	Dry Matter
DN	Digestible Nutrient
DP	Digestible Protein
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed Conversion Ratio
FFS	Full-Fat Seed

HORM	High Oil Residue Meal (Expelled Meal)
PUFA	Poly-unsaturated Fatty Acids
MM	Mixed Enzyme Soaked SEM Meal
MUFA	Mono-unsaturated Fatty Acids
Nd	Not Detected
NDF	Neutral Detergent Fibre
NR	Not Required
Nr	Not Reported
NRC	National Research Council
NT	Not Tested
PC70	Protein Concentrate (70% CP)
PC80	Protein Concentrate (80% CP)
PM	Bio-phytase Soaked SEM Meal
PUFA	Poly-unsaturated Fatty Acids
RT	Rainbow Trout (*Oncorhynchus mykiss*)
SEM	Expelled Solvent Extracted Meal
sem	Standard Error of the Mean
SM	superzyme-OM Soaked SEM Meal
SPC	Soy protein concentrate
TDC	True Digestibility Coefficient
TSE	Toasted SEM
WM	Water Soaked SEM Meal

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CHAPTER 1 INTRODUCTION

Novel plant-based protein and lipid sources are of particular interest in the aqua-feed industry due to a number of factors. These factors include an increase in aquaculture production, low availability of marine feed ingredients, and the rising costs and decreasing availability of current plant-based protein and lipid sources (Food and Agriculture Organization of the United Nations (FAO), 2012). To replace animal protein by-products in fish diets, including fishmeal, plant-based protein sources must have a crude protein of equal or greater than 65%, with a suitable amino acid profile that is high in limiting amino acids like methionine and lysine (Lim et al., 1998; Samocha et al., 2004; Lim et al., 2008). Plant protein sources do have value in fish diets but are often limited due to their high carbohydrate content (Gomes et al., 1995; Tibbetts et al., 2006). Plant-based protein sources should have a low anti-nutrient content, or have a process in place that can remove anti-nutrients from the product (reviewed by Jobling, 2016; reviewed by Krogdahl et al., 2010). Plant-based lipid sources must have a fatty acid profile that is high in n-3 fatty acids, have a low n-6 to n-3 ratio, and contain or provide precursors for biosynthesis of eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) for those species that can perform biosynthesis (reviewed by Tocher, 2015; reviewed by Turchini et al., 2008).

Camelina sativa is an oilseed that has been subjected to renewed interest within academic circles, particularly in relation to aquaculture feeds, due to its high crude protein content and its high concentration of n-3 fatty acids, especially α-linolenic acid (ALA; Zubr, 1997). ALA is the precursor to desaturation and elongation biosynthesis of EPA and DHA (Codabaccus et al., 2011). Therefore, higher levels of ALA and a low n-6 to n-3 ratio would be beneficial in order to increase the rate of bioconversion and in turn increase the EPA and DHA content obtained from

the diet of farmed animals (Harnack et al., 2009). When fed to swine, the crude protein content of camelina meal was high in arginine, but low in methionine and lysine (Kahindi et al., 2014). This finding suggests methionine and lysine could be a limiting factor that need to be supplemented with commercial amino acids (Espe et al., 2006; Hansen et al., 2007; National Research Council (NRC), 2011).

One of the major issues with using plant-based protein sources in carnivorous fish diets is the presence of anti-nutrients. Anti-nutrients are naturally produced chemicals that deter plants from being eaten by herbivores (reviewed by Gatlin III et al., 2007). They typically decrease palatability and/or affect the digestion and absorption of nutrients (Kaushik et al., 1995). The anti-nutrients in camelina seed and meals include glucosinolates, phytate, tannins, trypsin inhibitor, and fibre (Espe et al. 2006; Hansen et al., 2007; Kahindi et al., 2014). These anti-nutrients likely can be removed or inactivated with processing or pre-treatments before being added to fish diets similar to closely related oilseeds (Higgs et al., 1995). However, the current literature indicates research on the elimination of anti-nutrients from camelina by-products is limited (Rajapakse, 2015; Fraser et al., 2016). An additional benefit of today's technology is that plants, like camelina, can be genetically altered to reduce the anti-nutrients or to contain higher levels of nutritionally required components such as EPA and DHA (Betancor et al., 2015). Before a product can be used in a balanced diet, the digestibility of the ingredients must to be determined in order to calculate the digestible nutrient content to facilitate diet formulation.

Digestibility is defined as the percent of the total nutrient that is absorbed and utilized by the animal. It is determined by calculating the difference between the amount of nutrient ingested to

the amount of nutrient excreted. Digestibility values for *Camelina sativa* seed and its by-products have not been determined for use in Atlantic cod, Atlantic salmon, and rainbow trout diets. These three species are important or potentially important for aquaculture in the Atlantic Provinces of Canada. Camelina seed is mechanically processed on a commercial basis to produce a high valued organic vegetable oil for human consumption, resulting in a by-product of mechanically processed camelina meal (HORM). Determining a use for the resulting HORM by-product is a must for commercial processing facilities, the feed industry is a likely consumer of the HORM. Currently, novel camelina by-products produced from the HORM must be created in a lab-scale setting, as these products are not manufactured on a commercial basis. This current study produced eight novel camelina by-products, expelled solvent extracted camelina meal (SEM), toasted SEM, incubated SEM with water and three enzyme treatments, and two camelina protein concentrates. The digestibility of each product was tested and digestible nutrients were calculated. This data will be valuable for formulation of balanced diets for future growth studies and application in commercial feed formulations.

CHAPTER 2 LITERATURE REVIEW

2.1 STATE OF FEED USE IN AQUACULTURE

The global aquaculture industry produced 73.8 million metric tonnes (MMT) of fish, crustaceans, and molluscs in 2014, of which Canada produced 133,783 tonnes (Food and Agricultural Organization of the United Nations (FAO), 2016a). Aquaculture contribution to global food fish production has tripled from 13.4% in 1990, to 44.1% in 2014 (FAO, 2016a). Of this, 3.4 MMT were salmon, trout, and smolt, and 1,702 tonnes were cod, hake, and haddock, valued at around $CDN 20.1 billion and $CDN 8.0 million, respectively (FAO, 2016b). Aquaculture is a fast growing food production sector in the world. In 2014, Canada's total finfish aquaculture production was 93,656 tonnes ($CDN 650 million), of which salmon production represented around 84.3% (78,979 tonnes; $CDN 578 million) of the total production, followed by trout with an additional 7.2% (6,698 tonnes; $CDN 38 million; Department of Fisheries and Oceans Canada (DFO), 2016). Finfish production in the Atlantic Canadian Provinces, shown in Table 2.1, injects millions of dollars into the local rural economy.

In 2008, 81.2% of the global farmed fish and crustaceans were fed manufactured aquaculture feed. This production included 8.3% for marine fish, 7.0% for salmon and 3.0% for trout (FAO, 2012). During the same year, 29.2 million tonnes of aqua feeds for aquaculture were produced (FAO, 2012). It was reported that the global aquaculture feed production in 2014 reached 40 MMT worth around $CDN 22.2 billion, this was an increase in production of about 10.9 million metric tonne in 6 years (International Feed Industry Federation (IFIF), 2015). This increase in aquaculture feed production is due to the exponential growth of aquaculture fish production (FAO, 2014). Since the caught wild fish sector has remained stagnant over the past 10 years, the

Table 2.1. Canadian production value of aquaculture raised finfish in 2014 by province* (DFO, 2016).

Province	2014 Finfish Production (tonnes)	2014 Finfish Production Value ($CDN)
British Columbia	54,971	380,354,000
New Brunswick	17,184	117,944,000
Nova Scotia	6,824	49,664,000

*All other provinces either do not produce aquaculture finfish or are not reporting.

aquaculture industry has had to increase its production to provide an adequate fish protein source for human consumption (FAO, 2014). The aquaculture sector is expected to continue to grow, resulting in a larger aquaculture feed sector (FAO, 2014). Adequate nutrition contributes to around 50% of total fish production costs among farmed carnivores. The three main sources of nutrients in aquaculture feeds come from animal (aquatic and terrestrial), plant, and microbial sources, the amount of each is dependent of availability and cost for each region (FAO, 2012).

2.2 ANIMAL-BASED INGREDIENTS

There are several animal-based nutrient sources that can be used in aquaculture feed, including fishmeal and oil, terrestrial meat and bone meals, terrestrial animal fats, and blood meal (FAO, 2012; Lim et al., 2008). Fishmeal is utilised primarily for carnivorous fish species, due to its high protein content, amino acid profile, and digestibility. Fish oil is used due to its high levels of essential n-3 fatty acids, specifically EPA and DHA (reviewed by Turchini et al., 2009; NRC, 2011; Boyd, 2015; reviewed by Tocher, 2015). In 2014, 20.9 million tonnes of fish product was utilised for non-food uses, and seventy-six percent (15.8 million tonnes) of this was used in fishmeal and oil production (FAO, 2016a). Some of the major users of fish oil are diets for

salmon (37%), marine fish (25%), and trout (16%). For these fish, typical fishmeal dietary levels are 14%, 19%, and 6%, respectively (FAO, 2012).

The current supply of fishmeal cannot meet the ever increasing demand of the aquaculture industry. Exacerbating the issue is the increased demand for use within other animal sectors such as poultry, swine, ruminant, and companion animal industries (reviewed by Hardy, 2010; NRC, 2011; Boyd, 2015). Fish oil will be a limiting ingredient sooner than fishmeal because it is produced in a smaller quantity from the pressing process of fish to produce fishmeal (Boyd, 2015). Therefore the price is increasing for these products (Boyd, 2015). In October 2015, the wholesale price of fishmeal (65/66% protein) was around $US 1410-1430/tonne and crude bulk fish oil was $US 2000-2050/tonne (FAO, 2014; Bacon, 2015). Other marine animal-based feed ingredients are potential sources of essential amino acids, like methionine and lysine (Tacon, 1995; Naylor et al., 2009; NRC, 2011). These include crab meal, krill meal, and fish protein hydrolysates (derived from fish processing waste; Tibbetts et al., 2006).

Terrestrial animal protein sources include meat and bone meal, blood meal, feather meal, and poultry meal. Terrestrial animal fat sources, like poultry grease, tallow, lard, and animal/vegetable blends are also available for use in feeds, however they are high in saturated fatty acids which some fish find harder to digest, such as Atlantic halibut (*Hippoglossus hippoglossus*; Martins et al., 2009). These by-products are derived from slaughter plant processing wastes, therefore when using these ingredients there is the possibility of passing on disease to other livestock systems, particularly if they are poorly processed (Naylor et al., 2009). The value of fishmeal and oil is two to three fold higher than the terrestrial animal by-products such as meat and bone meal ($US 420-450/tonne), feather meal ($US 530-550/tonne), and

poultry meal ($US 600-620/tonne; Bacon, 2015). Yellow grease (tallow) is currently selling for around $US 335/tonne, which is about six times less expensive than fish oil (United States Department of Agriculture (USDA), 2015). Quality and availability of animal by-products are variable depending on the market trends and sources (Boyd, 2015).

2.3 SIX FACTORS AFFECTING PLANT-BASED INGREDIENTS IN CARNIVOROUS FISH DIETS

The high price of fishmeal and fish oil is driving the aquaculture industry to utilize plant-based ingredients as they are readily available and cost effective (reviewed by Lim et al., 2008). The most commonly used plant ingredients are soybean, corn, canola, and wheat (FAO, 2012). The demand for plant-based products is expected to increase, for instance, soy protein concentrate is expected to increase to over 2.8 million tonnes by 2020 (FAO, 2012). Six factors must be taken into consideration for a plant ingredient to be used in aquaculture feed. The first factor is low cost compared to currently used protein and lipid sources (reviewed by Tantikitti, 2014). Soybean meal (49% protein) and soybean oil currently sells for $US 332-392/tonne and $US 738/tonne, respectively, about four times cheaper as a protein source and two times cheaper as an oil source compared to fish products (Bacon, 2015; Commodity Basis, 2015). Currently, camelina is a novel oilseed, therefore the pricing and marketing of its products are not commercially well known or established. A commodity price index is unknown.

The second factor is protein content. For a plant-based product to be considered it must have a high crude protein content with an amino acid profile that is high in essential amino acids, such as lysine and methionine (reviewed by Glencross et al., 2007; Tantikitti, 2014). Soybean meal

(48% protein) is high in cysteine but limiting in lysine, methionine and threonine, which are essential amino acids in fish diets (Chowdhury et al., 2012). Processing soybean meal into a protein concentrate (SPC) increases the amino acid level relative to carbohydrate removed, but the amino acid profile remains limiting compared to herring meal (Jeong et al., 2016). Corn is more limiting in lysine compared to soybean, but higher in methionine levels (NRC, 2011; Miller, 2004). Corn is a popular feed ingredient due to its abundance, and it provides starch to act as a binder during extrusion and it can provide ingredients with protein concentrations as high as 75% (Empyreal 75, Cargill Corn Division, 2016; Barrows and Sealey, 2015). Camelina meal is around 39.0% crude protein, with a similar methionine and cysteine level to soybean meal and canola meal at 1.6% as fed (Thacker and Widyaratne, 2012; Ye et al., 2016). However, camelina meal is low in lysine (1.6% as fed), which should be considered when formulating rations (Thacker and Widyaratne, 2012).

The third factor is the fatty acid profile (reviewed by Turchini et al., 2009; Tantikitti, 2014). Plant oils tend to be high in n-6, n-9 and saturated fatty acids, such as linoleic acid (18:2n-6), oleic acid (18:1n-9), palmitic (16:0) and stearic acids (18:0), respectively (Turchini et al., 2011). Conventional plant oils do not contain long chain n-3 fatty acids, EPA and DHA, which are essential for fish health, growth and marketability (Codabaccus et al., 2011). However, certain plant oils, such as flaxseed oil, can provide other omega 3 fatty acids, such as ALA (53.4% of total lipid), which can be utilized as a precursor for EPA and DHA by some fish species (Rajaram, 2014; Codabaccus et al., 2011). The ability of the fish to bio-convert the ALA to EPA and DHA by elongation and desaturation enzymes is species dependent (Tocher, 2015), and environmental salinity dependent, as the enzyme bio-conversion was lower in marine fish

(camelina oil, Atlantic cod, 6.1% converted) compared to freshwater fish (camelina oil, Atlantic salmon, 25% converted, rainbow trout, 23% converted; Hixson et al., 2014). The internal milieu remains the same for freshwater and marine fish (osmolality, 300 mosmol/kg), this is achieved by morphological changes to the fish to allow it to either absorb salt and excrete water (freshwater) or absorb water and excrete salt (marine) (Hwang et al., 1989). The gene expression of Δ6 desaturase in the liver of Atlantic salmon fed rapeseed diets was lower in saltwater (smolt) treatment versus the freshwater (parr) (Codabaccus et al., 2011). The enzyme activity for elongation and desaturation of ALA is lower in the marine fish due to the higher salinity triggering a change in the digestive enzymes produced to allow for the changes in excretion and absorption of salt (Bell et al., 1997; Tocher et al., 2010; Sarker et al, 2011). It is hypothesized that due to the drinking of seawater during osmoregulation activity of marine fish, the transport and uptake of fatty acids changes for a reason that is currently unknown. Transgenic plants, on the other hand, can be modified to contain high levels of EPA and DHA to provide a terrestrial source of these long-chain polyunsaturated fatty acids without the need to bio-convert (Ruiz-Lopez et al., 2014). Camelina oil contains high levels of ALA (30.8% of oil), which could be utilized by the fish to convert to EPA and DHA (Thacker and Widyaratne, 2012).

Anti-nutrients are the fourth factor relevant to the choice of plant products for aquaculture feed (reviewed by Francis et al., 2001; Tantikitti, 2014). They include carbohydrate fractions (non-starch polysaccharides and oligosaccharides), seed proteins (antigenic, protease inhibitors, and lectins), oestrogenic compounds, phytic acid, and saponins (Matthäus and Zubr, 2000; Francis et al., 2001). Some of these naturally occurring chemical products are produced by plants to provide protection. A high anti-nutrient content can cause an array of negative impacts including,

but not limited to, reduced nutrient availability, palatability and growth, and cause intestinal irritation and liver damage (Ye, 2014; Hixson et al., 2014a). However, 'anti-nutrients' can have some beneficial effects, acting as antioxidants, immune boosters, pellet stabilizers, and prebiotics, dependant on the amount consumed (Matthäus and Zubr, 2000). Some anti-nutrients can be eliminated with processing techniques, such as heat-treatment or chemical removal. Alternatively, a plant can be bred to produce lower levels of problematic anti-nutrients. For example, canola is a cultivar of rapeseed that was bred to produce an oilseed with lower levels of glucosinolates and erucic acid (Canola Council of Canada, 2014). Some anti-nutrients present in camelina seed are glucosinolates (18.5 mmol/kg), phytic acid (29.9 g/kg), sinapine (2.32 g/kg), and condensed tannins (3.10 g/kg) (Russo and Reggiani, 2012). This study will look at reducing the glucosinolates and phytic acid using mechanical and enzymatic processes.

The fifth factor in choosing a plant product is feed ingredient palatability. High palatability encourages the fish to eat however, certain plants containing high glucosinolates and/or phytates have low palatability (reviewed by Tantikitti, 2014; Nyina-Wamwiza et al., 2010). To increase palatability, off-tasting flavours and smells can be masked or removed by processing methods, plant breeding, and/or with the addition of attractants to the feed formulation (Gatlin III et al., 2007). Gilthead seabream (*Sparus aurata* L.) have a reduced feed intake when fed diets containing soy and rapeseed protein concentrate compared to a fishmeal-based control diet (Kissil et al., 2000). Feed intake was decreased due to reduced palatability and it was suggested that the addition of feed attractant to diets containing these ingredients would be beneficial (Kissil et al., 2000). Similarly, feeding soy protein concentrate to rainbow trout reduced food intake due to palatability concerns (Stickney et al., 1996). Palatability is of some concern when

using camelina meals in feed due to the glucosinolate (18.5 mmol/kg) and sinapine (2.32 g/kg) present in the seed, which can cause a bad taste or smell to the feed (Russo and Reggiani, 2012; Ryhänen et al., 2007). Palatability was negatively affected in broilers fed camelina expeller meal (Ryhänen et al., 2007). However, no palatability effect occurred with pigs fed camelina expeller meal (Almeida et al., 2013). Atlantic salmon showed no difference in feed consumption when fed camelina oil and solvent extracted meal versus a control diet, therefore indicating camelina caused no difference to the palatability of the diet (Ye et al., 2016). The palatability between camelina products may be different and will be shown in this experiment via the feed consumption data collected in the experimentation.

The sixth factor is digestibility. Plant-based products need to be highly digestible to be suitable for fish feed, allowing the nutrients in the plant-based feed ingredients need to be highly available (reviewed by Gatlin III et al., 2007). Digestibility is the measure of the amount of nutrient provided from food that is able to be utilised by an animal (Lloyd et al., 1978). It is measured by calculating the difference between nutrient in food given and the nutrient that is excreted after digestion (Lloyd et al., 1978; Moyle and Cech, 2004). Based on the digestibility value of a food and the total amount of nutrients provided by the food, we can calculate the digestible protein and digestible energy values of the food provided, which are important in order to formulate balanced diets for fish (NRC, 2011). The digestibility of the food provided to animals effects the overall production and health of the animal, such as FCR, growth rate, reproduction, and gut health (Øverland et al., 2009). As providing the proper nutrients to a species allows it to have the energy to fuel the body cells to perform their proper functions (NRC, 2011).The apparent digestibility coefficients for dry matter can be lower due to the

presence of indigestible carbohydrate components, including crude fibre (Tibbetts et al., 2006). Dietary mineral availability may be affected due to the phytate present in plants (Matthäus and Zubr, 2000; Lall, 2002). The current digestibility data says that camelina expeller meal fed to broiler at 15 % inclusion has an energy digestibility of 63.7% (Thacker and Widyaratne, 2012). Swine showed a protein digestibility of between 52.9 to 60.1% for camelina expeller meal (Almeida et al., 2013). Camelina seed, meals and oil need to be investigated to see if they fit the requirements of a replacement plant-based product listed above. Camelina fits the protein and lipid requirements, but it is important to note, however, that digestibility of the resulting protein and lipid from the products of camelina seed needs to be tested to determine if the products meet the sixth factor. This is a current gap in the literature and merits further research and investigation within this research project.

2.4 MICROBIAL-BASED INGREDIENTS

Microbial products, derived from micro-organisms, such as bacteria, yeasts, and moulds, can be added to a product or diets to provide nutrients (Canadian Food Inspection Agency (CFIA), 2013). Microbial derived ingredients are classified as either, viable or non-viable (CFIA, 2013). Viable organisms are live micro-organisms that are directly fed or inoculated into the feed (CFIA, 2013), whereas non-viable are the metabolites or biomass of a product inoculated or exposed to a micro-organism, such as amino acids, vitamins, enzymes, or the yeast by-product from fermentation (CFIA, 2013). Microbial products typically are added to products and complete diets to improve the digestibility of the feed and/or the bioavailability of nutrients. The micro-organisms chosen for the particular diet or product breakdown naturally occurring chemical products in the feed, such as phytate, this makes phosphorus more bio-available to fish

(Cao et al., 2007; Tacon, 1995; Lall, 2002). Microbial ingredients may provide an additional benefit besides breaking down anti-nutrients. An example of this would be the yeast by-product from *Saccharomyces cerevisiae* which can provide ascorbic acid (vitamin C) and certain unidentified growth factors (Fossati et al., 2011). This research project will use microbial-based enzyme ingredients to incubate with water to reduce or remove anti-nutrients, like phytic acid, glucosinolates, and fibre.

2.5 PLANT-BASED REPLACEMENTS IN FISH DIETS

2.5.1 Plant-based usage

Protein concentrates and gluten meals are popular plant products used in aquaculture feeds to replace fishmeal due to their relatively high protein content (Miller, 2004). There is a movement to reduce marine products in carnivorous fish feeds to 50% or less of the diet (FAO, 2012). In 2010, Canadian salmon diets contained 41% plant products, 30% terrestrial animal by-products, and 29% marine by-products (17% fishmeal and 12% fish oil, respectively; Campbell, 2010; Table 2.2). Product usage is dependent on product availability and cost (Campbell, 2010). By comparison, in 2013, Norwegian fish diets consisted mostly of plant nutrient sources (plant proteins, 36.7%; plant oils, 19.2%) which made up the greatest percentage of commercial diets (Ytrestøyl et al., 2015). Marine nutrient sources (marine proteins, 18.3%; marine oils, 10.9%; 2013) are steadily decreasing in Norwegian commercial feeds, there was a decrease in the ratio of the amount of fish used to produce edible aquaculture raised fish in Norway between the years of 2010 (protein, 1.1; fat, 0.9) and 2013 (protein, 0.7; fat, 0.5; Ytrestøyl et al., 2015).

The main limitation of using plant-based ingredients in marine fish diets is they provide insufficient essential nutrients, such as limiting amino acids and omega 3 fatty acids (Rust et al.,

2011). In addition, the anti-nutrients present in plant products may further limit the ability of the fish to use the nutrients (Krogdahl et al., 2010; Rust et al., 2011). Therefore, research is needed to improve processing, decrease the cost of products, improve the nutrient profile, and to remove anti-nutrients. Studies need to investigate novel plants and/or plant breeding for novel traits. Oilseeds are the most promising replacements due to the potential of a protein and a lipid source being produced from one plant (Tocher et al., 2010).

Table 2.2. Estimates of raw feed ingredient inclusion in salmon diets by country (weighted average of all products; Campbell, 2010).

	Country			
Raw Material (% of Diet)	Canada	Norway	Chile	Scotland
Starch Source	13	13	13	13
Vegetable Oils	4	15	11	4
Vegetable Proteins	24	34	21	36
Poultry Oil	8	0	3	0
Land Animal Proteins	22	0	21	0
Fish Oil	12	15	12	24
Fishmeal	17	23	19	23
Average Feed Cost (%)	88	97	90	100

2.5.2 Plant protein in feeds

Characteristics a plant-based ingredient needs to possess to be considered as a protein source in fish diets, include plentiful supply, competitive price, ease of handling and addition to feeds, a high protein content, a good amino acid profile, high digestibility, and good palatability (Miller, 2004; Hemre et al., 1995). Gatlin III et al. (2007) reviewed nutrient targets a plant product must

have to meet the characteristics of a suitable alternative feed ingredient to fishmeal (Table 2.3). Grain sources are limited in their ability to provide a protein source for fish due to the high content of carbohydrate, fibre and starch, which can negatively affect digestibility (Hemre et al., 1995). Atlantic salmon, for example, can only tolerate up to 9% dietary starch before digestibility is negatively affected (Hemre et al., 1995).

Table 2.3. Nutrient content (as-fed basis) of fishmeal compared to the targeted range for an alternative feedstuff from grain or oilseed to meet the dietary requirements of carnivorous fish (reviewed by Gatlin III et al., 2007).

Nutrient	Fishmeal (%)	Target (%)
Crude Protein	65-72	48-80
Arginine	3.75	>3.0
Lysine	4.72	>3.5
Methionine	1.75	>1.5
Threonine	2.5	>2.2
Crude Lipid	5-8	2-20
Omega 3 Fatty Acids*	~2	>3.0
Non-Soluble Carbohydrates	None	<8
Nitrogen Free Extract	<1	<20
Starch	<1	<20
Fibre	<2	<6
Ash	7-15	4-8

*The total lipid content and fatty acid profile of various plant-based products are different.

Fish can utilize plant protein material to some extent. Atlantic salmon are able to effectively utilize diets where fishmeal is fully replaced by plant protein sources, such as corn gluten meal (CGM) and wheat gluten meal (WGM), with no decrease in fish health and only a small decrease

in fish growth (Espe et al., 2006). Atlantic cod growth and feed conversion ratio (FCR) was not significantly affected by inclusions of soybean meal (4.8% of diet), SPC (12.8% of diet), and WGM (17.8 % of diet) up to 50% of the protein (Hansen et al., 2007). When fishmeal was replaced with 80% plant protein sources (CGM, WGM, and soybean meal), the amount of fishmeal needed to produce edible fish protein was about four times less than the diet with no fishmeal replacement (Torstensen et al., 2008).

In rainbow trout diets when SPC replaced 100% of the fishmeal, the trout had the same growth rate as the fishmeal based diet when methionine is supplemented above 50% addition (Kaushik et al.,1995). Plant protein meals tend to be limiting in some essential amino acids for carnivorous fish diets, particularly methionine and lysine (Wilson, 2002; Espe et al., 2006; Hansen et al., 2007; Torstensen et al., 2008). The addition of crystalline amino acids to an unbalanced diet has reduced or eliminated the negative impact by improving the amino acid profile in plant based diets to meet the requirements of the fish (Espe et al., 2006; Hansen et al., 2007; Torstensen et al., 2008). Digestibility of plant-based crude protein in Atlantic cod, Atlantic salmon, and rainbow trout was not significantly different than that of crude protein from fishmeal (Hansen et al., 2007; Torstensen et al., 2008; Kaushik et al., 1995). However, the digestibility of starch decreased as the plant inclusion increases in the fish diet (Hemre et al., 2002; Hansen et al., 2007; Torstensen et al., 2008; Brinker and Reiter, 2011). Rainbow trout diets can substituted with 100% plant protein sources (wheat gluten meal, corn gluten meal, and soybean meal) without lowering diet digestibility. Digestibility of total diet protein, lipid, and phosphorus increases as the plant protein inclusion increases (Brinker and Reiter, 2011).

Feed intake of plant protein based diets is generally lower than fishmeal based diets, likely due to reduced palatability, feed texture, or negative anti-nutrient effects on the intestinal tract of the fish (Espe et al. 2006; Hansen et al., 2007; Torstensen et al., 2008; Kaushik et al., 1995). Plant proteins can be used in carnivorous fish diets provided the limiting amino acids have are supplemented, palatability is increased, and anti-nutrients are neutralized. The protein content of the plant-based products has to be within the range of fishmeal (65-75%) to be able to completely replace fishmeal, as the dietary requirements of fish do not change (Kaushik et al., 1995; Hansen et al., 2007; Torstensen et al., 2008). When the plant ingredient is processed to increase the protein content, undesirable components such as starch, fibre, carbohydrates, and anti-nutrients can be removed, but this can also remove desirable components such as lipids, fatty acids, amino acids, and vitamins and minerals (Kaushik et al., 1995; Hansen et al., 2007; Torstensen et al., 2008).

2.5.3 Plant lipid in feeds

The replacement of between 50 to 90% fish oil with vegetable oils does not affect survival, growth, and FCR of carnivorous fish. Rainbow trout fed 80 to 90% fish oil replacement by plant oil showed no difference in growth or FCR (Caballero et al., 2002). In turbot diets, the complete replacement of fish oil by linseed or soybean oil resulted in no difference in the digestibility or feed efficacy, but somatic growth was reduced (Regost et al., 2003). Atlantic salmon in freshwater fed diets containing rapeseed oil 100% replacing fish oil exhibited similar growth and survival.

The main concern replacing the fish oil with the plant oils is the reduction in dietary EPA and DHA content causing a lower deposition of these important polyunsaturated fatty acids in fish flesh (Regost et al., 2003; Hixson et al., 2013, 2014a,b). Atlantic salmon fed a fish oil diet had flesh that contained the highest levels of EPA, DHA and the best n-3:n-6 ratio compared to those fed plant oils (Codabaccus et al., 2011). In response to these findings, plant oils such as camelina and echium are being evaluated as potential fish oil replacements due to their high levels of n-3 C18 polyunsaturated fatty acids (PUFA), including as ALA (18:3 n-3) and stearidonic (18:4 n-3) acids (Hixson et al., 2013, 2014a,b; Codabaccus et al., 2011). The potential for biosynthesis of these fatty acids to n-3 long-chain PUFA (EPA and DHA) by some fish species via an enzyme desaturase and elongase bioconversion can be effective (Codabaccus et al., 2011; Lenihan-Geels et al., 2013; Figure 2.1). Echium oil had a high conversion rate due to its high level of naturally occurring stearidonic acid, which is a rate limiting factor in the bioconversion of ALA by Δ6 desaturase (Codabaccus et al., 2011; Lenihan-Geels et al., 2013). Atlantic salmon fed diets containing 20% echium oil had flesh with increased n-3 content, and an increase in the total PUFA concentrations closer to that of fish oil fed fish. This was due to a higher Δ6 desaturase activity in the liver and white muscle (Codabaccus et al., 2011). A similar, elongation and desaturase activity was reported in rainbow trout (Caballero et al., 2002). Therefore, a higher level of ALA (n-3) and/or stearidonic acid is important to match the higher inclusion of plant oil in fish diets.

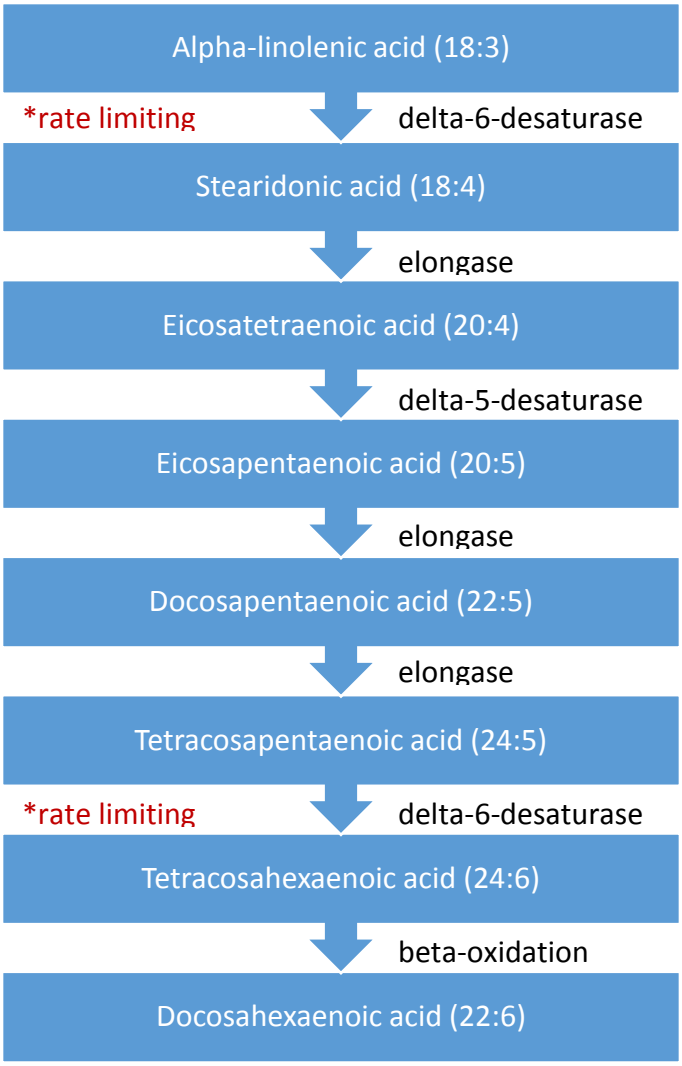

Figure 2.1. Enzymatic pathway of bioconversion of eicosapentaenoic acid and docosahexaenoic acid from alpha-linolenic acid (Lenihan-Geels et al., 2013).

2.6 PROCESSING OF OILSEED PLANT-BASED FEED INGREDIENTS

Improvements in the nutrient composition and digestibility or availability of the nutrients have been achieved using new technology and processing methods (Hansen and Hemre, 2013). Lipids and carbohydrates can be removed from plant meals, resulting in a product that is similar in crude protein content (65 to 72%) to fishmeal (Glencross et al., 2005). Vegetable oil is an important product of oilseed seed processing. Processing steps include cleaning, flaking,

conditioning, mechanical extraction via expelling and/or extrusion, and finally solvent extraction, in which the temperature can reach 100˚C (Figure 2.2; Carr, 1995; Zubr, 1997). These processes produce various products that could be used in fish feeds as protein and/or lipid replacements.

2.6.1 Expelling of seed

There are three methods of mechanical extraction of oil from oilseeds, expelling, cold-pressing, and extruder-expelling. To expel or press the seed a screw-press (Figure 2.2; Carr, 1995) can be used which consists of a rotating screw shaft in a cylindrical barrel and cage. The cage is made up of flat, steel bars that are evenly spaced to keep the cake in, while removing the oil (Carr, 1995; Unger, 1990). The rotating screw shaft moves the seed cake towards an adjustable choke that restricts the amount of cake that leaves the barrel (Carr, 1995). Some screw-presses can have throttle rings along the rotating screw shaft that allow the machine to press the seeds without flaking (Unger, 1990). When camelina oil is expelled from the press it has a yellow colour (Zubr, 1997). The oil can be filtered after being expelled as a pre-treatment before it is processed further for edible oils or other oil products, such as cosmetics, biodiesel, and engine lubricants (Carr, 1995). A by-product of pressing is high residual oil cake which can be milled to produce meals. These cakes and meals can be feed ingredients or can be processed further to remove more oil (Schaufler and Schaufler, 2013).

The oilseed can be pre-heated using steam (60 to 70˚C) before mechanical extraction (van Doosselaere, 2013). Heat is created via friction in the screw barrel which deactivates enzymes

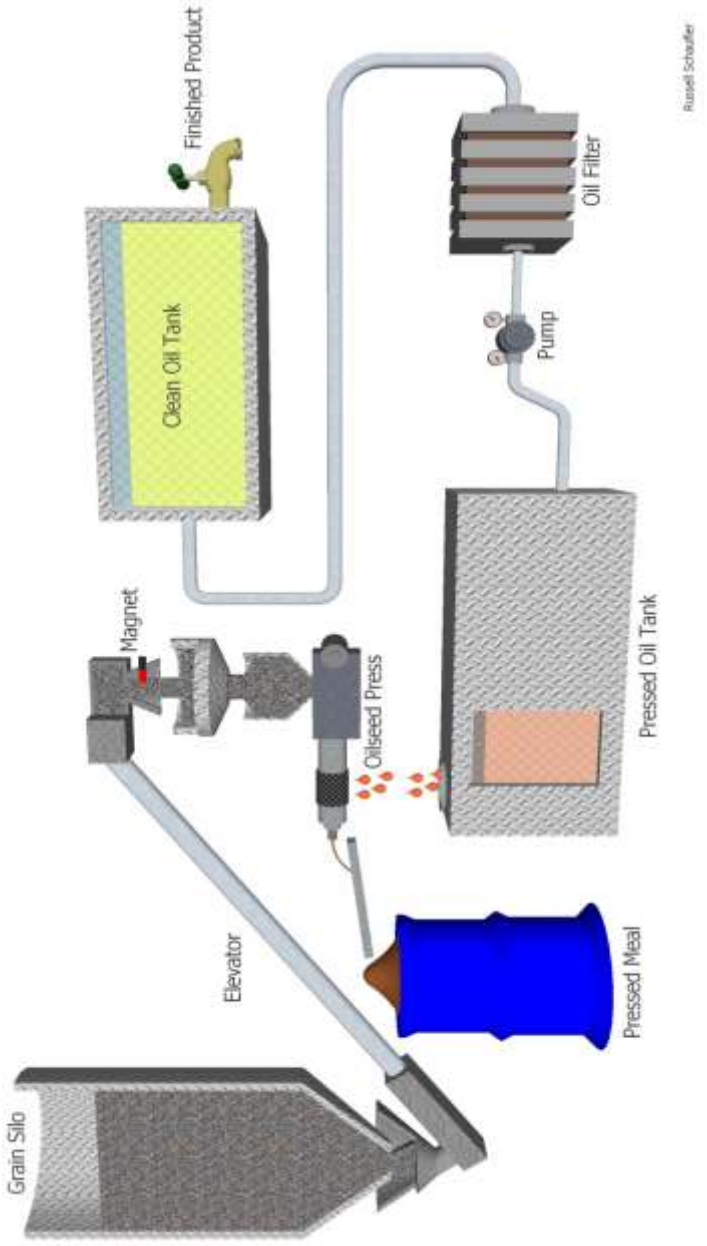

Figure 2.2. Small-scale expeller press setup for the processing of oilseeds to produce two products, the pressed meal and oil (Schaufler and Schaufler, 2013).

improving both protein quality and the texture of the meal (Kenkel and Holcomb, 2008; van Doosselaere, 2013). Cold-pressing involves crushing the seed in an expeller press that includes a chiller to maintain a temperature below 49˚C during the process (Schaufler and Schaufler, 2013). Temperatures above 49˚C start to degrade long chain polyunsaturated fatty acids (Ritter et al.,

2015). Cold-pressing improves the nutritional qualities of the oils and reduces phosphorus levels (Schaufler and Schaufler, 2013). A drawback of this method, however, is that the overall yield of the oil is reduced (de Mours et al., 2008).

2.6.2 Extrusion-expelling

The most efficient way to extract oil from an oilseed for use in an aquaculture feed is extrusion-expelling. Extruding squeezes the oil from the seed by mechanically breaking and pressing open the fat-containing cells. Extrusion-expelling involves pre-heating the oilseed in a mechanical press under high pressure (200 to 600 psi; Williams, 2010). The pressure is achieved using a tapered die to produce friction as the source of heat, raising the seed to around 135˚C (Blair, 2007; Kenkel and Holcomb, 2008). The extruder can be run dry or with the addition of steam to inactivate trypsin inhibitors which improves feed conversion ratio in pigs (Blair, 2007). The heat deactivates enzymes and destroys micro-organisms (Kenkel and Holcomb, 2008). On leaving the extruder the material expands, breaking the starch cells and releasing oil (Anderson, 2005). The extruded material is immediately expelled in a screw-press to separate the oil from the meal, increasing the yield. Also, the temperature and time of extrusion can be adjusted to influence digestibility and meal quality (Kenkel and Holcomb, 2008).

2.6.3 Solvent extraction

Direct solvent extracted material can be produced from oilseed with less than 30% oil without mechanical expelling (Kemper, 2013). By comparison, expelled solvent extraction removes more oil from products containing over 30% oil. Nearly all canola meal is produced with the use of

expelled solvent extraction (Anderson-Hafermann et al., 1993). During the process, the seed has to be expelled to produce an oil seed cake or meal before it is exposed to the solvent (Carr, 1995; Unger, 1990; Kemper, 2013). After extraction the product is called expelled solvent extracted meal or cake. The main objective is to use very little solvent to extract as much oil as possible from the oil seed cake (Carr, 1995). The oil is brought into contact with the solvent drawing it into solution (Carr, 1995; Unger, 1990). Particle thickness of the meal is important. The smaller the particle size, the more oil will be extracted as more ruptured cells come into contact with the solvent (Kemper, 2013). The cake or meal is placed in solvent for 30 to 120 minutes and heated to between 50 and 60˚C to increase the solubility rate (Unger, 1990; Kemper, 2013). The most commonly used solvent in industrial operations is n-hexane, at a ratio of 1:1 (meal:hexane; Kemper, 2013). Hexane is most commonly used due to the faster rate of extraction (Wakelyn and Wan, 2001). Petroleum ether, also known as commercial hexane or Skellysolve F, has a boiling point between 40 to 60˚C. It contains around 80% n-hexane (CAS#: 8030-30-6; Barthet and Daun, 2004; ChemBook, 2008). Petroleum ether is also a suitable solvent to extract oil from oilseeds, and is cheaper and less volatile than hexane. It was the best solvent to extract the oil from jojoba seed (*Simmondsia chinensis*) due to its lower cost than hexane and a similar yield. Jojoba seed contains about 50% oil by weight (Abu-Arabi et al., 2000). Hexane, by contrast, has been used almost exclusively in commercial production of canola meal in Canada (Rao et al., 2011). The solvent extraction process is typically not performed on extruded-expelled meals as it reduces the oil content to about 1% versus cold-pressing (20%) and expelling (8-12%) leaving more oil in the meal (Blair, 2007).

2.6.4 Protein meals

Protein concentrate and protein isolate products are created by further processing of the solvent extracted meals (Ohren, 1981). Typically the ground solvent extracted meal is mixed with various solutions such as alcohols, acids, and bases. The end product is a meal low in anti-nutritional components. For example, rapeseed and canola protein meals were lower in glucosinolates and fibre than in pressed meals (Landero et al., 2012). Moreover, the digestibility of these products by finfish was enhanced as the processing concentrated the protein (Higgs et al., 1995).

2.6.4.1 Protein concentrate

Protein concentrate contains at least 65% crude protein on a dry matter basis (Endres, 2001). The three most common production methods are either an aqueous-alcohol wash, an acid wash, or a heat denaturation water wash of the defatted or solvent extracted meal (Ohren, 1981; Berk, 1992). The aqueous-alcohol wash consists of the defatted meal being soaked in water and alcohol (either methanol, ethanol, or isopropyl alcohol) solution in which the sugars solubilize, but the proteins do not. The solution is then removed and the wet mash is dried then ground (Morr and Lin, 1970).

The acid wash involves soaking the defatted meal in solution at a pH of 4.2 to 4.5. This is a relatively difficult procedure because the soaked meal must be separated from the solution by rotary vacuum filters or a decanting centrifuge (Campbell et al., 1985). However this is a cheaper, less toxic, and less explosive procedure in comparison to other methods (Berk, 1992). Following the acid wash the defatted meal is neutralized at pH 7.5 to 12 for 30 to 60 minutes

(Ohren, 1981). The meal is separated from the solution using a decanting centrifuge (Berk, 1992). The slurry solution containing the protein is acidified to precipitate out the protein (Ohren, 1981). The acidified mixture is then placed in a decanting centrifuge, to separate the protein from the solution. The protein paste is then dried and ground (Berk, 1992). If ground barley was placed in a solution at pH 11.2 for 25 mins at a 1:10 ratio of barley to solution, then the resulting slurry was acidified (pH 5.4) the protein would precipitate out of solution. The final product had a 75 to 77 % crude protein and was called a precipitated protein isolate (Bilgi and Çelik, 2004).

In the third procedure, heat denaturation water wash, the defatted meal is cooked using steam then soaked with water to dissolve the sugars (Ohren, 1891). One study reported that rice flour protein can be denatured when a moist temperature of 80˚C is applied for 10 minutes (Ju et al., 2001). Denaturing the protein in rice flour causes it to become hydrophobic, allowing it to be separated out with a water soaking (Ju et al., 2001).

2.6.4.2 Protein isolate

Protein isolate is a product that must contain at least 90% crude protein on a dry matter basis (Endres, 2001). The procedure to produce this high level of crude protein is performed on the defatted or solvent extracted meal, and is an extension of the precipitated protein isolate procedure (Ohren, 1981; Bilgi and Çelik, 2004). The key step is ultrafiltration of the supernatant from the acidic precipitate stage (Figure 2.3). A cross-flow ultrafiltration column was used to produce a rapeseed protein isolate of 10kDa molecular weight (Yoshie-Stark et al., 2008).

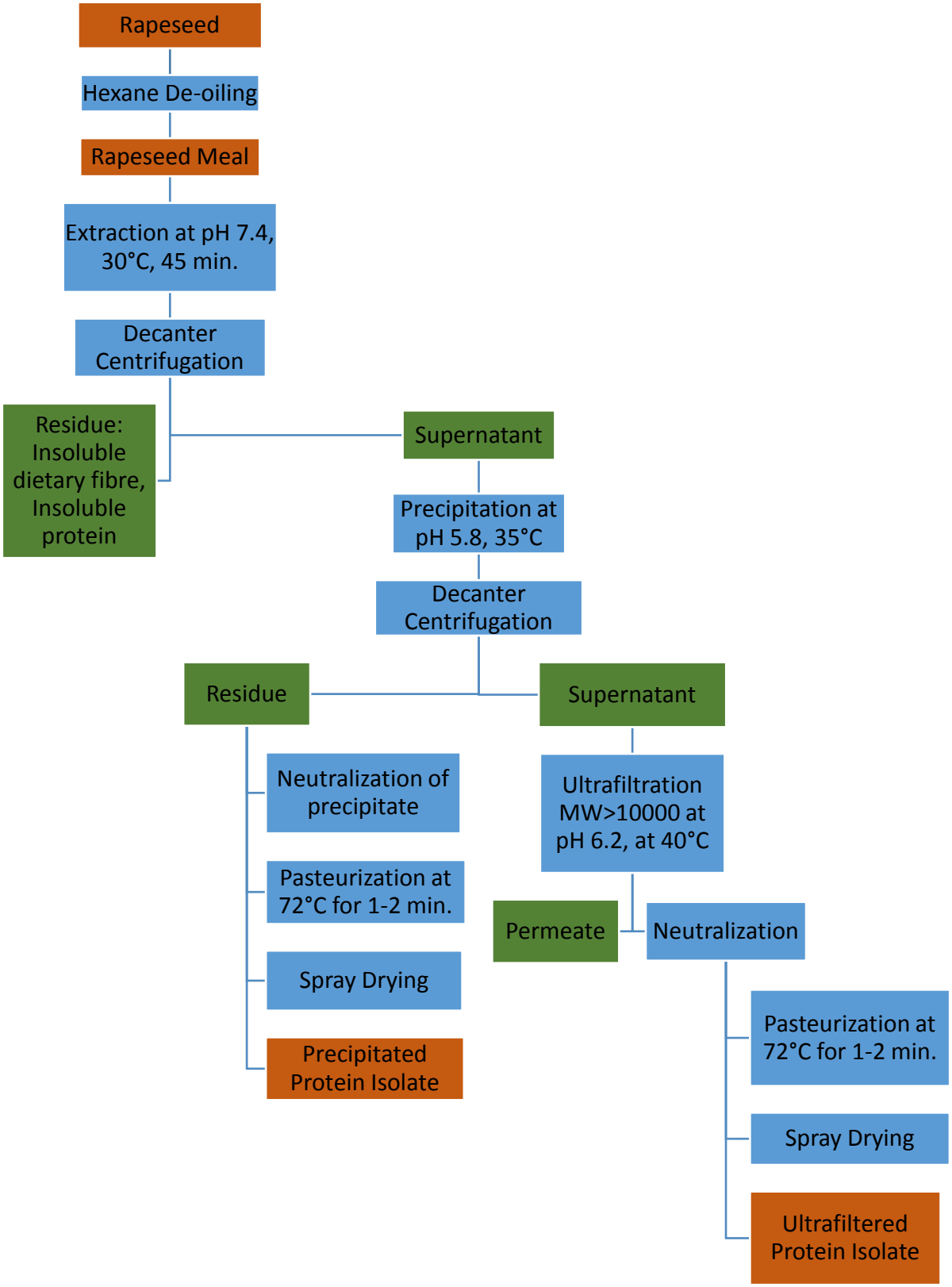

Figure 2.3. Protein extraction from rapeseed meal (Yoshie-Stark et al., 2008).

2.7 NUTRIENT REQUIREMENTS OF SELECT FISH SPECIES

In order to study the potential use of camelina by-products as a component for salmonid fish feed, it is essential to know their nutrient requirements. In this section, nutrient requirements of two salmonid species (Atlantic salmon and rainbow trout) and a marine fish (Atlntic cod) are briefy reviewed. All three species are considered carnivorous with a short, straight intestine digestive tract compared to herbivorous fish (NRC, 2011). The distal intestinal structures in fish hydrolyze and absorb water, minerals, protein, lipid, and carbohydrates (NRC, 2011).

Atlantic cod (family Gadidae) inhabits cool marine waters of the North Atlantic Ocean (DFO, 2015). Cod can grow to be 2 to 3 kg in weight and 60 to 70 cm in length (DFO, 2015). Wild cod feed on finfish and shellfish species (DFO, 2015). They are a relatively new aquaculture species, and could be important to the Atlantic Provinces due to their traditional markets. Nutrient requirements are not listed for Atlantic cod by the National Research Council (2011) and researchers have noted that the nutritional requirements for cod have not yet been fully identified (Lall and Nanton, 2002; Björnsdóttir et al., 2010). Feeding Atlantic cod between 50 to 60% protein, 13 to 20% lipid, and less than 15% starch was optimum for growth (Rosenlund et al., 2004). For juveniles (<300g) the optimum feeding levels were 49% crude protein and 20% lipid (Grisdale-Helland et al., 2008). Feeding lipid at 28% did not have an effect on feed efficiency, however lipid storage in the liver increased (Grisdale-Helland et al., 2008). The crude protein requirement of Atlantic cod was inversely related to body size, ranging from 47 to 52% at 40 to 107g to 36% at 400 to 900g (Árnason et al., 2010). Juveniles (250g) fed a high protein diet (65% crude protein; 16% crude lipid) or a low protein diet (54% crude protein; 31% crude lipid) there is a protein sparing effect (Hatlen et al., 2007). The digestible crude protein requirement for cod

was between 1.02 to 1.18 g/kg/d (N x 6.25), and that the digestible energy needed for maintenance functions was 53.8 KJ/kg/d (Hatlin et al., 2007). No data is available for either gross energy or crude protein digestibility coefficients of common ingredients.

Atlantic salmon (family Salmonidae) is an anadromous species (DFO, 2015). Rainbow trout is also in the family Salmonidae, and can be anadromous as Steelhead trout (DFO, 2015). Rainbow trout are land-locked freshwater fish, or the fish that remain and live in rivers and lakes (DFO, 2015). Both species are mostly opportunistic drift feeders, eating invertebrates, like plankton and insects, fish larvae, smaller fish and crustaceans (Johansen et al., 2011). The nutrient requirements of both species are similar (Table 2.4). There is a requirement for essential fatty acids but not lipid. Atlantic salmon grower diets should contain between 30 - 40% lipid, and rainbow trout should contain around 25% (Storebakken, 2002; Jobling et al., 2010). Feed efficiency between four species of salmonids differed, suggesting nutritional requirement also differ between species (Atlantic salmon, rainbow trout, lake trout (*Salvelinus namaycush*), and chinook salmon (*Oncorhynchus tshawytscha*); Azevedo et al., 2004). As the DP/DE ratio decreased in the diet, the ADC of dry matter and gross energy also decreased, and there was no effect on crude protein. Rainbow trout showed a decreased ADC of crude lipid as the DP/DE ratio decreased, however the Atlantic salmon did not (Azevedo et al., 2004). This indicated that as dietary lipid and carbohydrate content increased the digestibility of lipid decreased. These studies suggest rainbow trout could be fed a diet containing a DP/DE ratio between 18 to 24 g/MJ, but Atlantic salmon require a lower DP/DE ratio of between 18 to 20 g/MJ for good growth and nitrogen retention (Azevedo et al., 2004). Atlantic salmon therefore require a higher content of digestible lipid. Digestibility values for the Atlantic salmon and rainbow trout show

that there are differences in the digestibility of same feedstuff by these two fish species (Cho, 1992; Kim and Kaushik, 1992).

Table 2.4. Nutrient requirements of Atlantic salmon and rainbow trout from the National Research Council (NRC, 2011).

Nutrient	Atlantic Salmon	Rainbow Trout	Atlantic cod
Digestible energy	4490[a]	4180[b]	3770[c]
Digestible protein	36[a]	38[b]	38[c]
Amino acids (%)			
Arginine	1.8	1.5	4.4
Histidine	0.8	0.8	4.6
Isoleucine	1.1	1.1	4.2
Leucine	1.5	1.5	3.5
Lysine	2.4	2.4	5.9
Methionine	0.7	0.7	4.0
Methionine +	1.1	1.1	NR
Cysteine	0.9	0.9	NR
Phenylalanine +	1.8	1.8	4.5
Threonine	1.1	1.1	4.8
Tryptophan	0.3	0.3	4.9
Valine	1.2	1.2	2.4
Taurine	NR	NR	NR
Fatty acids (%)			
18:3n-3	1.0	0.7-1.0	NR
n-3 LC-PUFA	0.5-1.0	0.4-0.5	NR
18:2n-6	NT	1.0	NR

LC-PUFA; long chain polyunsaturated fatty acids
NR; Not Required
NT; Not Tested
[a]Cho (1992)
[b]Kim and Kaushik (1992)
[c]Grisdale-Helland et al. (2008)
Refstie et al. (2006); amino acids retention in Atlantic cod

2.8 DIGESTIBILITY

2.8.1 Definition of digestibility

Digestibility is the measure of the ability of an animal to breakdown nutrients in food (Lloyd et al., 1978). Its value is obtained by measuring incomplete absorption and breakdown of carbohydrate, fat, and protein and removing that fraction from the total nutrients provided (reviewed by Guillaume and Choubert, 2001). The incomplete usage of nutrients is caused by the inability to break down the cell walls of food and/or ingredients (Lloyd et al., 1978). Digestion in fish results from the breakdown of food via enzymatic and acidic secretions into the gut (Moyle and Cech, 2004). If a species lacks the enzymes or acids needed to hydrolyze cellulose, then processing of ingredients needs to be performed prior to ingestion in order to disrupt cell walls and tissues (Ellis et al., 2004). The measure of digestibility is used for screening of feedstuffs for potential value in diet inclusion and for formulating diets to maximize growth, provide the right amount of nutrients, and to reduce fecal waste (reviewed by Bureau et al., 2002; Allan et al., 2000). Measuring fish digestibility is difficult because the nutrients from the fecal matter can leach into the water before collection (Bureau et al., 2002).

There are two measures of digestibility; true and apparent. True digestibility accounts for endogenous and microbial losses of nutrients in the feces, as well as the absorption of nutrients. It can be calculated as the true digestibility coefficient (TDC) using the following equation (reviewed by Guillaume and Choubert, 2001):

TDC = ((ingested nutrient – (fecal nutrient – endogenous fecal nutrient)) / ingested nutrient) x 100

Endogenous losses are not directly of feed origin and include shed mucosal cells, intestinal secretions, and digestive enzymes produced by the fish and lost in the gut (Miller, 2004). TDC is not usually used for fish because endogenous fecal matter is very low due to the high protein content of fish diets, which lowers the fecal nitrogen output (Hardy and Barrows, 2002).

Apparent digestibility is the measure of the nutrients lost from the diet as fecal matter (reviewed by Guillaume and Choubert, 2001). This is the most popular method of measuring digestibility within livestock and animal research as it is easiest to obtain (Lloyd et al., 1978). The apparent digestibility coefficient (ADC) is a measure of digestibility. It is calculated using the following equation:

ADC = ((ingested nutrient – fecal nutrient) / ingested nutrient) x 100

Apparent digestibility coefficients (ADC) are the numeric representation of the apparent digestibility of a feed ingredient or diet.

2.8.2 Methods to determine ADC in diets

The ADC of a diet can be determined either directly or indirectly. The direct method is to measure the total diet or food given and the total fecal matter produced, while the indirect method uses a known amount of inert dietary marker in the feed (reviewed by Guillaume and Choubert, 2001).

2.8.2.1 Direct method of calculating ADC

The direct method of calculating digestibility is also known as the total collection technique. Compared to other methods, it is more time consuming and costly, but is the most reliable (Khan et al., 2003). Total collection requires force feeding an individual fish restrained in a metabolic chamber. Total feed inputs and all excreta outputs are collected and measured (Smith, 1971). The criticisms of this method are that feed absorption could be decreased by stress and that force feeding could increase the speed of passage through the intestine (Sadler, 1979; Grove et al., 1978). Animals have reduced feed consumption due to increased stress from handling or confined housing and the overall temperament of the animal plays a major role in the success in using this system (Khan et al., 2003). The direct method is calculated using the following equation (Khan et al., 2003):

Apparent digestibility (%) = [(total ingested nutrient – total excreted nutrient) / total ingested nutrient] x 100

2.8.2.2 Indirect measure of calculating ADC

The indirect method of measuring digestibility involves incorporating an inert dietary marker (NRC, 2011). It is assumed that the concentration of dietary marker remains constant in the feed and fecal matter throughout the collection period and that all dietary marker ingested will be present in the fecal matter (NRC, 2011). This method allows the fish to be reared using normal culturing practices with a larger population of fish (Maynard et al., 1979). There is no need to force feed fish and only a representative sample of feed and fecal matter must be collected instead of total collection (NRC, 2011). The indirect method is calculated using the following equation (Maynard et al, 1979):

Apparent digestibility (%) = 100 – (100 x ((% marker in feed / % marker in feces) x (% nutrient in feces / % nutrient in feed)))

2.8.2.2.1 Choice of digestibility markers

Chromic oxide (Cr_2O_3) is commonly used as an inert marker to measure the digestibility of the macronutrients. This is due to its effectiveness in previous aquaculture animal studies (Morales et al., 1999; Vandenberg and de la Noüe, 2001; Tibbetts et al. 2006). The ability to see the marker in the excreta as the green colour is used to trigger the start of fecal collection (Whitby and Lang, 1960). Studies use chromic oxide as a standard of comparison for other markers (Morales et al., 1999; Atkinson et al., 1984; Sigurgisladottir et al., 1992). There are alternative markers to chromic oxide used due to arguments that digestibility values obtained by using chromic oxide are variable with poor recovery (Riche et al., 1995). The addition of chromic oxide at 2% of the diet affects digestion and metabolism of glucose in tilapia and in rainbow trout (Shiau and Chen, 1993; Tacon and Rodriques, 1984). By contrast, chromic oxide was reliable in reproducing the same ADC values and that inclusion levels of 0.5% and 1% of the diet did not affect nutrient digestibility (Tacon and Rodriques, 1984). Inclusion of chromic oxide up to 1% of the channel catfish diet did not affect digestion and metabolism of glucose (Ng and Wilson, 1997). Common inclusion levels range from 0.5% to 1% of the diet (NRC, 2011).When chromic oxide is used at common inclusion levels there is generally considered to be no impact of its inclusion in the diet.

Acid-insoluble ash (AIA; Celite, diatomaceous earth, SiO_2, processed diatomites, Johns Manville Corporation, USA) can be used as an indicator, however the results tend to be inconsistent.

Celite added at 0.5% of diet overestimated nutrient digestibility in rainbow trout (Morales et al., 1999). AIA (celite) at 1% of the diet was a suitable marker to replace chromic oxide in rainbow trout diets, yeilding similar digestibility coefficients as chromic oxide (Atkinson et al., 1984). Acid washed sand has been used as an AIA marker, but the ADCs obtained were lower and the repeatability was erratic (Tacon and Rodriques, 1984). When using Sipernat 50 (SiO_2), precipitated synthetic magnesium and calcium silicates, (Degussa, Germany) at 1% inclusion to the rainbow trout diet, the resulting ADCs were similar to the chromic oxide values at a 0.5% inclusion (Vandenberg and de la Noüe, 2001).

Crude fibre has been used as a dietary marker in rainbow trout, but its effectiveness depends on the crude fibre composition of the diet, as some fibrous portions are digestible by fish (Morales et al., 1999; Stickney and Shumway, 1974). The digestible carbohydrate portions include the monosaccharides, disaccharides, and some oligosaccharides. The non-digestible carbohydrates include other oligosaccharides, hemicellulose, cellulose, pectin, β-glucans, and gums (Krogdahl et al., 2005). Crude fibre could be used as a dietary marker in practical farm settings (Tacon and Rodriques, 1984). Polyethylene has been used as a dietary marker, however, it yielded erratic results and ADCs were up to 6% lower in rainbow trout with inclusions of 0.5%, 1 %, and 2% (Tacon and Rodriques, 1984). The researchers found that 1% inclusion of polyethylene was the most accurate inclusion level for this marker. Microtracer Fe-Ni has also been used as a dietary marker. Studies found that the ADC were similar to the chromic oxide, but that the inclusion of 1% was the only inclusion rate that showed no erratic changes in the marker content through the digestive tract (Kabir et al., 1998).

Ytterbium (Yb_2O_3), lanthanum (La_2O_3), and yttrium (Y_2O_3) oxides can produce similar ADC results as chromic oxide in rainbow trout (Austreng et al., 2000). Yttrium oxide produced lower protein (2%), energy (4%), total lipid (3%), triacylglycerol (0.1%), and palmitic fatty acid (0.2%) ADC when added at 1% of the Atlantic salmon diet, compared to cholestane ($C_{27}H_{48}$) added at 1% (Carter et al., 2003). Barium oxide (BaO) was a suitable marker at 0.5% inclusion but not 1% (Riche et al., 1995). Using titanium oxide (TiO_2) on rainbow trout produced ADCs that were variable. The difference between ADC values was 0.4% to 5% when comparing values from titanium oxide to the chromic oxide (Weatherup and McCracken, 1998). When comparing chromic oxide, titanium oxide and AIA (Sipernat 50), the chromic oxide values were more consistent and accurate when comparing the analysis of marker in the feed (Vanderburg and de la Noüe, 2001).

Cholestane has been used to measure fatty acid digestibility (Sigurgisladottir et al., 1992; Carter et al., 2003). Cholestane produced digestibility values higher for lipid than yttrium oxide in Atlantic salmon (Carter et al., 2003). Cholestane added at 0.5% of the fatty acid content of the diet, gave similar digestibility values to chromic oxide, but standard deviation was lower compared to chromic oxide for all fatty acids but one (18:1n-9; Sigurgisladottir et al., 1992). Three of the fatty acids (14:0, 16:0, and 18:0) had significantly lower standard deviations using the cholestane compared to the chromic oxide. The study concluded that the cholestane would be a better marker for lipid and fatty acid digestibility evaluation. This is because of the lower deviation and the ease of not having to test for the marker separately from the fatty acid analysis as it is fat soluble and can be measured as a peak in the chromatography (Sigurgisladottir et al., 1992).

It has been suggested that a mixture of markers would provide more accurate ADC values for each nutrient (Hillestad et al., 1999; Vanderburg and de la Noüe, 2001; Martins et al., 2009). Using two dietary markers in Atlantic halibut diets has been explored, using chromic oxide (0.5% of diet) to follow the macronutrients, and cholestane (0.07% of diet) to follow the fatty acids (Martins et al., 2009).

In conclusion, chromic oxide is the most consistent and reliable dietary marker for the crude macronutrient measurement, including dry matter, protein, energy, and lipid (Weatherup and McCracken, 1998; Vandenberg and de la Noüe, 2001). This marker is the most recommended marker for use in digestibility studies. Cholestane provides fatty acid digestibility values that have a low standard deviation compared to chromic oxide, therefore this marker should be used to measure fatty acid digestibility (Sigurgisladottir et al., 1992). Using a mixture of markers is a good practise as more accurate values are obtained and the beneficial aspects of each marker are employed (Vandenberg and de la Noüe, 2001). Therefore, the literature indicates that a mixture of two markers, chromic oxide and cholestane, would be the best approach to use in a fish nutrition digestibility study and were adopted in this project.

2.8.2.3 Measuring ADC of a test ingredient

To calculate the digestibility or ADC of an ingredient in the diet, there are three different methods (reviewed by Guillaume and Choubert, 2001):

The first method for calculating digestibility is that the ingredient is fed alone to the fish and ADC calculated. It would be very hard to make a pelleted diet with only one ingredient for fish,

feed pellets require different ingredients to bind a pellet together and feed refusal is high for single ingredient feeds (reviewed by Glencross et al., 2007).

The second method for calculating digestibility is that the ingredient can be added to a diet of a known digestibility and then the ration is increased in proportion of the test ingredient to calculate ADC. It should be noted that the digestibility values obtained could be affected by the decreasing amount of another nutrient in another ingredient, giving a skewed digestibility value (Glencross et al., 2005).

Finally, the third method for calculating digestibility is that the ingredient is added to a basal diet with a known digestibility. The ingredient is added at a known percentage to the basal diet, the ADC is calculated and the difference determined is considered due to the test ingredient (Maynard et al., 1979). A higher rate of inclusion, typically between 20 to 40%, results in a more accurate digestibility value (Allan et al., 1999). However, high levels of the test ingredient may affect the ability to produce a quality pellet (Glencross et al., 2005). Poor quality pellets can cause loss of nutrients due to leaching (Cruz, 1996). There should be at least a four day acclimation period once the fish are switched onto the digestibility diets before the fecal collection is started, due to the high variation in ADC obtained during the first three days (Blyth et al., 2015). Testing various inclusion rates can show the possible interaction of the test ingredients inclusion in future diet formulations (Allan et al., 1999). When a digestibility study occurs, the weight gain and FCR can be measured during the trial. These metrics are indicators of an imbalance in the test ingredient. Therefore, if the growth and FCR during the digestibility show depression at a certain inclusion, then when a growth study is performed the tendency

would be to use inclusion rates below that inclusion rate during the digestibility study. The calculation for ADC of an ingredient is obtained from the following equation (Forster, 1999):

$$ADC = ((Nb + Nt) \times ADCtd - (Nb) \times ADCbd)) / Nt$$

Where:

Nb = nutrient level of basal diet (nutrient in basal diet x (100 – i))

Nt = nutrient level of test ingredient (nutrient in test ingredient x i)

i = inclusion rate of test ingredient

ADCtd = apparent digestibility coefficient of test diet

ADCbd = apparent digestibility coefficient of basal diet

2.8.3 Methods of fecal collection

Different methods can be used to collect fecal matter. These methods include, dissection, stripping, anal suction, fecal collection or decanting devices, and continuous effluent filtration (Windell et al., 1978; Nose, 1967; Austreng, 1978; Cho and Slinger, 1979; Choubert et al., 1979). Dissection requires removing the fecal matter by killing the fish and dissecting the intestine (Windell et al., 1978). Stripping requires applying pressure to the underbelly of the fish and pushing the fecal matter out with gentle pressure towards the anus (Nose, 1967). During anal suction, a glass tube is inserted into the anus and the contents of the rectum are removed through the use of suction (Nose, 1967). Dissection, anal suction and stripping can give lower digestibility values because the fecal samples can contain portions of the diet that have not been fully digested (Windell et al., 1978). The values may be affected by endogenous material, like scales, secretions, and urine, being collected along with the fecal sample (Vanderberg and de la Noüe, 2001). Anal suction can induce stress on the fish which may cause changes in digestibility,

induce vomiting, causing early evacuation of contents in the intestinal tract and damage the intestinal lining (Windell et al., 1978). Stripping can induce stress causing a change in ADC (Hajen et al., 1993a). Stripping can also be performed repetitively over a series of days, this can injure the intestinal membrane and alter ADC values over time (Vandenberg and de la Noüe, 2001). Manual stripping is not possible on some fish and crustaceans, due to the different physiological and morphological characteristics amoung fish species and the lower amount of fecal excretion in small sized fish (juvenile)(Glencross et al., 2005).

The sedimentation methods of decanting or column collection method place less stress on the fish, as many samples can be collected over a short period of time, and the samples collected are fully digested by the fish (Vanderberg and de la Noüe, 2001). Digestibility values of some diets may be affected due to loss of some fecal matter using the collector system (de la Noüe and Choubert, 1986). However, if the feces is undisturbed or unbroken from its pellet form, then nutrient leaching is minimal (Cho et al., 1985). Continuous effluent filtration can reduce the amount of leaching as fecal matter is removed from the water through filters, however the system is complex and special filtering equipment with rotating screens is needed or a conveyor belt with screens to capture feces as the effluent water passes over (Satoh et al., 1991; Cho and Slinger, 1979). These systems can be quite bulky and are both difficult and complex to set up.

2.8.3.1 Comparison of different methods of fecal collection

A comparison of fecal collection methods, including stripping, decanting column collection, and conveyor belt collection has been conducted on rainbow trout (Austreng, 1978; Cho and Slinger, 1979; Choubert et al., 1982; Vanderberg and de la Noüe, 2001). The stripping method gave the

lowest digestibility values out of the three methods (Vanderberg and de la Noüe, 2001). The column (decantation) method gave higher ADC values (about 10%) than the collection methods (continuous filtration, conveyor belt). The exception to this was lipid ADC which was similar for both method types (Vanderberg and de la Noüe, 2001). The higher values using the column collection could be due to the leaching of nutrient into the water while awaiting decantation (Glencross et al., 2005). When comparing the stripping and column (sediment) method energy ADC values were around 3% higher for the column method for barramundi (*Lates calcarifer*; Blyth et al., 2015). Carbohydrate ADC values that were compared between the column sedimentation method and the stripping method have been found to be far more differential (Glencross et al., 2005; Blyth et al., 2015), which could be caused by a lower fecal pellet quality allowing for increased leaching in the column method (Blyth et al., 2015). However, another study showed the digestibility values for crude protein and gross energy were about 8% higher for the stripping method over the column (sediment) method (Ramsay et al., 2000). This work used winter flounder (*Pleuronectes americanus*), therefore this shows that the method of fecal collection of choice could be dependent on species.

There is a difference between the stripping and column collection methods using rainbow trout (Glencross et al., 2005). Stripping gave lower ADC values compared to the column collection values, due to the lack of water exposure for nutrient leaching. Fecal matter collected with undigested food, bodily fluids (blood, urine), scales, skin mucosa, intestinal epithelium and digestive tract enzymes, can lead to lower digestibility values (Cho et al., 1985). Thus contamination was the main reason for the lower values observed in studies using the manual stripping method (Vandenberg and de la Noüe, 2001). Proteins are absorbed in the posterior

section of the intestinal tract, which means that fecal matter collection via the stripping method may not calculate the effect of the full digestion process (Sire and Vernier, 1992). High levels of stress caused by handling during stripping can affect the ADC values obtained, by adjusting metabolic functions, such as enzyme and mucous production (Hajen et al., 1993 a,b; Love, 1980).

Leaching is the greatest source of error in a water collection system (Percival et al., 2001). Digestibility values increased when fecal matter was removed from the posterior section of rainbow trout intestine and placed in water for a timeframe of one to four hours, where digestibility estimates increased on an hourly basis, 11.5% (two hours), 10.0% (three hours), and 3.7% (four hours; Windell et al., 1978). Very little change in digestibility values was found in soaking between four to sixteen hours (Windell et al., 1978). Leaching losses occurred between six and eighteen hours in saltwater, and in freshwater leaching losses occurred between six to twenty four hours (Hajen et al., 1993b; Kabir et al., 1998). With all water collection methods some leaching of nutrients occurs, therefore the ability to collect the fecal matter quickly is important.

Manual stripping will typically give good conservative ADC values, but may not reflect the full tract digestibility capacity of the fish due to presence of undigested material or endogenous material (Vanderberg and de la Noüe, 2001). As mentioned previously, this method cannot be applied to all fish, either due to species traits or size (Vanderberg and de la Noüe, 2001). The effluent water collection or decanting methods cause less stress to the fish, have high fecal recovery, fecal matter is easy to collect, and they provide good solid ADC values for formulation

(Cho and Slinger, 1979; Choubert et al., 1982). Some leaching will occur in these systems, however if fecal pellet is unbroken and collected in a timely manner, leaching should be minimal (Cho et al., 1985; Hajen et al., 1993a). The factors listed above suggests that the method of choice for collection depends upon the fish species, fish size, and trial size.

2.8.4 Calculation of digestible nutrient content

ADC values are useful to determine the ability of a feed ingredient to provide a species with the proper nutrients (Guillaume and Choubert, 2001). The ADC values are used to calculate digestible nutrient profiles of ingredients allowing formulation of balanced diets (Lloyd et al., 1978). Digestible nutrient (DN) is calculated by the following equation (Lloyd et al., 1978):

DN = (Amount of nutrient in ingredient / ADC of ingredient nutrient) / 100

The first step in introducing a new feed ingredient for dietary inclusion is to measure the digestibility of nutrients by the target organism (NRC, 2011). The digestibility of camelina seed and its by-products are unknown for most species including fish.

2.8.5 Difference in digestibility among species

Digestibility can differ among species. Atlantic salmon and rainbow trout for example, digest nutrients from plant products, like lupin and soybean meal, differently. Rainbow trout digested more phosphorus with a higher non-starch polysaccharide content than Atlantic salmon (Glencross et al., 2004). When fed soybean meal, the digestibility of nitrogen (6% greater), fat (8% greater), and energy (11% greater) were all higher in rainbow trout compared to Atlantic

salmon (Refstie et al., 2000). Nitrogen retention was highest in Atlantic cod (44.9%), followed by Atlantic salmon (39.4%), then rainbow trout (33.6%) when fed a commercial cod diet top-dressed with lipid (Grisdale-Helland et al., 2007). Energy retention was highest in the Atlantic salmon (52.2%) followed by the Atlantic cod and rainbow trout (44.9 and 44.8%, respectively). Phosphorus retention was similar for all species (65.9%; Grisdale-Helland et al., 2007).

2.8.6 Summary of digestion and digestibility

Digestibility is the measure of bioavailability of required nutrients for a species after the digestion process has occurred. These values are species-specific. To determine digestibility the nutrients provided in the diet must be measured, then compared to the nutrient excreted. The most efficient technique to determine digestibility is the indirect method using a dietary marker, such as chromic oxide. The sedimentation method to collect fecal matter using a collection column is the most simplistic system for a large digestibility trial, while the filtering collection (conveyor belt method) is the most accurate. Digestibility values of ingredinets obtained by these two methods are widely used to measure apparent digestible nutrients for research and feed formulation.

2.9 EFFECT OF PROCESSING ON DIGESTIBILITY

2.9.1 Use of enzymes to improve digestibility

Enzyme pre-treatment of potential feed ingredients can improve the nutrient profile and overall digestibility of the ingredients (Dalsgaard et al., 2012; Sajjade and Carter, 2004). The enzymes β-glucanase, xylanase, and protease used either individually or as a mixture improved the

digestibility of dry matter, protein, starch, and lipid by rainbow trout (Dalsgaard et al., 2012). For soybean meal, the addition of glucanase and protease increased digestibility by about 5% for all nutrients tested, while xylanase increased protein digestion by about 8% (Dalsgaard et al., 2012). For rapeseed meal, the addition of xylanase increased lipid and dry matter digestibility by about 2%. The mixed enzyme treatment had no effect on soybean meal, but did improve rapeseed meal digestibility of lipid and dry matter by about 2% (Dalsgaard et al., 2012). Atlantic salmon growth and FCR were unaffected by inclusion of phytase in diets, but phosphorus ADC increased by 10% (Sajjade and Carter, 2004). Plants contain phytic phosphorus, therefore the deposition of phosphorus indicates that the phytic acid that binds minerals such as zinc, iron and calcium, is being removed or degraded in the feed (Kaushik, 2001). Pre-treating a soy protein concentrate with phytase reduced the phytic acid from 9.3 g/kg to 0.5g/kg, which improved the ADC in Atlantic salmon of protein (3%), phosphorus (15%), and minerals (calcium 7%, magnesium 13%, zinc 30%; Storebakken et al., 1998). The addition of enzymes can improve digestibility of less expensive feed ingredients releasing essential nutrients without the need for addition of expensive concentrated nutrients such as single amino acid supplements, minerals, and vitamins (Storebakken et al., 1998). A mixed dietary enzyme, such as Energex™ (multi carbohydrase including hemicellulase, β-glucanase, and pectinase), Bio-Feed™ Pro (bacterial protease), and alpha galactosidase (α-galactosidase; Novo Nordisk, Bagswaerd, Denmark) treatment increased the protein efficiency ratio for rainbow trout from 2.38 to 2.58 fed a finely ground dehulled lupin based diet (50% inclusion, *Lupinus angustifolius*; Farhangi and Carter, 2007). Two commercial enzymes used in this study are Superzyme-OM and Bio-phytase 5000G (Canadian Bio-Systems Inc., Calgary, Alberta). The two enzyme mixes used have been widely used as enzyme feed supplements for monogastrics animals, such as poultry and swine.

2.9.2 Effect of heat treatment on digestibility

Heat treatment improved digestion by common carp (*Cyprinus carpio*) and coho salmon (*Oncorhynchus kisutch*) and degraded anti-nutrients such as trypsin inhibitor (Davies and Gouveia, 2010; Arndt et al., 1999). A dry roasting of pea meal (*Pisum sativum*) increased the digestibility of dry matter (3%), protein (6%), lipid (8%), and energy (5%) by common carp (Davies and Gouveia, 2010). Autoclaving soy defatted flour at 121˚C for 20 minutes reduced trypsin inhibitor from 181 to 1.8 TUI/mg (Arndt et al., 1999). This decrease in trypsin inhibitor increased digestibility of protein by about 4%. Extrusion heat between 100 to 150˚C had no effect on energy, protein and amino acid digestibility in rainbow trout, but a moisture addition of 25 to 30% during extrusion increased the cysteine digestibility by about 3% (Sørensen et al., 2002).

Excess heat can cause a Maillard reaction, a chemical reaction of heated sugars and amino acids, which is indicated by a browning colouration, smell or flavor change of the product (Bastos et al., 2012). Maillard reaction decreased the digestibility by 5% of a fish protein isolate by rainbow trout (Plakis et al., 1985). Importantly, lysine digestibility was reduced by 19% causing it to become a limiting factor to growth, whereas digestibility of other amino acids was reduced by 4% (Plakis et al., 1985).

2.9.3 Effect of water soaking on digestibility

Six hour water soaking at a 1:10 wt/vol ratio of *Bauhinia purpurea* L. seeds, decreased the content of phenolics by 65%, tannins (71%), phytic acid (37%), and oligosaccharides (raffinose (15%), stachyose (10%), and verbascose (19%)) in the ground seed meal (Vijayakumari et al.,

2007). In-vitro digestibility was unchanged with this removal of anti-nutrients during water soaking (Vijayakumari et al., 2007). Protein digestibility in pigs was unaffected by water soaking, but reduced or removed glucosinolates from rapeseed (from 20 mmol/kg to 2.1) and rapeseed press cake (from 18.5mmol/kg to 0.3; Schöne et al., 1997). This reaction occurs after the seed has been crushed allowing the naturally occurring enzyme myrosinase to interact with the glucosinolates, creating breakdown products such as isothiocyanates and nitriles (Duncan, 1991). Protein and lipid is released from anti-nutrient binding and becomes more bioavailable for digestion and absorption (Francis et al., 2001). The total removal of glucosinolates improves energy digestibility by 2 MJ/kg, as observed with canola meal fed to rainbow trout (Mwachireya et al., 1999). Whole rice grain soaked in water for 24 hours at 45˚C reduced the phytic acid content by 77%, improving the bioavailability of the minerals, such as zinc and iron by 4.78% and 5.92%, respectively, but *in vitro* protein digestibility decreased by 7.3% (Albarracín et al., 2015). This could be due to the water soluble amino acids being decanted with the water. Improved digestibility by rohu (*Labeo rohita*) of dry matter (up to 30%), protein (up to 7%), fat (up to 30%), and ash (up to 40%) for rohu (*Labeo rohita*) following soaking sal seed meal (*Shorea robusta*) for 16 hours (Mukhopadhyay and Ray, 1997). Also, tannin content was reduced from 3.4% to 0.7%, which likely increased the digestibility of sal seed meal (Mukhopadhyay and Ray, 1997). The *in vitro* digestibility of water soaked material does not show positive results for protein digestibility, while *in vivo* digestibility results have shown possible increases in protein digestibility. Therefore, this study will evaluate the effect of water soaking solvent extracted camelina meal *in vivo* fish digestibility experiments.

2.10 *CAMELINA SATIVA*

2.10.1 General background

Camelina sativa (camelina), also known as false flax, gold of pleasure, Dutch flax or linseed dodder, is a cruciferous oilseed plant with yellow flowers belonging to the family *Brassicaceae* (Ní Eidhin et al., 2003). Use of camelina dates back to the Bronze Age (1500-400 B.C.) and into the Iron Age (400 B.C. – 500 A.D.) in Europe and Scandinavia, where it was used for human consumption (Zubr, 1997). Camelina is a low input crop (low-nitrogen demand), does not require pesticides or herbicides, and can grow in semi-arid regions (Shukla et al., 2002; Zubr, 1997). It can be harvested with a combine adjusted to harvest rapeseed (Zubr, 1997). The harvested seed should have less than 11% water content, and should be dried down to less than 8% water for proper storage and subsequent processing (Zubr, 1997).

2.10.2 Nutrient composition

The extruded camelina meal (high oil residue meal) has an amino acid profile with the potential to be used in fish feeds (Table 2.5). The higher protein by-products produced from this meal potentially have increased amino acid levels (Cogan et al., 1967). Four amino acid concentrations needed in the protein product to replace fishmeal are arginine > 3.0%, lysine > 3.5%, methionine > 1.5%, and threonine > 2.2% (reviewed by Gatlin III et al. 2007). The amino acid concentration of the extruded camelina meal met this threshold for arginine (3.22%), but not lysine (1.80%), methionine (0.68%), or threonine (1.49%; Kahindi et al., 2014). Therefore, when using camelina as a basis for feed, these amino acids would only need to be added into the diet in small amounts. All of these amino acids are commercially available.

Expelled camelina meal has a high fibre content (NDF, 28.1%; ADF, 18.6%; as fed; Table 2.5) which could be a problem in carnivorous fish diets causing a lower digestibility and a laxative effect as did flaxseed expelled meal tested on Atlantic cod (Tibbetts et al., 2006). Glucosinolate levels in camelina meal are relatively high (34.4 µmol/g; Table 2.5), about four-fold greater than

Table 2.5. Nutrient composition (as fed basis) of camelina meal compared to soybean, canola and fishmeal and oil products.

Nutrient	Feed Ingredient			
	Fishmeal	Soybean Meal[a]	Canola Meal[a]	Camelina Meal[a]
Moisture (%)	7.1[b]	8.6	8.0	8.6
Crude protein (%)	70.6[b]	46.2	35.4	33.0
Ash (%)	17.2[b]	5.8	7.0	5.3
Ether extract (%)	12.2[b]	1.1	3.1	11.1
Neutral detergent fibre (%)	Nr	8.5	21.1	28.1
Acid detergent fibre (%)	Nr	3.9	18.0	18.6
Calcium (%)	Nr	0.3	0.7	0.2
Phosphorus (%)	Nr	0.7	1.1	0.7
Total glucosinolates (µmol/g)	Nr	0.2	8.8	34.4
Essential amino acids (%)				
Arginine	4.6[b]	3.1	2.2	2.6
Histidine	1.6[b]	1.2	1.0	0.8
Isoleucine	2.9[b]	1.9	1.4	1.1
Leucine	4.8[b]	3.5	2.7	2.3
Lysine	4.8[b]	3.0	2.3	1.6
Methionine + Cysteine	2.5[b]	1.5	1.8	1.6
Phenylalanine	2.6[b]	2.3	1.5	1.4
Threonine	2.9[b]	1.9	1.7	1.6
Valine	3.4[b]	2.5	2.2	2.1

[a]Thacker and Widyaratne, 2012
[b]Nagel et al., 2012
nr = not reported

canola and 100 fold greater than soybean. Glucosinolates reduce palatability and nutrient absorption (Benn, 1977). However, they can be removed via processing to produce a protein concentrate or protein isolate where sugars and fats are removed to increase the protein level (Ohren, 1981). Glucosinolates can be reduced in HORM and SEM through the process of water soaking. The fibrous components can be removed by a pre-treatment with multiple enzymes (Denstadli et al., 2011).

Use of *Camelina sativa* seed, oil, and meal is promising as the oil contains high level polyunsaturated fatty acids, in particular ALA (Table 2.6). Some species of fish are able to chain elongate ALA to omega 3 long-chain PUFA which fish require for normal health and related functions (Tocher et al., 2010). The omega-3 fatty acids in camelina meal are several-folds higher compared to soybean and canola meals. This causes the omega-3 to omega-6 ratio to be about five to nine-fold lower than canola or soybean meals (Table 2.6). The higher omega-3 content provided to fish by the camelina meal could be beneficial to increase omega-3 deposition of fish flesh (Lenihan-Geels et al., 2013). Erucic acid content in camelina oil is 2.6% of the fatty acid composition (Hixson et al., 2013). Erucic acid fed to rats at a level of 30% of the fatty acid content can cause fatty degeneration of the heart, kidney, adrenal gland and thyroid (Kako and Vasdev, 1979). The European Union has laws to limit the use of oils, fats, or blends with a level of erucic acid greater than 5% of the total fatty acid profile (Council Directive 76/621/EEC, 1976). The erucic acid level of camelina oil (3.4% of oil) is within the 5% limit (Table 2.6), indicating that it should cause no problems. Genetically altering plants is a way to reduce or remove the erucic acid. Plant breeders produced canola oil, a rapeseed modified with low erucic acid (Canola Council of Canada, 2014).

Table 2.6. Fatty acid composition (as fed basis) of camelina oil compared to soybean, canola and fishmeal and oil products.

Nutrient	Feed Ingredient			
	Fishmeal	Soybean Meal[a]	Canola Meal[a]	Camelina Meal[a]
Fatty acid (% of oil)				
Oleic acid (C18:1 n-9)	7.1[b]	14.6	58.6	15.0
Linoleic acid (C18:2 n-6)	0.4[b]	54.4	26.4	20.9
Alpha-linolenic acid (C18:3 n-3)	0.7[b]	9.6	3.0	30.8
Erucic acid (C22:1 n-9)	Nd	Nd	Nd	3.4
EPA (C20:5 n-3)	14.2[b]	Nd	Nd	Nd
DHA (C22:6 n-3)	10.5[b]	Nd	Nd	Nd
Saturated fatty acids	Nr	21.5	10.1	11.1
Monounsaturated fatty acids	Nr	14.6	61.0	32.4
Polyunsaturated fatty acids	Nr	63.9	29.4	56.1
n-3 fatty acids	Nr	9.6	3.0	32.3
n-6 fatty acids	Nr	54.4	26.4	23.5
n-3 to n-6 ratio	0.1[b]	5.7	8.9	0.7

[a]Thacker and Widyaratne, 2012
[b]Deng et al., 2014
nd = not detected
nr = not reported

2.10.3 Previous research into *Camelina sativa* in monogastric nutrition

Prior to the present project, most research on *Camelina sativa* has been performed on terrestrial monogastric animals. Broiler chickens fed diets with 5 and 10% replacement of soybean meal with *Camelina sativa* expeller meal exhibited improved feed conversion ratio and an increased n-3 fatty acid profile in homogenised leg meat (Pekel et al., 2016). By contrast, growth rate of male broilers was inhibited when fed camelina meal (5% inclusion) possibly due to the high level of glucosinolates (22.9 μmol/g; Ryhänen et al., 2007). In another trial on broilers, 100 % replacement of canola meal with camelina expeller meal reduced digestibility, feed intake, feed

conversion ratio, and weight gain, but increased the omega-3 fatty acid profile of the abdominal fat pad (Thacker and Widyaratne, 2012). The camelina meal had a higher content of fibre (NDF 28.1%; ADF 18.6%), and glucosinolate (34.4 μmol/g) compared to the canola meal (NDF 21.1%; ADF 17.9%; glucosinolates 8.8 μmol/g) and soybean meal (NDF 8.5%; ADF 3.9%; glucosinolates 0.2 μmol/g) which contributed to the poor growth performance (Thacker and Widyaratne; 2012). By contrast, lower inclusion rate of camelina meal for between 9 to 12% of the diet did not affect the weight gain, feed intake, or feed conversion ratio.

Layer hens fed extruded camelina meal at 10% of the laying hen diet for 12 weeks improved both egg shell strength and the total omega-3 fatty acid profile, with DHA content increasing almost three-fold (Kakani et al., 2012). The ALA from the camelina meal was converted by the hen to DHA resulting in an enhanced content of this long chain omega-3 fatty acid in egg yolks. Feeding camelina to layers at up to 10% of the diet did not reduce the feed consumption, hen body weight, or egg production (Kakani et al., 2012). These results indicate that a diet of camelina is closely linked to increased levels of omega-3 fatty acids and DHA. Similarly, young turkeys fed diets containing up to 5% camelina meal exhibited no negative effects on body weight and feed conversion ratio. In addition camelina oil was substituted for other vegetable oil at a 1:1 ratio without any effect on young turkeys (Frame et al., 2007).

Pigs fed extruded camelina meal (high oil residue meal) had a high digestible energy (4180 kcal/kg), but low digestibility of protein (57.5% DP) and amino acids (essential amino acids, 55.7%; non-essential amino acids, 62.8%; Kahindi et al., 2014). The high NDF (31.5%), phytate (6.4%), tannins (2.0%), and glucosinolate (36.3μmol/g) levels in the meal may have decreased

the palatability and absorption of nutrients (Kahindi et al., 2014). Phytate and tannins bind to the amino acids and inhibit the digestive enzymes to breakdown protein into the amino acids for absorption (Woyengo and Nyachoti, 2011; Robbins et al., 1987). The high level of NDF decreases the absorption of amino acids by increasing the endogenous production of enzymes, mucus, and sloughed enterocytes preventing absorption of nutrients and increasing the passage rate of food through the digestive tract (Nyachoti et al., 1997). Glucosinolates decrease iodine absorption, which effects thyroid function and hormones released by the thyroid gland (Schöne et al., 1990).

2.10.4 *Camelina sativa* seed and by-products in fish nutrition

Replacing fish oil with 80% camelina oil did not affect the growth of Atlantic cod (Hixson et al., 2013). However, it did decrease the long chain polyunsaturated fatty acids, including EPA and DHA in skeletal muscle, compared to the fish oil diet. The change in muscle fatty acid profile was a reflection of the fatty acid profiles in the diet. The camelina oil can replace fish oil in Atlantic cod diets, but in order to retain the long chain polyunsaturated fatty acids in the fish flesh, between 1 to 2.7% of fish oil must remain in the diet. Gene markers have been identified in Atlantic cod signifying that desaturase and elongase enzymes were working to form long chain polyunsaturated fatty acids from the omega 3 sources in the camelina oil diets (Xue et al., 2015).

Replacing 100% of the fish oil with camelina oil had no effect on the growth of Atlantic salmon and rainbow trout (Hixson et al., 2014a; Hixson et al., 2014b). But, growth did decrease when all sources of fish oil available were removed, including from the fishmeal (Hixson et al., 2014a). In both Atlantic salmon and rainbow trout the DHA and EPA levels were reduced in the fish dark

muscle tissue (Hixson et al., 2014a; Hixson et al., 2014b). DHA biosynthesis in the muscle tissue of 100% camelina oil replacement fed fish was evident (Hixson et al., 2014b). Other work indicated camelina oil could entirely replace fish oil in Atlantic salmon practical diets without any negative effects to the hindgut (Ye, 2014). While the expelled solvent extracted camelina meal (SEM) could only be included at 8% in smolt diet and 10% in parr diets. Hindgut enteritis was found in fish with reduced growth, at greater than 15% inclusion of SEM (Ye, 2014).

Feeding *Camelina sativa* expeller pressed meal to broilers and turkeys at 10 to 15% of the diet has negative effects on growth and feed conversion ratio (Ryhänen et al., 2007; Thacker and Widyaratne, 2012; Kakani et al., 2012). However, it has positive effects on the fatty acid profiles of human consumable fish filets, and hen eggs by increasing the omega 3 and ALA levels. Processing or mechanical modification of the camelina expeller meal to remove the fibre, carbohydrates or anti-nutrients components of the meal has the potential to improve the nutritional value (Mwachireya et al., 1999). Plant breeding could improve the nutrient composition for specific species. Transgenic *Camelina sativa* seed with a high EPA content (24% of fatty acids), was fed to Atlantic salmon at 17.5% of diet without any effect on growth, but an increase in lipid digestibility and uptake was observed (Betancor et al., 2015). An increase in fatty acid digestibility, and an increase in EPA content in the pyloric caeca tissue occurred compared to the fish oil treatment. Camelina oil could be a highly valuable product added to monogastric diets to improve the omega-3 fatty acid profile of the meat or egg products (Thacker and Widyaratne, 2012; Kakani et al., 2012). Further research using fish should be preceded by development of digestible nutrient content for effective diet formulation. Digestibility values

need to be established before investigation of the seed and its by-products can be further evaluated. The lack of data on digestibility presents a current gap in the literature.

2.11 SYNTHESIS OF THE LITERATURE ADDRESSING UNKNOWN TO ADVANCE THE STATE OF KNOWLEDGE

Due to an increasing need for aquaculture feeds, costs have increased and availability has decreased of popular ingredients, such as fish oil and fishmeal. Replacement of these ingredients with plant based protein meals and oils is a priority. *Camelina sativa* is an oilseed that has potential in aquaculture feed due to its high protein content and its high level of α-linolenic acid. Alpha-linolenic acid present in camelina oil is the precursor fatty acid in the desaturation and elongation process by fish to form EPA and DHA. Camelina meal has an amino acid profile high in arginine, but may be limiting in lysine and methionine.

Valuable by-products from camelina seed processing include oil, expelled meal, solvent extracted expelled meal, and protein concentrate and/or isolate. The products with high crude protein and/or high lipid have the greatest potential for fish diets. However, these products can contain anti-nutrients that affect nutrient absorption, gut transit time, and palatability. Camelina seed is high in glucosinolates, fibre (mucilage), and phytate. These may be removed from the seed and meals via processing or enzymatic pre-treatment thus improving product quality.

Digestibility coefficients for camelina seed and its by-products need to be defined. Processing of feed ingredients to remove glucosinolates, to remove phytates, and to enhance digestibility of carbohydrates, lipid, and protein are strategies with potential to increase the value of the

camelina. Solvent extracted pre-expelled camelina meal pretreated by water soaking with or without dietary enzymes was investigated as part of this thesis to determine their effect on digestibility. The current study determined the apparent digestibility of various *Camelina sativa* products. Focus was placed on potential commercial products from the seed that could be used aquaculture feeds. Atlantic salmon, rainbow trout, and Atlantic cod were used as test species.

2.12 OBJECTIVES

The objectives of this research were:

1. Develop potential camelina by-products using lab-scale equipment.
2. Determine the digestibility and digestible nutrients of camelina by-products by Atlantic salmon, rainbow trout, and Atlantic cod.
3. Minimize glucosinolates, mucilage, and phytate from the solvent extracted expelled meal using processing methods and enzyme incubations.
4. Evaluate the changes in nutrient composition and digestibility of potential camelina feed ingredients subjected to different processing methods.
5. Compare the camelina by-product for Atlantic cod, Atlantic salmon and/or rainbow trout based on digestible nutrients.

2.13 HYPOTHESES

The hypotheses of this research were:

1. High quality by-products from camelina seed will range in protein (70 to 30% CP) and lipid (99 to 1% CF) contents. New application of processing technology will produce by-products suitable for utilization by fish.

2. The digestibility of the nutrients in camelina by-products will be different; and above 70% digestible. Effective oil processing technology will yield highly digestible camelina oil. Digestible nutrient content will differ among the three fish species.
3. The major proportion of glucosinolates, mucilage, and phytic acid contents will be reduced by incubation. The mixed enzyme pre-treatment will cause decrease in glucosinolates, mucilage, and phytic acid .
4. Digestible nutrient concentration will be different for each camelina by-product.
5. The camelina oil will contain the highest concentration of digestible energy and digestible lipid for all species. The SEM will have the highest concentration of digestible crude protein for all three species. Digestible nutrient will likely differ among the three fish species.

CHAPTER 3 MATERIALS AND METHODS

3.1 CAMELINA SEED AND PROCESSING

The *Camelina sativa* (Calena) was grown at Lyndhurst Farms in Canning, Nova Scotia. It was harvested in August of 2009. The seed was cleaned using a Petkus 400 seed cleaner. Cleaned seed was used throughout this study. Full-fat camelina seed was ground in a small domestic coffee grinder (Black and Decker, Model CBG100SC, 118 ml) before inclusion into fish diets. To maintain the seed at room temperature, grind duration was limited to 20 seconds, and two grinders were used in rotation. Excessive heating was not a factor during this process as the seed as heat buildup was tested once, twenty minutes into grinding, using a heat gun.

Nine other camelina by-products were produced to evaluate digestibility by fish. They were high oil residue camelina meal (HORM), extruded solvent extracted camelina meal (SEM), camelina oil, toasted SEM (TSE), water soaked SEM (WM), Bio-phytase enzyme, water soaked SEM (PM), superzyme-OM enzyme, water soaked SEM (SM), Bio-phytase and superzyme-OM enzyme, water soaked SEM (MM), and protein concentrate (PC; Figure 3.1).

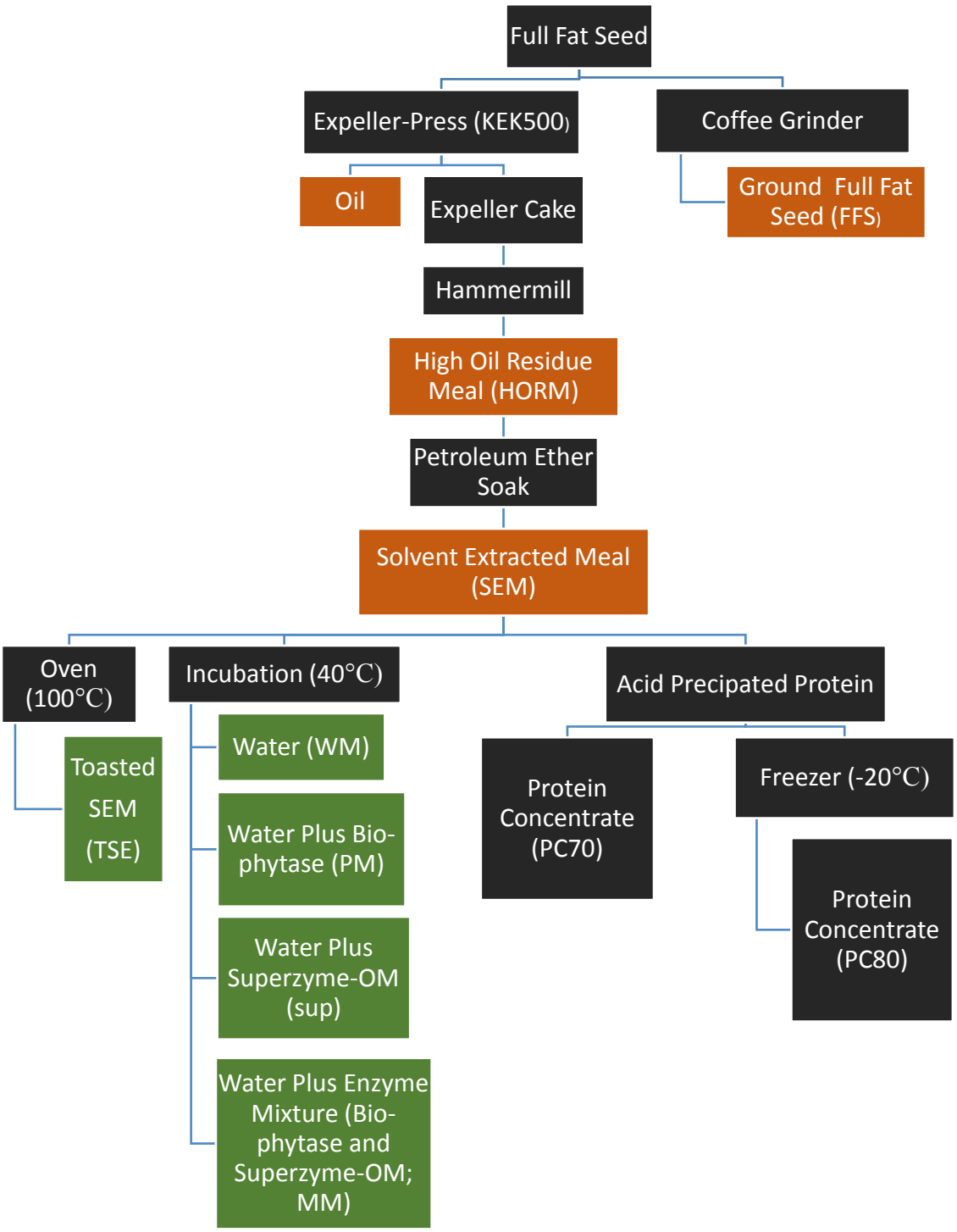

Figure 3.1. Flow chart of *Camelina sativa* seed processing. By-products shown in orange were tested on Atlantic cod, Atlantic salmon and rainbow trout. By-product shown in green were tested only on rainbow trout.

3.1.1 Production of high oil residue camelina meal (HORM) and camelina oil

High oil residue meal (HORM) and an oil product were processed in a commercial expeller-press with a capacity of 500 kg per hour (KEK500; Egon Keller GMBH and Co.). The machine was located at Atlantic Oilseed Processing Ltd., Prince Edward Island, and was used mainly to process canola seed. The temperature of the meal and oil at the 3mm expeller die reached an average of 90°C. The oil expelled from the seed was filtered through a 1 mm mesh to remove particulates. The high protein residue was in the form of a large flake cake. The cake was hammer-milled, using a 5 mm screen, into the high oil residue meal (HORM) on site in Prince Edward Island. Three days after preparation, the HORM and camelina oil were delivered to the Dalhousie University Agricultural Campus (AC, May 2010), and stored at <12°C. Ethoxyquin (60% ethoxyquin, 40% silica; Santoquin mixture 6, Novus International (Canada), Inc., Ontario, Canada) was added to the meal and oil one day after arrival at AC, at 0.2% of the predicted lipid content to preserve the ingredients for bulk storage. Ethoxyquin was added to the meal in large 1 ton mixer (L.V. Feeds mixer #) at the Chute Animal Nutrition Centre (CANC), Dalhousie University Agricultural Campus. Ethoxyquin was added to the oil using a drill with a paint stir attachment. Oxidative stability of the oil was not tested. HORM (9.9% crude fat) was stored in the CANC at room temperature in 1000 kg tote canvas bags. The oil was a 1136 litre oil storage plastic tank tote, provisioned with a metal frame to be moved with a forklift, at room temperature in the CANC for one week, then about 250 litres was bucketed off into 15 litre plastic pails with air-tight lids and stored at -20°C.

3.1.2 Production of expelled solvent extracted camelina meal (SEM)

Each batch of expelled solvent extracted meal (SEM) was produced from the high oil residue meal (HORM). Petroleum ether (Fisher Chemical, Certified ACS, CAS# 8032-32-4) was added to the HORM at a 3:1 (v/w) ratio (2.4 L:800 g) in a 4L glass beaker. The mixture was placed in a fume hood and stirred every 15 minutes for one hour to allow full contact of the meal with the ether. Then, the petroleum ether containing the oil was carefully poured off and discarded. The ether soaked meal was then spreadout on an absorbent pad (Fisherbrand™ Absorbant Underpads; 50.80 x 60.96 cm; Fisher Scientific) in the fume hood, and was then patted dry with another absorbent pad. The meal was left to air dry overnight (16 hours) at room temperature (21°C) in the fume hood. The oil content of the resulting meal was <5%, the endpoint crude fat content was 3.2% in the SEM. Petroleum ether effectively removes triglycerides from the meal (Higgs and Dong, 2000; Sargent et al 1989). The remaining lipid could be phospholipids, due to the difficulty to remove these without degumming the product beforehand (Wan and Wakelyn, 1997).

3.1.3 Product production from removal of anti-nutrients from SEM

A heat treatment and four enzyme hydrolysis treatments were performed on the SEM to remove anti-nutrients.

3.1.3.1 Production of toasted SEM (TSE)

This product was created to test if heat treatment could improve the digestibility of energy containing components and to remove anti-nutrients (tannins and trypsin). In the commercial production of canola meal (Newkirk, 2009), toasting reduces or removes anti-nutrients such as

phenols, tannins, L-dopa, and phytic acid (Iyayi et al., 2008). The SEM was toasted at 102.3°C for 30 minutes in a drying oven (JPW Design and Manufacturer, Model ST333ATUL208V9KW) on an aluminum plate (23 cm diameter).

3.1.3.2 Production of incubated products from SEM

Four products were made to evaluate the effect of enzyme treatment on the nutrient profile and digestibility of the SEM. They were: 1) water incubated SEM (WM), 2) Bio-phytase (PM) plus water incubated SEM, 3) Superzyme™-OM (SM) plus water incubated SEM, and 4) a mixture of these 2 enzymes (MM) plus water incubated with SEM. The SEM had the highest crude protein and was able to be up-scaled in production for this study. The water and enzymes treatments were chosen based on their ability to hydrolyze certain carbohydrate components. The enzymes chosen to reduce problematic concentrations of carbohydrates, and other compounds including glucosinolates, phytate, mucilage, and fibre within camelina seed.

The addition of water activated a naturally occurring enzyme (myrosinase) in the plant material that can hydrolyze the glucosinolates. Incubation of SEM in only water for 24 hours tested the effect of the water soaking. Water was used in all other treatments subjected to the addition of commercial enzyme products. The nutrient profiles and digestibility coefficients of SEM were measured using only the water or the water plus enzymes. Various ratios of tap water to meal were tested. A 1:7 ratio is the lowest ratio that would allow the mixture to be stirred easily. Addition of water to the SEM caused the mixture to expand and became very sticky due primarily to the mucilage present. Hence a higher ratio of water was needed. SEM in water was incubated for 24 hours with enzymes at addition rates shown in Table 3.1 then oven dried (35-

40°C). Both enzymes are dry products that would be activated in a wet environment. Four beaker batches of each treatment were made.

Table 3.1. Four incubation treatments used for expelled solvent extracted meal (SEM).

Treatment	Ratio (g meal:ml water:g enzyme)	Treatment abbreviation
SEM:Water	1:7:0	WM
SEM:Water:Bio-phytase[1]	1:10:0.6	PM
SEM:Water:Superzyme™-OM[2]	1:7:3	SM
SEM:Water:Bio-phytase:superzyme™-OM	1:7:0.6:3	MM

SEM, expelled *Camelina sativa* seed through KEK500 press then solvent extracted to a crude fat of 3.2% using petroleum ether.
[1]Bio-phytase; Canadian Bio-systems Inc., Calgary, Alberta; 5000 phytase FYT units/g enzyme
[2]Superzyme™-OM; Canadian Bio-Systems Inc., Calgary, Alberta; (enzyme g/diet); 2800 cellulase CMC units/g, 400 mannanase MAN units/g, 50 galactanase GAL units/g, 1000 xylanase XYL units/g, 600 glucanase GLU units/g, 2500 amylase FAA units/g, 200 protease HUT units/g.

The Bio-phytase enzyme contained 5000 FYT phytase units/g (releases 1 mole of inorganic phosphate per minute from sodium phytate at a pH 5.5 and 37°C. It hydrolyzes phytic acid and increases the digestibility of phosphorus and calcium (Canadian Bio-systems Inc., 2015). The dosage of the Bio-phytase was 0.006g per 100g of SEM. The ratio of water was higher with this product, as the expansion of the SEM was greater when using the phytase enzyme, therefore more water was needed for ease of stirring.

Superzyme™-OM is a multi-carbohydrase containing cellulase at 2800 CMC cellulose units/g; releases 1 mg of glucose (reducing sugar) per hour at pH 4.6 and 37°C. Mannanase at 400 MAN mannanase units/g (releases 1 μmole of mannose (reducing sugar) per minute at pH 4.0 and 40°C). Galactanase at 50 GAL galactanase untis/g (releases 1 μmole of galactose (reducing sugar) per minute at pH 4.0 and 40°C). Xylanase at 1000 XYL xylanase units/g (releases 1 μmole of xylose (reducing sugar) per minute at pH 4.5 and 40°C). Glucanase at 600 GLU glucanase unts/g (releases 1 mg of maltose (reducing sugar) per minute at pH 5.0 and 50°C).

Amylase at 2500 FAA amylase units/g (breaks down 5.26 mg of starch in one hour at pH 5.0 and 40°C). Protease at 200 HUT protease units/g hemoglobin units on a tyrosine basis (produces hydrolysate with an absorbance at 275nm that is the same as that of a solution containing 1.1 mg/ml of tyrosine in 0.006N HCl). This product has the ability to break down a wide range of digestible and indigestible carbohydrates and releases protein in feeds. It is formulated for high lipid diets containing oilseed products. The dosage of the Superzyme™-OM was 0.03g per 100g of SEM. The ratio of water was similar to the water soaking only treatment, as the expansion of the SEM was similar when using the Superzyme™-OM enzyme.

Both Bio-phytase and Superzyme™-OM were used to prepare the combined enzyme mixture. The combination treatment was used to evaluate if a more complex mixture of enzymes would improve the digestible nutrients in SEM. These enzymes within Superzyme™-OM target different carbohydrates and may act synergistically with the phytase. The mixture dosage was 0.006g of Bio-phytase + 0.03g of Superzyme™-OM per 100g of SEM (Table 3.1). The ratio of water was similar to the water soaking only treatment, as the expansion of the SEM was similar when using the combined enzyme.

3.1.3.2.1 Process of incubation

For each batch of the four treatments, 300g of the SEM was placed in a 4 L glass beaker and mixed with the required treatment enzyme (see Table 3.1). The enzyme and SEM was mixed together dry for 1 minute with a glass stir rod then a set volume of tap water (21°C, pH 7.4) was added. The treatments were then mixed again for 5 minutes with the glass stir rod until all the SEM and enzyme was water soaked. The beakers were placed in a pre-heated drying oven (JPW

Design and Manufacturer) at 35-40°C for 24 hours. The meal was then spread on parchment paper on a 4'x4' aluminium sheet pan and dried at 40-45°C for about 48 hours. After drying, the products were crumbled by hand into small pieces, and then ground into a meal using a small-scale hammermill (Christy and Norris Limited, Lab Mill, Serial # 11881, Chelmsford, England) with a 1 mm screen. Each batch of material prepared yielded 284 ± 3g of dry ingredient, however there was some loss due to the meal sticking to the pans during the drying process. The four batches of each incubated meal were pooled prior to use in diets (3.5 beakers were used in feed production).

3.1.4 Production of camelina protein concentrates (PC70)

The intent was to create and test a product with a high 70 to 75% crude protein similar to the current industry plant protein sources like soybean and corn protein concentrates. However, insufficient camelina product was produced for a digestibility trial due to very low yield rates (10% yield). The procedure was a modified version of a barley protein concentration (Bilgi and Çelik, 2004).

SEM was mixed with sodium hydroxide (NaOH; 0.06 mol/L) at a 1:10 (g: ml) ratio of meal to solution (50 g of meal: 500 ml of petroleum ether per batch). The meal and solution mixture was stirred continuously for 25 minutes at room temperature (21°C) with a glass rod. After 25 minutes the mixture was placed in 250 ml cups then centrifuged (SANYO Harrier 18/80) at 6000 revolutions per minute (4220 xg) for 20 minutes at 20°C. After centrifugation, the upper liquid layer was decanted into a glass beaker and the meal was discarded. The initial pH of the liquid layer was 11.3. This was adjusted to a pH of 5.4 using hydrochloric acid (HCl; 6 mol/L) to precipitate the protein. In addition, the liquid turned colour from brown to pale yellow. The

upper liquid layer was removed and re-centrifuged at 6000 rpm for 20 minutes at 20°C. The upper liquid layer was decanted and discarded and the remaining protein concentrate on the bottom was scraped out of the cups and placed on an 11.4 cm diameter aluminium plate. The protein concentrate was then frozen at -80°C, and then freeze dried (ThermoFisher Scientific, FR-Drying Digital Unit, Model # MODULYOD-115). After drying the protein concentrate was ground for about 20 seconds in a small coffee grinder and stored at -20°C. From start of process using 50g of SEM, the end protein concentrate meal (69.7% crude protein) yielded 5g. Since only 50g of the protein concentrate with a 70% crude protein (PC70) was produced, it was used to evaluate the nutrient profile of the product. The yield of PC70 was 10% of the original material.

3.1.4.1 Production of enriched protein concentrates (PC80)

To get a higher crude protein content in the protein concentrate, a procedure for creating the PC70 was followed. After the solvent extracted meal was soaked in the basic solution it was frozen at -20°C for one week. After 1 week, the mixture was thawed, and continued through the previous procedure. From the start of the process using 200g of SEM, the end protein concentrate meal (82.3% crude protein; PC80) yield was 21.8g. A similar 10% yield as the PC70 procedure, however with a higher crude protein content. The yield of PC80 was 11% of the original material.

3.1.4.2 POS Bio-Science protein concentrate

A protein concentrate containing 52% crude protein was produced by POS Bio-Science (Saskatoon, Saskatchewan). Using a modified version of the method of Bilgi and Çelik (2004),

300kg of HORM was mixed with 6000L of soft water (25°C) at pH 11.2 (using 50% NaOH) for 1 hour then centrifuged (6000 revolutions per minute; 4220 xg). After centrifugation the solid matter was discarded, and the liquid fraction was adjusted to a pH of 5.4 using HCl (6 mol/L). The solution was allowed to sit for 15 minutes, and then centrifuged. The liquid material was discarded, and the solid material was washed with water and adjusted to pH 9. The product was then spray dried. Subsequently the material was washed with methyl pentane, centrifuged, and desolventized by allowing the solid material to sit in a fume hood for 24 hours. The final protein product contained 52% crude protein. The final yield was 5.8kg.

3.2 DIGESTIBILITY STUDIES

One trial was conducted on each of Atlantic cod and Atlantic salmon, and two trials on rainbow trout (Table 3.2).

Table 3.2. Ingredients tested in four digestibility trials on three fish species.

Ingredient	Atlantic cod	Atlantic salmon	Rainbow trout	
			Trial 1	Trial 2
Full-fat seed	X	X	X	
Oil	X	X	X	
HORM[1]	X	X	X	
SEM[2]	X	X	X	X
Toasted SEM			X	
SEM incubated with Water				X
SEM incubated with Bio-phytase[3]				X
SEM incubated with Superzyme™-OM[4]				X
SEM incubated with Enzyme MM[5]				X

[1]HORM, high oil residue camelina meal
[2]SEM, extruded solvent extracted camelina meal
[3]Bio-phytase; Canadian Bio-systems Inc., Calgary, Alberta; 5000 phytase FYT units/g enzyme
[4]Superzyme™-OM; Canadian Bio-Systems Inc., Calgary, Alberta; (enzyme g/diet); 2800 cellulase CMC units/g, 400 mannanase MAN units/g, 50 galactanase GAL units/g, 1000 xylanase XYL units/g, 600 glucanase GLU units/g, 2500 amylase FAA units/g, 200 protease HUT units/g.
[5]Enzyme MM, using both Bio-phytase and superzyme™-OM at the same time.

3.2.1 Feed Formulation

The inclusion rate of the test ingredients was 30% of the basal diet, except for the camelina oil, which was added at 20% in order to preserve pellet quality. The basal diet in the Atlantic cod trial was modified from Tibbetts et al. (2006) by using two digestibility markers versus one in the original diet formulation. The cod diets used some of the same ingredient sources tested by Tibbetts et al. (2006; Table 3.3). The basal diet used for the Atlantic salmon and rainbow trout trials was a modified from Glencross et al. (2004; Tables 3.4 and 3.5), it was modified by using two digestibility markers versus one in the original diet formulation. Full-fat seed, camelina oil, HORM, and SEM were tested in all three fish species, Atlantic cod, Atlantic salmon, and rainbow trout. An additional ingredient tested in the first rainbow trout trial was toasted SEM (Table 3.5). The second rainbow trout trial tested water incubated enzyme treated SEM meals (Table 3.6). The ingredients for the diets were weighed out and mixed in a 19 L Hobart mixer (Model A-200-T). Chromic oxide was added to the diets, as an indigestible marker, at 1 % of the basal diet for the Atlantic cod, and rainbow trout trials, and 0.5 % of the basal diet for the Atlantic salmon trial. Cholestane was added as a marker for fat absorption, at 0.25% of the calculated amount of both fish and camelina oil (g/kg of diet) of each diet. Cholestane was not added to the enzyme trial diets, due to the low level of crude fat in the camelina test products. The vitamin and mineral premixes were prepared separately and mixed for 5 minutes in the Hobart mixer. These premixes were then added to the rest of the ingredients for mixing as required. The fish oil and camelina oil were pre-heated in a stainless steel bowl (5 L volume) on a hot plate (Fisher Scientific stirring hot plate) to around 80°C to ensure they were all liquified, then added to the diets. The dry ingredients were mixed for 5 minutes, and then the oils were added. The complete mash was mixed for 5 minutes. After mixing for 10 minutes, the diets were

Table 3.3. Diet formulation (g/kg) and calculated composition (as fed basis) of the Atlantic cod digestibility diets.

	Experimental Diets (g/kg)				
Ingredients	Basal	Full-fat seed	Oil	HORM	SEM
Camelina source	-	300.00	200.00	300.00	300.00
Fishmeal	475.25	332.68	380.20	332.68	332.68
Fish oil	63.67	44.36	50.70	44.36	44.36
WGM[1]	49.50	34.65	39.60	34.65	34.65
CPSP-G[2]	49.50	34.65	39.60	34.65	34.65
Wheat middling	165.61	115.82	132.22	116.04	116.10
Whey powder	69.31	48.52	55.45	48.52	48.52
Krill hydrolysate	19.80	13.86	15.84	13.86	13.86
Corn starch (pre-gel)	55.45	38.82	44.36	38.82	38.82
Vitamin mixture[3]	19.31	13.52	15.45	13.52	13.52
Mineral mixture[4]	19.31	13.52	15.45	13.52	13.52
Choline chloride	2.97	2.08	2.38	2.08	2.08
Chromic oxide	10.00	7.00	8.00	7.00	7.00
Cholestane[5]	0.32	0.52	0.75	0.30	0.24
Calculated Composition (%)					
Dry matter	91.9	92.8	84.2	92.2	92.1
Crude protein	48.8	42.1	39.0	45.4	46.2
Gross energy (kcal/kg)	4895	5269	5783	4830	4729
Crude fat	12.6	20.8	30.1	12.1	9.7

HORM, high oil residue camelina meal; SEM, extruded solvent extracted camelina meal

[1]WGM, wheat gluten meal

[2]CPSP-G, Concentré protéique soluble de poisson (soluble fish protein concentrate; Sopropêche, France)

[3]Vitamin mixture (IU or g/kg of premix); vitamin A, 900,000 IU; vitamin D_3, 400,000 IU; vitamin E (dl-alpha tocopheryl acetate), 25,000 IU; vitamin K (menadione sodium bisulphate), 3.0 g; thiamin, 3.0 g; riboflavin, 4.0 g; pantothenic acid (as d-calcium pantothenate), 12.0 g; biotin, 0.1 g; folic acid, 1.0 g; vitamin B_{12}, 0.003 g; niacin, 15.0 g; pyridoxine, 4.0 g; ascorbic acid, 30.0 g; carrier (wheat middlings).

[4]Mineral mixture (g/kg of premix); manganous oxide, 23.0 g; zinc oxide, 70.0 g; copper sulfate, 6.0 g; potassium iodide, 2.0 g; carrier (wheat middlings).

[5]Cholestane added at 0.25% of the calculated fat content.

Table 3.4. Diet formulation (g/kg) and calculated composition (as fed basis) of the Atlantic salmon digestibility diets.

	Experimental Diets (g/kg)				
Ingredients	Basal	Full-fat seed	Oil	HORM	SEM
Camelina source	-	300.00	200.00	300.00	300.00
Fishmeal	757.21	529.36	605.06	529.50	529.26
Fish oil	77.23	53.99	61.71	54.00	53.98
Corn starch (Pre-gel)	105.31	73.62	84.16	73.65	73.61
α- cellulose	45.96	32.75	37.20	32.75	33.11
Vitamin mixture[1]	4.96	3.47	3.96	3.47	3.47
Mineral mixture[2]	1.98	1.38	1.58	1.38	1.38
Choline chloride	1.98	1.38	1.58	1.38	1.38
Chromic oxide	5.00	3.50	4.00	3.50	3.50
Cholestane[3]	0.37	0.55	0.75	0.37	0.31
Calculated Composition (%)					
Dry matter	95.7	93.0	91.7	92.7	92.4
Crude protein	52.7	43.8	38.5	40.6	47.2
Gross energy (kcal/kg)	5112	5554	5959	5036	4913
Crude fat	14.6	22.1	30.1	14.6	12.2

HORM. High oil residue camelina meal; SEM, extruded solvent extracted camelina meal.
[1]Vitamin mixture (IU or g/kg of premix); vitamin A, 2,500,000 IU; vitamin D_3, 250,000 IU; vitamin E (dl-alpha tocopheryl acetate), 25,000 IU; vitamin K (menadione sodium bisulphate), 1.7 g; thiamin, 2.5 g; riboflavin, 4.2 g; pantothenic acid (as d-calcium pantothenate), 8.3 g; biotin, 0.17 g; folic acid, 0.8 g; vitamin B_{12}, 0.005 g; niacin, 25.0 g; pyridoxine, 2.0 g; ascorbic acid, 75.0 g; inositol, 58.3 g; ethoxyquin, 20.8 g; carrier (wheat middlings).
[2]Mineral mixture (g/kg of premix); manganous oxide, 15.0 g; zinc oxide, 25.0 g; copper sulfate, 2.5 g; magnesium sulfate, 16.6 g; ferrous iron, 10.0 g; carrier (wheat middlings).
[3]Cholestane added at 0.25% of the calculated fat content.

Table 3.5. Diet formulation (g/kg) and calculated composition (as fed basis) of the rainbow trout digestibility diets.

Ingredients	Experimental Diets (g/kg)					
	Basal	Full-fat seed	Oil	HORM	SEM	Toasted SEM
Camelina source	-	300.00	200.00	300.00	300.00	300.00
Fishmeal	754.02	527.81	603.22	527.81	527.81	527.81
Fish oil	76.90	53.83	61.52	53.83	53.83	53.83
Corn starch (Pre-gel)	104.87	73.41	83.90	73.41	73.41	73.41
α- cellulose	44.96	31.18	35.50	31.36	31.42	31.42
Vitamin mixture[1]	4.94	3.46	3.95	3.46	3.46	3.46
Mineral mixture[2]	1.97	1.38	1.58	1.38	1.38	1.38
Choline chloride	1.97	1.38	1.58	1.38	1.38	1.38
Chromic oxide	10.00	7.00	8.00	7.00	7.00	7.00
Cholestane[3]	0.37	0.55	0.75	0.37	0.31	0.31
Calculated Composition (%)						
Dry matter	95.7	93.0	91.7	92.7	92.4	92.4
Crude protein	52.7	43.8	38.5	40.6	47.2	47.2
Gross energy (kcal/kg)	5112	5554	5959	5036	4913	4913
Crude fat	14.6	22.1	30.1	14.6	12.2	12.2

HORM, high oil residue camelina meal; SEM, extruded solvent extracted camelina meal

[1]Vitamin mixture (IU or g/kg of premix); vitamin A, 2,500,000 IU; vitamin D_3, 250,000 IU; vitamin E (dl-alpha tocopheryl acetate), 25,000 IU; vitamin K (menadione sodium bisulphate), 1.7 g; thiamin, 2.5 g; riboflavin, 4.2 g; pantothenic acid (as d-calcium pantothenate), 8.3 g; biotin, 0.17 g; folic acid, 0.8 g; vitamin B_{12}, 0.005 g; niacin, 25.0 g; pyridoxine, 2.0 g; ascorbic acid, 75.0 g; inositol, 58.3 g; ethoxyquin, 20.8 g; carrier (wheat middlings).

[2]Mineral mixture (g/kg of premix); manganous oxide, 15.0 g; zinc oxide, 25.0 g; copper sulfate, 2.5 g; magnesium sulfate, 16.6 g; ferrous iron, 10.0 g; carrier (wheat middlings).

[3]Cholestane added at 0.25% of the calculated fat content.

Table 3.6: Digestibility diet formulation (g/kg) and calculated composition (as fed basis) of the expelled solvent extracted (SEM) enzyme treated diets tested on rainbow trout.

Ingredients	Experimental Diets (g/kg)					
	Basal	SEM	SEM incubated with			
			Water	PM[1]	SM[2]	MM[3]
Camelina source	-	300.00	300.00	300.00	300.00	300.00
Fishmeal	754.02	527.81	527.81	527.81	527.81	527.81
Fish oil	76.90	53.83	53.83	53.83	53.83	53.83
Corn starch (Pre-gel)	104.87	73.41	73.41	73.41	73.41	73.41
α- cellulose	45.33	31.73	31.73	31.73	31.73	31.73
Vitamin mixture[4]	4.94	3.46	3.46	3.46	3.46	3.46
Mineral mixture[5]	1.97	1.38	1.38	1.38	1.38	1.38
Choline chloride	1.97	1.38	1.38	1.38	1.38	1.38
Chromic oxide	10.00	7.00	7.00	7.00	7.00	7.00
Calculated Composition (%)						
Dry matter	95.7	92.4	92.4	92.4	92.4	92.4
Crude protein	52.7	47.2	47.2	47.2	47.2	47.2
Gross energy (kcal/kg)	5112	4913	4913	4913	4913	4913
Crude fat	14.6	12.2	12.2	12.2	12.2	12.2

[1]PM, Bio-phytase; Canadian Bio-systems Inc., Calgary, Alberta; 5000 phytase FYT units/g enzyme
[2]SM, Superzyme-OM; Canadian Bio-Systems Inc., Calgary, Alberta; (enzyme g/diet); 2800 cellulase, 400 mannanase, 50 galactanase, 1000 xylanase, 600 glucanase, 2500 amylase, 200 protease.
[3]MM, Use of both enzymes Bio-phytase and Superzyme-OM.
[4]Vitamin mixture (IU or g/kg of premix); vitamin A, 2,500,000 IU; vitamin D_3, 250,000 IU; vitamin E (dl-alpha tocopheryl acetate), 25,000 IU; vitamin K (menadione sodium bisulphate), 1.7 g; thiamin, 2.5 g; riboflavin, 4.2 g; pantothenic acid (as d-calcium pantothenate), 8.3 g; biotin, 0.17 g; folic acid, 0.8 g; vitamin B_{12}, 0.005 g; niacin, 25.0 g; pyridoxine, 2.0 g; ascorbic acid, 75.0 g; inositol, 58.3 g; ethoxyquin, 20.8 g; carrier (wheat middlings).
[5]Mineral mixture (g/kg of premix); manganous oxide, 15.0 g; zinc oxide, 25.0 g; copper sulfate, 2.5 g; magnesium sulfate, 16.6 g; ferrous iron, 10.0 g; carrier (wheat middlings).

steam pelleted using a lab scale California pellet mill fitted with a 3 mm die. The pellets were dried in a drying oven (JPW Design and Manufacturer) at 49°C for 2 hours. Diets were stored at -20°C in woven fibreglass feed bags tied at the top with twine until needed for feeding.

3.2.2 Experimental tanks and fecal collection technique

Fecal collection was based on a sedimentation system described by Tibbetts et al. (2006; Figure 3.2). The system was a flow-through with 18 tanks, each tank had a diameter of 72 cm and a

volume of 126 L. Tanks were cone shaped to allow fecal matter to settle quickly at the centre of the tank. Water inflow to each tank was 0.32 to 0.97 L/min (trial dependent; Table 3.7). Treatment was randomly assigned to each tank in the system. Each treatment was assigned 3 replicate tanks; therefore the Atlantic cod and Atlantic salmon trial only used 15 of the 18 tanks.

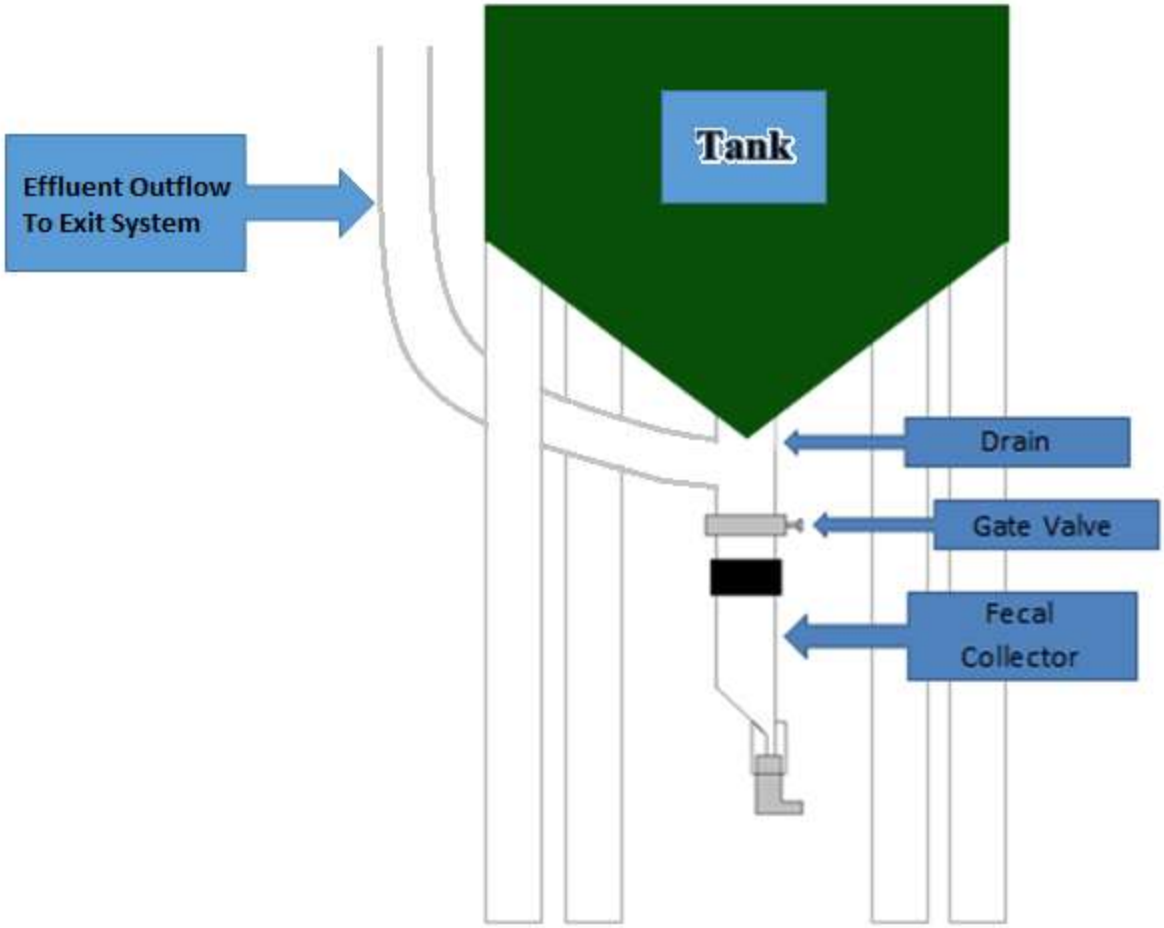

Figure 3.2. The fecal sedimentation collection tank modified from Tibbetts et al. (2006).

Diets were hand-fed to apparent satiation twice daily. Fecal collection began when the fecal matter turned green in colour due to the presence of chromic oxide. Fecal collection was performed from a settling column attached to the bottom of each tank. After the afternoon feeding (16:00 h), the tank sides, drains and collectors were scrubbed and the tanks partially

drained to make sure there was no residual feed in the system to contaminate the fecal samples. The collectors were then attached to each tank and the gate valves were opened overnight. In the morning (08:00 h) gate valves were closed, the fecal matter from the collectors was drained and placed into 2 L plastic containers. The fish were then fed their morning feeding. The fecal samples were centrifuged (4000 rpm for 20 minutes at 4°C), and the supernatant was decanted off. The feces was then stored in 120 mL sample cups in a -20°C freezer until analysis was performed.

Water temperature and oxygen levels were checked once daily in the morning an hour after feeding. The timing of measurement was to avoid stress to the fish before the meal was fed there by reducing the influence on feed consumed. The water quality, fish size and numbers were different for each experiment (Table 3.7). The Atlantic cod were obtained from Memorial University of Newfoundland, Ocean Sciences Research Centre. The rainbow trout and Atlantic salmon were obtained from the general fish population at the Aquaculture Centre of the Dalhousie University Agricultural Campus. Trials were conducted insequence in the same system, at the Dalhousie University Agricultural Campus, Aquaculture Centre. Maintenance and care of fish was carried out according to the Canadian Council on Animal Care (CCAC) guidelines (ISBN #: 0-919087-43-4; CCAC, 2005). The fish acclimated to the basal diet, without the two dietary markers, for one week before the trial began. The fish were weighed in batches (5 fish per batch) at the beginning and at the end of the trial after a minimum of 150 g of wet fecal matter was collected from each tank. Batch weighing was performed on a tared scale without the use of anaesthetic. Fish were placed into the tared bucket of water and the weight was recorded. The fish were then placed into the respective tank slowly by transferring them from the bucket

back into the tank. Feed was placed into individual feed pots assigned for each tank and weighed at the beginning and end of the trial. Fish were fed to apparent satiation twice daily by hand at 09:00h and 16:00h.

Table 3.7. Water quality (mean ± SE) during each trial and number of fish for each tank per trial.

	Atlantic cod	Atlantic salmon	Rainbow Trout	Rainbow Trout (Enzyme)
No. of fish/tank	25	25	20	15
Ave. initial weight of fish (g)	58 ± 5.7	50 ± 3.4	153 ± 6.7	404 ± 3.0
Salinity	Saltwater (31 ppt)	Freshwater	Freshwater	Freshwater
Flow rate per tank (L/min)	0.32	0.54	0.65	0.97
Temperature (°C)	7.1 ± 0.8	11.1 ± 0.6	9.8 ± 0.5	11.5 ± 0.5
Dissolved Oxygen Saturation (‰)	106 ± 2	104 ± 2	108 ± 1	110 ± 2

3.3 ANALYTICAL PROCEDURES

The feed ingredients, diets and the fecal samples were analysed in duplicate. Samples were analysed until less than a 5 % error was found between duplicates. To determine dry matter of the fecal samples, they were weighed before and after freeze drying. Dry matter content of the ingredients and diet samples was determined by weighing before and after drying in a drying oven (Fisher Scientific, Isotemp, Model # 750G) using the Association of Official Analytical Chemists (AOAC) method no. 934.01 (2005). After drying and prior to further analysis, feeds, ingredients and feces were ground to a particle size of 1mm using a coffee grinder (Black and Decker, Model CBG100SC, 118 ml). Feed and fecal samples were ashed in a muffle furnace (Fisher Scientific; Isotemp) at 550°C for 18 hours (AOAC 2005; method no. 942.05) then subjected to a perchloric acid digestion, to determine the chromic oxide by spectrophotometry (Fenton and Fenton, 1979). Crude protein, (N x 6.25), was determined by the Dumas method (Ebeling, 1968) using a Leco nitrogen determinator (model FP-528, LECO Corporation; AOAC

2005; method # 990.03). Crude fat was determined using an ANKOM Hydrolyzing Unit and an ANKOM XT15 Extractor using petroleum ether as the solvent (AOAC 2005; method Am 5-04). Gross energy was determined using an isoperibol oxygen bomb calorimeter (model 6300, Parr Instrument Company) equipped with a water recirculation system (model 6520A, Parr Instrument Company). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) in ingredients were analysed using ANKOM A200 Fibre Analyzer and Fibre bag Technology (ANKOM Technology Methods 5 and 6, 2014, respectively). Glucosinolates in ingredients were analysed by high-performance liquid chromatography (HPLC; Lange and Schumann, 1995) at the Saskatoon Research Centre (Agriculture and Agri-Food Canada, Saskatoon, SK, Canada). Phytate was determined by HPLC by MCN BioProducts, Inc (Saskatoon, SK, Canada) using the method of Newkirk and Classen (1998). Fatty acids, lipid classes, and fatty acid methyl esters (FAME) were measured as described in Hixson et al. (2014a). A chloroform:methanol extraction was performed (Parrish, 1999). The lipid classes were determined using a Iatroscan and silica Chromarods (Parrish, 1987). The FAME were measured using Hilditch reagent and thin layer chromatography (Morrison and Smith, 1964). Amino acid composition was analysed at the University of Manitoba (Animal Science Department, Winnipeg, Manitoba, Canada) using an amino acid analyzer (Model S2100, S4300; SYKAM Germany), after sample preparation by alkaline hydrolysis using a buffer (pH 5.4) for tryptophan (Hugli and Moore, 1972), by performic acid for cysteine and methionine (oxidized hydrolysis), all others via acid hydrolysis (aspartate, threonine, serine, glutamate, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, and NH3; AOAC 1995; method # 994.12). Mineral analysis (calcium, phosphorus, sodium, potassium, magnesium, manganese, copper, and

zinc) was performed using argon-plasma spectrometry (AOAC 2003; method #968.08), after ashing and HCl acid hydrolysis.

3.3.1 Wood Stick Test

The WM and enzyme treatments were mixed as in section 3.1.3.2 of the paper. The process was stopped just before the drying process and the wood stick test was measured on the wet meal mixtures. Four wooden sticks (30.5 cm long by 5 cm wide by 2.5 cm deep) were pre-weighed. Each was stirred into one of the four mixtures for 10 seconds. Each stick was then removed and weighed. The difference or weight of meal on the stick was calaulated by subtracting the original weight of the stick from the final weight of the stick. To compare the difference between the WM and the three enzyme treatments, the percent of the difference using the value from the stick test of the WM treatment was the baseline 100%. Calculated using the following equation:

Percent of Difference = (S x 100%) / WS

Where:
S = Meal on Stick Weight (g)
WS = Meal on Stick Weight of the WM treatment (6.6 g)

3.4 DIGESTIBILITY CALCULATIONS

To calculate the dry matter digestibility of the control and test diets the following equation from Tibbetts et al. (2006) was used:

Diet dry matter digestibility (%) = 100 – (100 x (a / b))

Where:
a= chromic oxide in diet
b= chromic oxide in feces

To calculate the other nutrient digestibilities in the control and test diets the following equation from Maynard et al. (1979) was used:

Diet ADC (%) = 100 – (100 x (b / d) x (c / a))

Where:
a= nutrient in feed
b= chromic oxide in feed
c= nutrient in feces
d= chromic oxide in feces

To calculate the digestibility of the test ingredient used in the test diets, the following calculation from Forster (1999) was used:

Ingredient ADC (%) = ((B + I) x T – (B) x C) x I^{-1}

Where:
B = nutrient provided by basal diet
I = nutrient provided by test ingredient
T= test diet ADC
C= control diet ADC

To calculate the digestible nutrient content in each of the test ingredients, the following equation from Lloyd et al. (1978) was used:

Digestible nutrient content = (N x D) / 100

Where:
N= amount of nutrient in test ingredient (dry matter, crude protein, gross energy, crude fat)
D= Ingredient ADC

3.5 STATISTICAL PROCEDURES

The trials were completely randomized experiments. The model statement was as follows:

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

Where:
Y_{ij} = Digestibility or digestible nutrient.
μ = the overall mean digestibility or digestible nutrient.
τ_i = the effect of diet or ingredient.
ε_{ij} = the random error.

Means ± standard error of the mean (SEM) were calculated for diet and ingredient digestibilities (dry matter, crude protein, crude fat, and gross energy), and ingredient digestible nutrients (digestible dry matter, digestible protein, digestible fat, and digestible energy). These values were analysed using analysis of variance (ANOVA) on the mixed model of SAS 9.2 (Littell et al., 1996), using diet treatment as the main effect. The fixed effect is the diet fed. The random effect is tank. Significant differences were apparent if the probability value (p-value) was less than 0.05. If significant differences did occur, then the data was analysed using LSMEANS and PDIFF to determine where the differences occurred ($P \leq 0.05$). Normality was tested using the Darling Anderson plot test (SAS Institute, 1995). Statistical evaluations were limited to within experimental comparisons, although comparisons of digestibility and digestive nutrients obtained were examined among experiments.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 PRODUCT DEVELOPMENT

Eleven products were developed during this project: 1) ground full fat seed (FFS), 2) high oil residue expelled meal (HORM), 3) oil, 4) solvent extracted meal (SEM), 5) toasted SEM (TSE), 6) SEM incubated with water (WM), 7) SEM incubated with water and Bio-phytase (PM), 8) SEM incubated with water and Superzyme-OM (SM), 9) SEM soaked with water and both enzymes (mix), 10) 70% crude protein concentrate (PC70), and 11) 80% crude protein concentrate (PC80). The processing of the full-fat camelina seed served three main functions; remove the oil, remove anti-nutrients (primarily, fibre components, glucosinolates and phytates), and to improve digestion of nutrients by enzyme incubation of the extruded solvent extracted camelina meal (SEM). The PC70 and PC80 had protein concentrations similar to, or greater than fishmeal (75%; herring meal; Tibbetts et al., 2006). However, their digestibilities were not measured since their production could not be up scaled in the laboratory to yield enough protein products for a feeding trial. This is just a minor set-back as commercially made plant-based protein concentrates are now produced in large-scale facilities. These protein concentrates include soy protein concentrate and Empyreal 75 (corn protein concentrate). Our process produced a similar product nutritionally, but needed a larger centrifuge to increase production. The protein concentrates were a light brown or tan colour. The protein concentrate meal was a much finer powder (40 mesh; 0.42 mm) compared to the SEM (6 mesh; 3.36 mm) due to the protein particles being precipitated out into a solution from a larger particle sized material.

The recovery of this protein meal was 10% of the weight of SEM processed. Each 100g batch of SEM processed yielded only 10g of recoverable protein concentrate, a protein yield of 6.97g of protein per 40.6g of protein originally in the SEM. The recovery percentage, 17.2% is similar to the percent protein yield from *Amaranth mantegazzianus* flour when using an acid pre-treatment conventional method (19.1% protein yield; Castel et al., 2012). However, the protein yield is lower than that of dehulled solvent extracted rapeseed flour (protein yield 52.5%; total solid yield 2.12kg per 6.8kg; Liu et al., 1982). The camelina protein concentrate yield was probably lower because the starting SEM was not dehulled and/or the larger particle size of the SEM product. The SEM was a meal, not a flour. Reduced particle size and dehulling would expose more of the products cells to the acid solution to extract the proteins, but was not tested here.

The oil processing methods consisted of mechanical extrusion, and mechanical extrusion followed by solvent extraction. Only mechanically extruded oil was tested because not enough of the solvent extracted oil was produced. During mechanical extrusion of the camelina seed, the oil and meal cake reached a temperature of 90°C, caused by friction at the die orifice. A small die was needed to match the small size of the camelina seed (2 to 3 mm long, 1 to 2 mm wide; tear drop shaped). The colour of the oil collected from the extruder (KEK 0500) was bright yellow. The meal cake was a medium brown colour (Figure 4.1) and had a strong cabbage taste and smell. Following hammer milling the resulting meal exhibited no change in either colour, taste or smell. Once the meal was solvent extracted it changed colour to a lighter brown or tan colour (Figure 4.1). This colour change indicates a change in the nutrient content of the meal. The oil that was removed by the solvent was still the same bright yellow colour as the oil was from the mechanical extrusion procedure (Figure 4.2). Both oils showed good clarity after filtration to

remove particulate matter. The meal products with the high crude fat content had a more vivid colour (Figure 4.1), which I speculate to be the tocopherols (vitamin E) present in the camelina oil, as high levels of tocopherols have been reported to contributed to the yellow colour of unrefined camelina oil (117 mg tocopherols/100g; Sizova, 2014). Vitamin E is known to be a natural anti-oxidant, therefore this is a beneficial component of camelina oil. The presence of the vitamin E provides the fatty acids with a natural protection from oxidation. In mechanically expelled camelina meal (4.9% crude fat), 0.07mg tocopherols/100g was found (Aziza et al., 2010), showing that the level of tocopherols had been decreased by the mechanical removal of camelina oil.

Figure 4.1. Samples of *Camelina sativa* seed, expelled cake, expelled meal, and expelled solvent extracted meal (SEM; in order of appearance from left to right), colour change is notable between the expelled meal and SEM

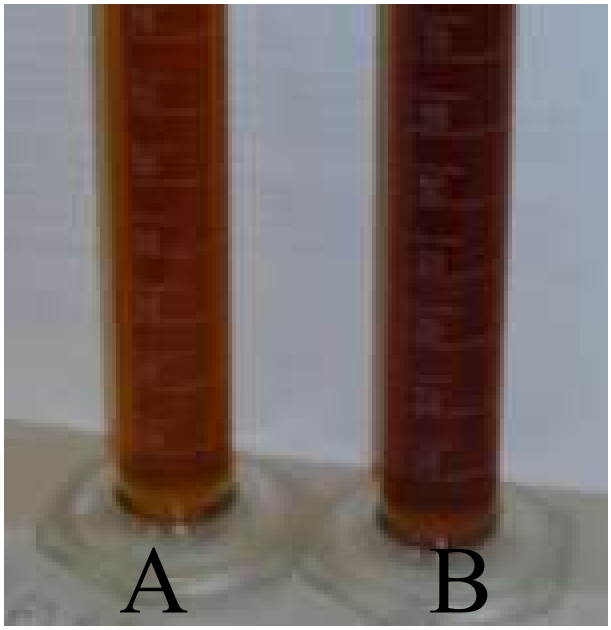

Figure 4.2. Photo showing red colour of filtered (A) and unfiltered (B) camelina oil.

The only other physical characteristic that changed after treatment of the SEM was a decrease in viscosity in the SM and MM treatments (Table 4.1). The high viscosity is caused by mucilage, which is a hard to measure directly. However, the high viscosity of the product decreased with the SM and MM treatments was demonstrated by the lesser amount of wet camelina material adhering to the wooden stick. The mucilage degradation was estimated from the reduced amount of NSP (non-starch polysaccharide) in defatted flax seed meal. In this meal the total NSP was degraded from 271g/kg to 178g/kg using a mixture of enzymes including cellulose, pectinase, xylanase, glucanase, mannanase, and cellulose (Slominski et al., 2006). This is a decrease of about 66% in the NSP content. The Superzyme-OM enzyme mixture included all the enzymes used in the research on flax, therefore similar results were expected. Our results from the stick test indicated the 32 to 35% reduction of the NSP. However, NSP levels should be quantified in future studies.

Table 4.1. Difference in wood stick test to demonstrate loss of mucilage in the SM and MM treatments compare to the WM treatment.

Meal on Stick	WM[1]	PM[2]	SM[3]	MM[4]
Amount (g)	6.6	5.4	2.3	2.1
Percent Difference from WM (%)	100	-82	-35	-32

[1]WM, water incubated SEM; [2]PM, phytase incubated SEM; [3]SM, superzyme incubated SEM; [4]MM, mixed enzyme incubated SEM; n = 3.

4.2 INGREDIENT PROFILES OF CAMELINA BY-PRODUCTS

The composition of the products from the camelina seed changed due to processing (Tables 4.2, 4.3, 4.4, 4.5, and 4.6). The crude protein content of the products was inversely related to oil content. Comparing the FFS with SEM there was an increase in crude protein from 26.8 to 40.6% (Table 4.3) and a crude fat decrease from 36.7 to 3.2% (Table 4.4), respectively. The increase in protein was expected, and the size of the increase was expected with the percentage of lipid removed and concentration of the other nutritional components. The difference between the crude lipid of FFS (36.7%) and the SEM (3.2%) is 33.2% (Table 4.4). The total difference of the crude protein between the FFS (26.8%) and SEM (40.6%) is 13.8% (Table 4.3). The predicted difference would be that crude protein would still be 26.8% of the residue meal, therefore the predicted increase would be about 17.9%. The actual increase (13.8%) is 4.1% lower than the predicted. Some loss of nutrient would occur via the removal of suspended smaller nutrient components in the solvent during the decanting procedure to drain off the petroleum ether. Compared to soybean meal (fat, 2.0%; crude protein, 47.3%) and soy protein concentrate (fat, 0.3%; crude protein. 68.7%) similar changes to the protein and lipid may occur (Tibbetts et al., 2006).

Table 4.2. Nutrient composition of camelina seed, oil, and meals showing gross energy provided (as-fed basis).

Nutrient Composition	Test Ingredient										
	FFS[1]	Oil	HORM[2]	SEM[3]	PC70[4]	PC80[5]	TSE[6]	WM[7]	PM[8]	SM[9]	MM[10]
Dry Matter (%)	94.7±0.18	99.3±0.23	93.0±0.13	92.4±0.04	94.1±0.36	98.6±0.33	92.3±0.17	95.1±0.04	91.8±0.28	94.9±0.44	94.8±0.13
Gross Energy (kcal/kg)	6190±53	9197±102	4744±64	4410±58	5994±45	6513±38	4270±72	4438±56	4456±46	4456±42	4456±52
Ash (%)	3.8±0.02	-	5.9±0.01	6.3±0.03	3.4±0.01	1.8±0.01	6.4±0.01	6.5±0.02	7.2±0.01	6.9±0.01	7.0±0.01
Calcium (%)	0.3±0.03	-	0.5±0.00	0.4±0.00	0.1±0.00	0.0±0.00	0.5±0.01	0.6±0.01	0.7±0.01	0.6±0.01	0.6±0.02
Phosphorus (%)	0.7±0.00	-	1.0±0.00	1.0±0.01	0.5±0.00	0.3±0.00	1.1±0.00	1.1±0.01	1.1±0.01	1.1±0.00	1.1±0.00
Sodium (%)	ND	-	0.00±0.00	0.0±0.00	0.9±0.01	0.4±0.00	0.0±0.00	0.1±0.00	0.1±0.00	0.1±0.00	0.0±0.00
Potassium (%)	1.0±0.01	-	1.4±0.01	1.5±0.01	0.8±0.00	0.3±0.01	1.5±0.01	1.5±0.00	1.5±0.00	1.5±0.01	1.5±0.01
Magnesium (%)	0.3±0.02	-	0.4±0.00	0.4±0.00	0.1±0.00	0.0±0.00	0.4±0.00	0.5±0.00	0.5±0.01	0.5±0.00	0.5±0.00
Manganese (ppm)	23.0±0.1	-	35.0±0.12	38.0±0.13	7.0±0.01	ND	38.0±0.11	40.0±0.14	40.0±0.09	41.0±0.16	40.0±0.21
Copper (ppm)	9.0±0.03	-	11.0±0.04	14.0±0.07	11.0±0.05	15.0±0.08	12.0±0.06	14.0±0.04	17.0±0.07	15.0±0.11	15.0±0.06
Zinc (ppm)	47.0±0.2	-	72.0±0.42	78.0±0.36	42.0±0.16	33.0±0.13	75.0±0.20	78.0±0.23	80.0±0.19	87.0±0.25	102.0±0.18
ADF (%)	23.6±0.28	-	18.3±0.48	18.0±0.85	13.9±0.17	18.1±0.29	17.6±0.02	16.4±0.31	18.4±0.34	18.7±0.20	17.2±0.27
NDF (%)	37.1±0.17	-	40.0±0.31	33.9±1.15	22.5±1.32	0.0±0.00*	36.5±2.83	24.0±0.79	31.7±0.75	35.0±0.71	29.0±0.69

Mean values ± sem (n=3); - = Not Tested due insufficient material/cost evaluation; ND=Not Detected; [1]FFS, full fat seed (ground); [2]HORM, extruded meal (high oil residue); [3]SEM, extruded solvent extracted meal (low oil residue); [4]PC70, protein concentrate 70% crude protein; [5]PC80, protein concentrate 82% crude protein; [6]TSE, toasted extruded solvent extracted meal; [7]WM, water incubated SEM; [8]PM, phytase incubated SEM; [9]SM, superzyme incubated SEM; [10]MM, mixed enzyme incubated SEM. * NDF value is not possible due to the presence of an ADF value, as NDF contains ADF (NDF = ADF + acid detergent solubles).

Table 4.3. Protein and amino acid compositions of camelina seed and its by-products (as-fed basis).

Protein Composition	Test Ingredient									
	FFS[1]	HORM[2]	SEM[3]	PC70[4]	PC80[5]	TSE[6]	WM[7]	PM[8]	SM[9]	MM[10]
Crude Protein (%)	26.8±0.10	38±0.11	40.6±0.31	69.7±0.13	82.3±0.26	40.2±0.06	41.3±0.02	42.1±0.10	41.6±0.13	41.4±0.11
Amino Acid (%)										
Aspartate	2.2±0.02	3.0±0.01	3.2±0.03	6.1±0.03	-	3.1±0.02	3.3±0.01	3.3±0.00	3.3±0.02	3.4±0.02
Threonine	1.1±0.01	1.5±0.01	1.6±0.01	3.0±0.00	-	1.6±0.01	1.7±0.00	1.7±0.00	1.7±0.01	1.7±0.01
Serine	1.3±0.00	1.7±0.01	1.9±0.01	3.5±0.01	-	1.9±0.01	1.9±0.02	2.0±0.00	2.0±0.01	2.0±0.01
Glutamate	4.6±0.01	6.2±0.02	6.6±0.06	12.6±0.03	-	6.6±0.05	7.0±0.02	7.0±0.01	7.1±0.04	7.1±0.03
Proline	1.4±0.01	1.9±0.00	2.1±0.03	4.0±0.01	-	2.2±0.02	2.3±0.00	2.3±0.01	2.3±0.01	2.2±0.03
Glycine	1.3±0.01	1.8±0.01	1.9±0.00	3.5±0.00	-	1.9±0.01	2.1±0.00	2.1±0.00	2.1±0.03	2.1±0.01
Alanine	1.2±0.00	1.6±0.00	1.7±0.01	3.1±0.01	-	1.7±0.01	1.9±0.00	1.9±0.00	1.9±0.01	1.9±0.00
Cysteine	0.6±0.01	0.7±0.00	0.8±0.02	0.9±0.01	-	0.7±0.06	0.8±0.00	0.9±0.01	0.9±0.01	0.9±0.00
Valine	1.2±0.01	1.8±0.02	1.7±0.00	3.6±0.03	-	1.6±0.01	2.0±0.03	2.0±0.02	2.0±0.01	2.0±0.02
Methionine	0.5±0.01	0.6±0.01	0.6±0.02	1.3±0.01	-	0.6±0.05	0.6±0.00	0.7±0.01	0.7±0.00	0.6±0.00
Isoleucine	0.8±0.02	1.2±0.01	1.2±0.01	2.5±0.03	-	1.2±0.01	1.4±0.03	1.4±0.00	1.4±0.01	1.4±0.02
Leucine	1.6±0.01	2.3±0.02	2.4±0.01	5.0±0.01	-	2.4±0.02	2.6±0.01	2.6±0.01	2.7±0.01	2.7±0.03
Tyrosine	0.7±0.01	0.9±0.01	1.0±0.00	2.0±0.00	-	1.0±0.01	1.0±0.01	1.0±0.00	1.0±0.01	1.0±0.00
Phenylalanine	1.0±0.02	1.5±0.01	1.5±0.00	3.2±0.00	-	1.5±0.01	1.6±0.00	1.6±0.01	1.6±0.01	1.6±0.02
Histidine	0.8±0.01	1.0±0.02	1.1±0.00	2.1±0.00	-	1.1±0.01	1.2±0.00	1.2±0.01	1.2±0.02	1.2±0.00
Lysine	1.2±0.01	1.7±0.01	1.8±0.00	3.0±0.01	-	1.8±0.01	1.8±0.02	1.8±0.01	1.9±0.00	1.8±0.00
Arginine	2.2±0.01	3.1±0.02	3.3±0.04	6.6±0.02	-	3.2±0.02	3.2±0.00	3.3±0.00	3.2±0.00	3.3±0.01
Tryptophan	0.3±0.01	0.3±0.01	0.4±0.00	0.8±0.00	-	0.4±0.00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
Ammonia (NH_3) (%)	0.6±0.00	0.8±0.01	0.8±0.01	1.5±0.00	-	0.8±0.01	0.9±0.00	1.0±0.00	1.0±0.00	0.9±0.00

Mean values ± sem (n=3); - = Not Tested, data not yet returned; [1]FFS, full fat seed (ground); [2]HORM, extruded meal (high oil residue); [3]SEM, extruded solvent extracted meal (low oil residue); [4]PC70, protein concentrate 70% crude protein; [5]PC80, protein concentrate 82% crude protein; [6]TSE, toasted extruded solvent extracted meal; [7]WM, water incubated SEM; [8]PM, phytase incubated SEM; [9]SM, superzyme incubated SEM; [10]MM, mixed enzyme incubated SEM

Table 4.4. Fat composition of oil in camelina seed and its by-products (as-fed basis).

Fat Composition	Test Ingredient										
	FFS[1]	Oil	HORM[2]	SEM[3]	PC70[4]	PC80[5]	TSE[6]	WM[7]	PM[8]	SM[9]	MM[10]
Crude Fat (%)	36.7±0.26	99.6±0.49	9.9±0.14	3.2±0.63	2.1±0.58	0.4±0.46	3.6±1.13	5.3±0.17	3.6±0.15	4.6±0.02	5.3±0.14
Fatty Acids (% of total fatty acid methyl ester)											
14:00	0.1±0.00	0.1±0.01	0.1±0.00	0.1±0.00	-	-	-	0.2±0.01	0.2±0.00	0.4±0.01	0.1±0.00
α15:0	0.1±0.00	0.0±0.00	0.0±0.00	0.0±0.00	-	-	-	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
15:00	0.0±0.00	0.1±0.00	0.1±0.00	0.1±0.00	-	-	-	0.1±0.00	0.1±0.01	0.1±0.00	0.0±0.00
16:00	7.2±0.08	7.7±0.04	8.6±0.01	11.1±0.02	-	-	-	11.6±0.01	12.0±0.03	12.2±0.04	11.7±0.02
16:1ω7	0.1±0.07	0.2±0.01	0.2±0.00	0.1±0.01	-	-	-	0.3±0.01	0.5±0.01	0.7±0.02	0.3±0.00
16:1ω5	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	-	-	-	0.1±0.00	0.0±0.00	0.0±0.00	0.0±0.00
α17:0	0.0±0.00	0.1±0.01	0.1±0.00	0.0±0.00	-	-	-	0.1±0.01	0.1±0.00	0.2±0.00	0.0±0.00
16:2ω4	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	-	-	-	0.0±0.00	0.1±0.01	0.0±0.00	0.0±0.00
17:00	0.1±0.00	0.1±0.00	0.1±0.01	0.1±0.00	-	-	-	0.1±0.01	0.1±0.00	0.1±0.01	0.0±0.00
17:01	0.1±0.00	0.1±0.01	0.1±0.00	0.1±0.01	-	-	-	0.0±0.00	0.1±0.00	0.1±0.00	0.0±0.00
18:00	2.3±0.01	2.3±0.03	2.4±0.01	2.5±0.02	-	-	-	2.6±0.3	2.7±0.06	2.9±0.07	2.7±0.05
18:1ω9	13.8±0.08	14.4±0.05	15.0±0.06	16.1±0.16	-	-	-	15.9±0.17	15.7±1.03	16.2±1.06	15.7±0.48
18:2ω7	1.1±0.02	1.3±0.01	1.7±0.01	1.4±0.04	-	-	-	1.9±0.01	2.3±0.02	2.2±0.01	2.1±0.03
18:2ω6	19.8±0.13	23.7±0.11	24.5±0.09	27.2±0.29	-	-	-	27.2±0.20	27.1±1.09	26.6±0.87	27.3±0.44
18:3ω3	33.5±0.23	28.3±0.08	27.9±0.12	26.7±0.07	-	-	-	24.2±0.37	23.4±0.84	23.4±1.02	24.7±0.62
18:4ω3	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	-	-	-	0.0±0.00	0.0±0.00	0.1±0.00	0.0±0.00
20:00	0.5±0.02	1.7±0.01	1.4±0.00	1.1±0.01	-	-	-	1.2±0.01	1.1±0.01	1.0±0.01	1.2±0.0
20:1ω9	12.8±0.07	12.1±0.06	10.4±0.02	7.8±0.03	-	-	-	8.3±0.06	7.9±0.07	7.8±0.04	8.3±0.06
20:2ω6	1.8±0.02	1.8±0.01	1.7±0.00	1.4±0.03	-	-	-	1.4±0.04	1.5±0.05	1.3±0.00	1.5±0.04
20:3ω3	1.2±0.01	0.8±0.00	0.9±0.00	0.5±0.01	-	-	-	0.7±0.01	0.7±0.02	0.7±0.01	0.5±0.01
22:00	0.2±0.00	0.4±0.01	0.3±0.00	0.1±0.0.0	-	-	-	0.2±0.03	0.1±0.00	0.1±0.00	0.1±0.01
22:1ω9	2.4±0.02	3.3±0.04	0.3±0.00	0.1±0.01	-	-	-	1.8±0.01	1.7±0.03	1.4±0.00	1.7±0.02
24:00	0.0±0.00	0.2±0.01	0.1±0.00	0.0±0.00	-	-	-	0.1±0.00	0.0±0.00	0.1±0.01	0.1±0.00
24:01	0.2±0.03	0.7±0.00	0.4±0.01	0.2±0.00	-	-	-	0.4±0.00	0.2±0.01	0.2±0.00	0.2±0.00

Mean values ± sem (n=3); - = Not Tested, too low to test/cost efficiency; [1]FFS, full fat seed (ground); [2]HORM, extruded meal (high oil residue); [3]SEM, extruded solvent extracted meal (low oil residue); [4]PC70, protein concentrate 70% crude protein; [5]PC80, protein concentrate 82% crude protein; [6]TSE, toasted extruded solvent extracted meal; [7]WM, water incubated SEM; [8]PM, phytase incubated SEM; [9]SM, superzyme incubated SEM; [10]MM, mixed enzyme incubated SEM.

The gross energy content decreased as the oil (9198 kcal/kg) was removed from the seed (6190 kcal/kg) and meals (Table 4.2; SEM, 4410 kcal/kg). The protein concentrates PC 70 and PC80 had high gross energy values of 5994 kcal/kg and 6513 kcal/kg, respectively. The higher level of gross energy in protein compared to carbohydrates could account for the difference (Miller, 2004). This is also seen in the soybean meal (crude protein, 47.3%; carbohydrate, 33.3%), soy protein concentrate (crude protein, 68.7%; carbohydrate, 18.0%) and soy protein isolate (crude protein, 85.6%; carbohydrate, 0.0%), where the carbohydrates drop as the protein concentration increases (Tibbetts et al., 2006). The carbohydrate value includes fibre, sugar and starch.

Acid detergent fibre (ADF) decreased in the full fat seed (23.6%) after extrusion to produce high oil residue meal (18.3%; Table 4.2), the ADF did not change among most of the rest of the products. The PC70 ADF was reduced to 13.9%, likely caused by the removal of the non-water soluble fibres such as cellulose in the residue meal during the acid soak. The ADF was 18.1% in the PC80. The NDF change from PC70 to PC80 was not expected. Since both the ADF and NDF values include cellulose the removal of all other components could have reduced the NDF to ADF levels, but not to zero percent. ANKOM F57 filter bags were used for the NDF and ADF analysis, which has a pore size of 25 microns. Triplicate sample runs of the NDF bags resulted in empty sample bags for the PC80 sample. As none of the heat seals were broken open, there is no explanation for this result of zero NDF. Since NDF is equal to ADF plus the acid detergent solubles, NDF cannot be zero if you have a value for ADF, as the PC80 does in Table 4.2.

The process of freezing the PC80 before the centrifuging the final protein precipitate from the basic solution caused the plant cell walls to be ruptured. This should allow additional protein

components to be solubilized into the basic solution and be removed in the protein concentrate. The process known as freeze-fractioning or freeze shattering, is a laboratory technique to break apart cell walls for plant studies without using exogenous enzymes (Wasteneys et al., 1997).

The product with the highest crude protein and the lowest crude fat was the protein concentrate (PC80) (82.3% crude protein, Table 4.3; 0.4 % crude fat, Table 4.4). The protein content of PC80 was greater than fishmeal (75% CP; herring meal; Tibbetts et al., 2006) and soy protein concentrate (56.6% CP; Chowdhury et al., 2012). The PC70 was high in arginine (6.6%; Table 4.3) and threonine (3.0%) compared to fish meal (3.84% and 2.58%, respectively; Jeong et al., 2016) and soy protein concentrate (3.9% and 1.8%, respectively; Chowdhury, 2012). However, lysine (3.0%; Table 4.3) and methionine (1.3%) are lower in concentration compared to fishmeal (5.56% and 2.70%, respectively; Jeong et al., 2016). When comparing PC70 to soy protein concentrate (1.3% methionine + cysteine; Chowdhury et al., 2012) the methionine concentration is higher (Table 4.3). The PC70 has a more concentrated amino acid profile than soy protein concentrate. Since PC80 has a higher concentration of crude protein, the amino acid concentrations should be higher than PC70 however the profile is unknown due to lack of quantity for analysis. The PC80 needs to be evaluated further to examine the amino acids change due to the freeze shattering that occurred in the procedure. The PC70 has the best potential for use as a concentrated protein source for aquaculture feed based on the amino acid profile concentration. Unfortunately, both protein concentrates could not be scaled up efficiently by any of the laboratory attempts to increase the yield from a 10:1 ratio of SEM to PC80. A larger centrifuge or a spray drier would have made it possible to make larger batches. Until a

commercial profitable process can be developed these P70 and P80 protein concentrates remain unusable.

The product most suitable for upscaling to commercial production based on yield with the highest crude protein content was the SEM (41% crude protein; Table 4.3). The process used to make SEM is very similar that used to make canola meal. The crude protein content of SEM was lower than fishmeal (75%; Tibbetts et al., 2006) and soy protein concentrate (56.6%; Chowdhury et al., 2012). This product was higher in neutral detergent fibre (NDF) compared to the PC70 (10% less; Table 4.2) and PC80 (0%), which can result in lower feed conversion (FCR), growth, and digestibility, especially if the indigestible fibre content is high in non-starch polysaccharide (NSP; Denstadli et al., 2011). The PC70 had a drop in ADF and NDF which would occur due to the loss of fibre in the residue meal discarded after the acid treatment.

Subjecting the original full-fat seed to mechanical pressing, solvent extraction, and protein extraction methods increased the amino acid concentration (Table 4.3) and decreased the fat content (Table 4.4). The product tested with a highest concentration of amino acids on an as-fed basis was the protein concentrate (PC70; 69.7% crude protein). The amino acid profile of the PC80 was not measured due to insufficient product produced during testing. The PC70 methionine concentration (1.3%) was double that of the SEM (methionine, 0.6%; Table 4.3) product, which was expected due to the increase in crude protein content without changing the amino acid profile. The amino acid profile of PC70 (methionine, 1.3%; cysteine, 0.9%; lysine, 3.0%; Table 4.3) has better potential for carnivorous fish diets than soy protein concentrate (methionine + cysteine, 1.3%; lysine, 2.7%; Chowdhury et al., 2012), due to higher

concentrations of limiting amino acids. This could be a more cost effective protein source if it can be made, compared to soy protein concentrate, depending on the growth factors, performance and price of the PC70, allowing for lower inclusions of additional amino acids to the complete feed formulation. Once a single amino acid is depleted from incorporation into lean tissue growth, the entire chain of protein synthesis stops and other amino acids provided in the diet are not utilized until the limiting amino acid becomes available (Rosenberg, 2012). Consequently, specific amino acids are added to commercial feeds to allow more complete protein usage from less expensive protein ingredients. The addition of single amino acids that are limiting can be a cost effective way of improving the protein usage in feed. The lysine (3.0%; Table 4.3) in the PC70 may be the limiting amino acid in future formulation, as it is lower than fishmeal (5.56%; Jeong, 2016) and canola meal. Comparing SEM (crude protein, 40.6%; methionine, 0.6%; cysteine, 0.8%; lysine, 1.8%) to solvent extracted canola meal (crude protein, 34.0%; methionine, 0.71%; cysteine, 0.86%; lysine, 2.02%; Landero et al., 2011), the amino acids profile is slightly lower for the SEM. Comparing PC70 (crude protein, 69.7%; methionine, 1.3%; cysteine, 0.9%; lysine, 3.0%) to canola protein concentrate (crude protein, 72.4%; methionine, 1.5%; cysteine, 1.5%; lysine, 3.5%; Thiessen et al., 2004), the amino acids are similar in methionine levels, but lower in cysteine and lysine. Methionine (1.3%) may be a limiting amino acid for fish due to the lower concentration when compared to fishmeal (2.7%; Jeong, 2016).

For rainbow trout feeds, inclusion rate of soy protein concentrate can exceed 95% of the crude protein content, but only with supplementation of limiting essential amino acids (methionine and lysine; Zhang et al., 2012). Therefore, since the PC70 contains a higher amino acid content than

the soy protein concentrate, it should be investigated further once a process or equipment scale up was found to increase production efficiently. The maintenance amino acids reported from post-smolt Atlantic salmon, showed a requirement of 18.0 mg $kg^{-0.7}/d^{-1}$ lysine and 4.3 mg $kg^{-0.7}/d^{-1}$ methionine (Helland et al., 2010). Maintenance amino acids are the amino acids needed to maintain normal body functions, this means no extras like growth or reproduction. The lysine requirement of salmonids has been reported a 1.8% of diet (NRC, 2011).

Two other processes in commercial production of soy protein concentrate, aqueous alcohol wash and heat denaturation water extraction, should be tested on SEM. Larger equipment or different equipment designs may be the key to increase the yield of PC70 or PC80. Canola protein concentrate also suffered from low yields improved by updated technology, such as centrifuges with higher G forces (Campbell et al., 2016). For this research, I did not have access to equipment, such as a rotary vacuum filter or a spray drier, which are typically used in commercial production of acid washed soy protein concentrate. SEM was used to investigate the ability of fish to digest the crude protein and other nutrients available from camelina, however this may change for PC70 and PC80 due to the higher crude protein with lower concentrates within these two products (Table 4.3).

Gross energy content (Table 4.2) decreased as the crude fat content of the products was reduced (Table 4.4). The product with the highest crude fat and gross energy was the camelina oil (99.6% and 9197 kcal/kg, respectively). The FFS was the product next highest in crude fat (36.7%), however it did not have the next highest gross energy (6190 kcal/kg). PC80 had the next highest

gross energy (6513 kcal/kg), but also had the lowest crude fat (0.4%). The higher gross energy in the PC80 compared to the FFS, was associated with the higher gross energy in crude protein content compared to carbohydrate. Gross energy is the highest in fats, such as oil, therefore we would expect a high gross energy value for the camelina oil. Protein is higher in energy than carbohydrate, this is why a high protein product would have a higher gross energy level (Hall et al., 2013).

An important aspect of the lipid profile in camelina is the high level of ALA (28.3% of lipid in oil; Table 4.4), which is the precursor for the biosynthesis of EPA and DHA by the desaturation and elongation enzymatic process in Atlantic salmon and rainbow trout (Caballero et al., 2002; Codabaccus et al., 2011). The higher content of 20:2 n-9, 22:4 n-6, 22:5 n-6, and 24:6 n-3 in the liver and muscle of rainbow trout (*Oncorhynchus mykiss*) indicates intermediate metabolites of PUFA synthesis were being formed while fish were fed rapeseed and soybean oil where there was no dietary DHA and EPA (Caballero et al., 2002). The presence of the desaturase and elongase enzyme gene expression in the liver and white muscle of Atlantic salmon (*Salmo salar*) being fed canola and echium oils compared to fish oil suggests an active biosynthesis (Codabaccus et al., 2011). These studies established chain elongation of EPA and DHA is possible in salmonids.

The fatty acid profiles of each of the products from the original FFS during mechanical and chemical extraction of camelina oil are shown in Table 4.4 and 4.5. Differences in crude fat between products in Table 4.4 were consistent with the amount of residual oil left after processing. For example, SEM (crude fat 3.2%) is lower than FFS (crude fat 36.7%). Total lipid

decreased as processing removed the oil content from the seed, as did the total omega 3 fatty acids and the omega 3 to 6 ratio (Table 4.5). Omega 6 fatty acids increased while omega 3 fatty acids decreased with mechanical and chemical removal of camelina oil from the seed and meals. In turn this changed the omega 3 to 6 ratio. Some of this change could be due to the heat (90°C) during the seed extrusion and chemical degradation of the omega 3 fatty acids during solvent extraction (Canvin, 1965; Byfield and Upchurch, 2007; Sargent et al., 1989; Higgs and Dong, 2000). Temperature increases of 20°C during the growth of the plants changed the fatty acid profile by decreasing the amount of unsaturated fatty acids, such as linolenic (rapeseed 6% decrease; flax 20% decrease) and linoleic (rapeseed 5% decrease; flax 10% decrease), with increase in fatty acids such as oleic acid (20% increase) (Canvin, 1965). Increased temperature activates a naturally occurring microsomal omega-3 linoleate desaturase enzyme in soybeans exposed to a temperature increase of about 10°C, this caused a decrease in the linolenic acid by 39 to 50% (Byfield and Upchurch, 2007). The 18:3 (linolenic acid) makes up about 96.5% (Table 4.4 and 4.5) of the omega 3 content of the FFS, therefore when the temperature is increased during extrusion of the seed, the enzyme would be activated reducing the omega 3 in the resulting HORM and oil camelina by-products.

The characteristics of the lipid in camelina products changed by processing. The triacylglycerol decreased and phospholipids increased in the camelina oil (49.9% of lipid; 8.5% of lipid; respectively; Table 4.5) and HORM (36.9% of lipid; 27.4% of lipid; respectively), as the oil content was removed via extrusion from the FFS (76.1% of lipid; 9.2% of lipid; respectively). Phospholipids are natural antioxidants, they are also known as gum, they are part of the plant cell

Table 4.5. Lipid classes of oil in camelina seed and its by-products (as-fed basis).

Fat Classification	Test Ingredient							
	FFS[1]	Oil	HORM[2]	SEM[3]	WM[4]	PM[5]	SM[6]	MM[7]
Lipid Class (% of total lipid)								
Total Lipid	27.0±2.35	100.0±1.48	6.8±1.53	5.5±1.29	5.7±1.05	5.0±0.22	4.8±1.96	3.9±1.16
Triacylglycerols	76.1±1.62	49.9±1.24	36.9±1.88	29.4±0.23	29.7±0.74	41.7±2.71	24.6±0.39	14.1±0.82
Phospholipids	9.2±0.86	8.5±0.62	27.4±1.49	32.3±2.06	27.7±1.03	15.7±0.47	31.7±1.49	44.6±1.67
Fatty Acids (% of total fatty acid methyl ester)								
∑SFA	11.4±0.24	12.5±0.31	13.0±0.06	15.0±0.17	15.9±0.19	16.4±0.27	16.8±0.14	16.1±0.21
∑MUFA	31.6±1.09	33.4±1.34	31.2±1.36	28.9±0.73	29.9±0.86	29.5±0.66	29.8±1.53	29.5±1.22
∑PUFA	56.9±2.32	54.6±1.04	55.7±2.14	56.1±1.36	54.1±2.49	54.0±1.47	53.2±1.62	54.4±2.13
∑ω3	34.7±0.49	29.1±1.81	28.9±1.06	27.2±1.76	24.9±1.52	24.5±1.27	24.7±0.58	25.2±1.01
∑ω6	21.9±1.28	25.5±0.96	26.5±1.51	28.8±1.38	28.8±1.64	28.8±1.82	28.0±1.07	28.9±1.27
ω3/ω6	1.6±0.09	1.1±0.07	1.1±0.04	0.9±0.01	0.9±0.02	0.9±0.01	0.9±0.01	0.9±0.02

Mean values ± sem (n=3); [1]FFS, full fat seed (ground); [2]HORM, extruded meal (high oil residue); [3]SEM, extruded solvent extracted meal (low oil residue); [4]WM, water incubated SEM; [5]PM, phytase incubated SEM; [6]SM, superzyme incubated SEM; [7]MM, mixed enzyme incubated SEM; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

wall and they are polar, therefore during the extrusion process the phospholipids may remain in the meal as they are insoluble in oil and are only soluble in water (Zhang et al., 1994). The opposite relationship was found with the solvent extraction process and the production of SEM from HORM, this could be caused by the solvent of choice, petroleum ether, which is a non-polar or neutral solvent (ChemBook, 2008). Phospholipids as polar molecules, will not solubilize into the solvent, so cannot be removed with this method. Triacylglycerol will solubilize as they are neutral molecules (Sargent et al., 1989; Higgs and Dong, 2000). The phospholipid contributes as a source of phosphorus, therefore the resulting drop in the concentration of phospholipid in PM (15.7%; Table 4.5) may be caused by action of the enzyme phytase making phosphorus available or degrading the phospholipid (Viveros et al., 2000). Although the phytase was in the MM treatment, a similar decrease was not observed. The drop in the PM is interesting and warrants further investigation.

The anti-nutrients increased as protein increased including glucosinolates (SEM, 38.1μmoles/g; FFS, 26.0 μmoles/g) and phytate (SEM, 0.7%; FFS, 0.5%; Table 4.6). They can be removed with further processing (Ohren, 1981) or enzyme treatments (Storebakken et al., 1998). Table 4.6 shows that heating had not effect on the glucosinolate concentration in the SEM. The glucosinolates however were totally removed using the WM incubation of SEM. The SEM was a ground product that was exposed to water, causing an activation of the naturally occurring enzyme myrosinase to interact with the glucosinolates for breakdown products such as isothiocyanates and nitriles (Duncan, 1991). Water soaking reduced glucosinolates from rapeseed (from 20 mmol/kg to 2.1) and rapeseed press cake (from 18.5mmol/kg to 0.3; Schöne et

Table 4.6. Glucosinolate and phytate composition of camelina seed, oil, and meals (as-fed basis).

	Test Ingredient										
	FFS[1]	Oil	HORM[2]	SEM[3]	PC70[4]	PC80[5]	TSE[6]	WM[7]	PM[8]	SM[9]	MM[10]
Glucosinolates (μmoles/g meal)											
9-Methyl-Sulfinyl-Nonyl	7.1±0.04	-	10.1±0.10	10.4±0.07	9.4±0.06	-	10.8±0.03	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
10-Methyl-Sulfinyl-Decyl	16.1±0.11	-	23.1±0.18	23.6±0.17	22.7±0.17	-	24.7±0.03	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
11-Methyl-Sulfinyl-Undecyl	2.7±0.02	-	4.0±0.03	4.1±0.07	4.4±0.01	-	4.3±0.00	0.0±0.0.0	0.2±0.00	0.0±0.00	0.0±0.00
Total	26.0±0.13	-	37.2±0.06	38.1±0.03	36.5±0.09	-	39.7±0.03	0.0±0.00	0.2±0.00	0.0±0.00	0.0±0.00
PMtate (%, as fed)	0.5±0.01	-	0.6±0.00	0.7±0.01	-	-	-	0.4±0.00	0.0±0.00	0.7±0.00	0.0±0.00
IP6[11]	0.5±0.01	-	0.6±0.00	0.6±0.01	-	-	-	0.1±0.00	0.0±0.00	0.6±0.00	0.0±0.00
IP5[12]	0.0±0.00	-	0.0±0.00	0.0±0.00	-	-	-	0.0±0.00	0.0±0.00	0.1±0.00	0.0±0.00
IP4[13]	0.0±0.00	-	0.0±0.00	0.0±0.00	-	-	-	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00

Mean values ± sem (n=3); - = Not Tested due insufficient material/cost evaluation; ND=Not Detected; [1]FFS, full fat seed (ground); [2]HORM, extruded meal (high oil residue); [3]SEM, extruded solvent extracted meal (low oil residue); [4]PC70, protein concentrate 70% crude protein; [5]PC80, protein concentrate 82% crude protein; [6]TSE, toasted extruded solvent extracted meal; [7]WM, water incubated SEM; [8]PM, phytase incubated SEM; [9]SM, superzyme incubated SEM; [10]MM, mixed enzyme incubated SEM; [11]IP6, Inositol hexaphosphate; [12]IP5, Inositol pentakisphosphate; [13]IP4, Inositol tetraphosphate.

al., 1997). The MM or SM treatments which consist of multiple enzymes that target carbohydrate portions, showed no additional effect on the removal of the glucosinolates. These were selected to target the carbohydrate portions of the SEM, such as mucilage. The enzymes cause a possible release of bound nutrients from the plant cell walls, from the hydrolysis of carbohydrate portions, like mucilage (Miller, 2004). Mucilage was decreased by enzyme treatments as shown (Table 4.1). Phytate was removed using a phytase enzyme incubation (PM and MM). Phytase catalizes the hydrolysis of phytic acid to bio-available phosphorus (Kaushik, 2001). Pre-treating a soy protein concentrate with phytase reduced the phytic acid from 9.3 g/kg to 0.5g/kg (Storebakken et al., 1998). Table 4.6 shows that a similar decrease of phytic acid occurred in SEM.

4.3 EFFECT OF PROCESSING ON DIGESTIBILITY

4.3.1 Digestibility of various camelina products

The apparent digestibility coefficients (ADC) differed among fish species and differed significantly among the camelina ingredients produced. The FFS had the highest digestibility of dry matter and crude fat by Atlantic salmon (70.5% and 87.6%; respectively; Table 4.7). The FFS dry matter and crude fat digestibility for Atlantic cod was 60.5% and 64.6%, respectively, and rainbow trout was 58.3% and 58.5%, respectively. The high dry matter digestibility of FFS in Atlantic salmon reflects the higher crude fat digestibility in this species. The crude protein digestibility of the FFS was high for all species. The Atlantic cod (91.0%) and Atlantic salmon (89.1%) had similar crude protein digestibility, while the rainbow trout crude protein digestibility of the FFS (80.9%) was lower. The gross energy digestibility of FFS was low, but still acceptable, for all three species. The gross energy digestibility by the Atlantic cod (73.0%) and

Atlantic salmon (75.6%) were similar, while the rainbow trout gross energy digestibility of the FFS (64.6%) remained lower.

Table 4.7. Digestibility of camelina feed ingredients by Atlantic cod (*Gadus morhua*), Atlantic salmon (*Salmo salar*), and rainbow trout (*Oncorhynchus mykiss*).

		Test Ingredient ADC (%)			
Nutrient	Species	FFS[1]	Oil	HORM[2]	SEM[3]
Dry Matter (%)	Cod	60.5 ± 0.69^{z}	63.0 ± 5.73	55.1 ± 1.14^{y}	59.3 ± 0.16^{z}
	Salmon	70.5 ± 0.87^{bz}	88.1 ± 2.71^{a}	66.8 ± 0.48^{bcy}	63.5 ± 0.95^{cx}
	Trout	58.3 ± 1.56^{cy}	80.9 ± 1.79^{a}	66.6 ± 0.84^{bz}	59.7 ± 0.35^{cy}
Crude Protein (%)	Cod	91.0 ± 0.28^{z}	n/a	90.8 ± 0.41^{z}	89.7 ± 0.19^{y}
	Salmon	89.1 ± 0.47	n/a	88.0 ± 0.77	87.6 ± 0.37
	Trout	80.9 ± 2.35^{y}	n/a	88.0 ± 1.66^{z}	86.7 ± 0.75^{z}
Gross Energy (%)	Cod	73.0 ± 0.37^{y}	71.7 ± 2.10	73.3 ± 0.45^{zy}	74.5 ± 0.32^{z}
	Salmon	75.6 ± 1.27^{bz}	92.7 ± 1.78^{a}	75.3 ± 0.42^{bcz}	71.8 ± 0.49^{cy}
	Trout	64.6 ± 2.22^{cy}	87.7 ± 1.47^{a}	70.9 ± 0.75^{bz}	67.9 ± 1.24^{bczy}
Crude Fat (%)	Cod	64.6 ± 2.48^{cx}	53.0 ± 3.53^{d}	81.7 ± 0.67^{by}	98.7 ± 0.24^{az}
	Salmon	87.6 ± 3.10	97.6 ± 1.70	92.1 ± 3.95	95.7 ± 4.10
	Trout	58.5 ± 0.44^{dx}	90.8 ± 0.19^{a}	76.3 ± 1.05^{bz}	66.7 ± 0.72^{cy}

Mean ± sem; n = 3
[a-d], means with different letters are significantly different among ingredients within each species including oil ($p \leq 0.05$).
[z-x], means with different letters are significantly different among ingredients within each species omitting the oil from the ANOVA ($p \leq 0.05$).
ADC, apparent digestibility coefficient
[1]FFS, full fat seed (ground)
[2]HORM, extruded meal (high oil residue)
[3]SEM, extruded solvent extracted meal (low oil residue)

Dry matter digestibility of camelina high oil residue meal (HORM) was highest in the Atlantic salmon (66.8%) and rainbow trout (66.6%; Table 4.7). The dry matter digestibility of HORM was about 12% lower for Atlantic cod (55.1%). It is important to note that the Atlantic salmon and rainbow trout were grown in fresh water, while the Atlantic cod were in salt water. Saltwater fish drink sea water during the functioning of osmoregulation, which causes an increase in the

salt content of the fecal matter thus skewing the values by adding extra ash to the sample (Cleveland and Hickman, 1968; Hajen et al., 1993 a,b; Grisdale-Helland and Helland, 1998; Tibbetts et al., 2004). In order to correct for saltwater the organic matter digestibility (dry matter – ash) must be reported instead of the dry matter digestibility, due to the high amount of ash produced by the excreted salt in marine fish faeces (Hajen et al., 1993b; Grisdale-Helland and Helland, 1998; Tibbetts et al., 2004). This was not performed on the Atlantic cod digestibility data, therefore the dry matter digestibility values may be lower due to the increased salt content. The protein, energy and fat digestibilities should not be affected (Hajen et al., 1993a).

Due to the removal of the oil from the FFS during processing to produce the HORM, the carbohydrates would have increased. Digestibility of cooked potato starch in Atlantic cod decreased from 40 to 26%, as the starch concentration increased in diet from 0 to 30% (Hemre et al., 1989). The crude protein and gross energy digestibility of the HORM was similar for Atlantic cod (90.8% and 73.3%; respectively), Atlantic salmon (88.0% and 75.3%; respectively), and rainbow trout (88.0% and 70.9%; respectively). Atlantic salmon and rainbow trout have a similar and high ability to digest soybean meal protein (between 94.4 and 99.0%, respectively; Glencross et al., 2004). The crude protein digestibility for the HORM in the current study was similar to these values. The crude fat digestibility of HORM was highest in the Atlantic salmon (92.1%), followed by Atlantic cod (81.7%), and lowest in the rainbow trout (76.3%). It is not clear why the species difference occurred.

Dry matter and crude protein digestibility of solvent extracted camelina meal (SEM) was similar for all species: Atlantic cod (59.3% and 89.7%; respectively), Atlantic salmon (63.5%

and 87.6%; respectively), and rainbow trout (59.7% and 86.7%; respectively; Table 4.7). The cod and salmon had similar digestibility of gross energy (74.5% and 71.8%; respectively) and digestibility of crude fat (98.7% and 95.7%; respectively) in the SEM. Trout by comparsion, exhibited lower digestibility of gross energy (67.9%) and crude fat (66.7%). SEM could be included in Atlantic salmon, Atlantic cod and rainbow trout diets. Rainbow trout had a similar digestibility of gross energy (76.4%) compared to solvent extracted rapeseed meal (Burel et al., 2000). Digestibility of crude protein (90.9%) and dry matter (70.8%) were slightly higher in Burel et al. (2000) than reported for the current study.

Based on the ingredient digestibility data, the best product for the Atlantic cod would be SEM, because it has the highest digestibility of dry matter, gross energy, and crude fat for this species. The product of choice for Atlantic salmon based on digestibility would be the FFS, as it had the highest digestibility of dry matter, and gross energy digestibilities, without loss of digestibility of crude protein. The rainbow trout had the lowest digestibilities of the FFS and its meal by-products. Rainbow trout appear to need a lower DP to DE ratio than Atlantic salmon and Atlantic cod, and that salmon and cod can digest higher amount of energy compared to trout (Glencross et al., 2004; Grisdale et al., 2007).

Removal of camelina oil from the HORM to produce SEM resulted in a decrease in ingredient dry matter ADC in salmon and cod by around 3 and 6%, respectively (Table 5.7). This decrease could be due to the increase in the proportion of anti-nutrients as oil is removed. Increased concentration of mucilage (Table 4.1), glucosinolates (1μmol/g), phytate (0.1% increase, Table 4.6), and phospholipids (about 5% increase; Table 4.5) were present in the SEM compared to

HORM. Atlantic cod exhibited a decrease in oilseed protein digestibility as lipid content increased (Tibbetts et al., 2011). Soybean meal (46-47% crude protein) had a 92.3% ADC compared to flaxseed meal (31% crude protein) with an ADC of 52.6%, while canola meal (38-39% CP) decreased by an intermediate amount with an ADC of 79.5% (Tibbetts et al., 2011). Flaxseed is high in mucilage (8% w/w; 9% extracted for 4 hours at 100°C) compared to canola and soybean, therefore digestibility is expected to be lower. Mucilage encapsulates the nutrients in feed, inhibiting absorption in the digestive tract (Mazza and Biliaderis, 1989; Marambe et al., 2013). Processing that involves water soaking to activate naturally occurring enzymes in this plant material can decrease the mucilage content, thus increasing the digestibility of flaxseed (Duncan, 1991; Francis et al., 2001). Water soaking treatment of SEM was performed to increase the digestibility of camelina products similar to the mucilage removal of ground flaxseed meal that increased *in vitro* protein digestibility by 51% (Marambe et al., 2013).

Digestible nutrient content of the ingredients were similar among species, however there were significant differences among created products (Table 4.8). These digestible nutrient values can be used to formulate rations for growth trials. As the amount of nutrient in the ingredient increased, the digestible nutrient value for the product increased. For example, the crude protein in HORM (38.0%) increased after solvent extraction (SEM, 40.6%) by 2.6% (Table 4.3). This caused the digestible crude protein in SEM to be between 34 to 36% among species, compared to HORM at 32 to 34% (Table 4.8). Based on the digestible protein content, the SEM provided the highest amount of digestible crude protein to the Atlantic cod (34.1%), Atlantic salmon (35.6%), and rainbow trout (35.2%). Based on digestible energy, the camelina oil was the best source of DE for Atlantic cod (6596 kcal/kg), Atlantic salmon (8524 kcal/kg), and rainbow trout (8063

kcal/kg). The lower digestibility of camelina oil by the cod causes the lower digestible energy values of products compared to the salmonid species tested. Digestible energy in the camelina meals ranged from 3365 to 3570 kcal/kg in the HORM, while SEM ranged from 3167 to 3284 kcal/kg. These digestible energy values for camelina meals are similar to soybean meal (3655 kcal/kg), and higher than canola meal (2627 kcal/kg; Tibbetts et al., 2006).

Table 4.8. Digestible nutrient level of the *Camelina sativa* test ingredients tested on Atlantic cod (*Gadus morhua*), Atlantic salmon (*Salmo salar*), and rainbow trout (*Oncorhynchus mykiss*).

		Test Ingredients			
Nutrient	Species	FFS[1]	Oil	HORM[2]	SEM[3]
Digestible Dry Matter (%)	Cod	57.3 ± 0.65^{z}	62.5 ± 5.69	51.2 ± 1.06^{x}	54.8 ± 0.15^{y}
	Salmon	66.8 ± 0.82^{bz}	87.5 ± 2.69^{a}	62.1 ± 0.44^{bcy}	58.6 ± 0.88^{cx}
	Trout	55.2 ± 1.47^{cy}	80.3 ± 1.78^{a}	61.9 ± 0.78^{bz}	55.1 ± 0.32^{cy}
Digestible Crude Protein (%)	Cod	23.0 ± 0.07^{x}	n/a	32.6 ± 0.15^{y}	34.1 ± 0.07^{z}
	Salmon	23.9 ± 0.13^{x}	n/a	33.4 ± 0.29^{y}	35.6 ± 0.15^{z}
	Trout	21.7 ± 0.63^{x}	n/a	33.4 ± 0.63^{y}	35.2 ± 0.30^{z}
Digestible Energy (kcal/kg)	Cod	4515 ± 23^{bz}	6596 ± 193^{a}	3476 ± 21^{cy}	3284 ± 14^{cy}
	Salmon	4680 ± 78^{bz}	8524 ± 163^{a}	3570 ± 20^{cy}	3167 ± 22^{dx}
	Trout	3995 ± 138^{bz}	8063 ± 135^{a}	3365 ± 36^{cy}	2992 ± 55^{dx}
Digestible Crude Fat (%)	Cod	24.9 ± 0.96^{bz}	52.5 ± 3.49^{a}	8.8 ± 0.07^{cy}	4.0 ± 0.01^{cx}
	Salmon	33.8 ± 1.20^{bz}	96.7 ± 1.69^{a}	9.9 ± 0.42^{cy}	3.9 ± 0.17^{dx}
	Trout	21.5 ± 0.16^{bz}	90.8 ± 0.19^{a}	7.6 ± 0.10^{cy}	2.1 ± 0.02^{dx}

Mean ± sem; n = 3

[a-d], means with different letters are significantly different among ingredients within each species including oil ($p \leq 0.05$).

[z-x], means with different letters are significantly different among ingredients within each species omitting the oil from the ANOVA ($p \leq 0.05$).

ADC, apparent digestibility coefficient

[1]FFS, full fat seed (ground)

[2]HORM, extruded meal (high oil residue)

[3]SEM, extruded solvent extracted meal (low oil residue)

The camelina oil product had the highest ADC for crude fat and gross energy for the Atlantic salmon (97.6% and 92.7%, respectively), followed by rainbow trout (90.8% and 87.7%, respectively). Atlantic cod, however, had the highest ADC for crude fat when fed the SEM product (98.7%) and the lowest ADC was in the oil product (50.0%). Digestible fat and energy were lower for the Atlantic cod (52.5%; 6596 kcal/kg) compared to the Atlantic salmon (96.7%; 8524 kcal/kg) and rainbow trout (90.9%; 8063 kcal/kg; Table 4.8). Water salinity could be causing a change in the digestive pH and enzyme activity levels creating the lower digestibility in the cod compared to the salmon and trout. The digestive ability of Atlantic salmon and rainbow trout, differs between fresh water and salt water environments, especially the starch digestion which decreased by about 5% for fish in salt water (Krogdahl et al., 2004). Chyme pH is different between Atlantic salmon in fresh water (stomach pH 5.1) versus seawater (stomach pH 4.5-4.7), which negatively affect the protein to energy ratios required as digestive enzyme activities change with the pH (Krogdahl et al., 2015). The magnitude of the change in digestibility in salmon was relatively minor between 1-3% lower in saltwater (Krogdahl et al., 2004). Atlantic cod at a low salinity (14‰) showed no change in protein digestibility compared to cod raised a seawater salinity of 28‰, as cod do not survive in freshwater the protein digestibility was not tested to show a difference to freshwater salmonids (Dutil et al., 1997).

All tanks of fish gained weight among all species evaluated. Weight gain (Appendix A2) was highest in the higher oil products, and rainbow trout had better FCR values for the camelina products compared to the salmon. This inferior FCR in the salmon was due to a lowered weight gain compared to the trout. These FCR values were not typical of balanced diets. Digestibility diets are not balanced for nutrient requirements of the fish, as they are designed to highly expose

a single ingredient to the fish. These diets cannot be used for an extended period of time for this reason. If the amount of nutrient required is not met then the growth and FCR values would be affected due to limiting nutrients. Growth and FCR were not parameters that these diets are designed to test, however the data collected on these parameters from a digestibility experiment can help determine if the ingredient was palatable. Palatability was higher in the high lipid products as the feed consumption and weight gain was greater for the higher lipid camelina by-products. Perhaps the higher concentration of glucosinolates (38.1 μmol/g), phytate (0.7%) and fibre in the SEM compared to the full-fat seed or higher lipid by-products (26.0 μmol/g; 0.5%, respectively) lowered the palatability. This problem may be overcome by enzyme treatment of the ingredient (Caballero et al., 2002).

4.3.2 Effect of processing treatments on digestibility of SEM by rainbow trout

To reduce anti-nutrients including glucosinolates and fibre from the SEM, several treatments were performed: dry heat, water soaking, and enzyme incubation with Bio-phytase, Superzyme™-OM, or a combination of the two.

4.3.2.1 Effect of heating (toasting) on SEM

The dry heat (toasting) at 100°C for 30 minutes did not affect the digestibility of the diet (Appendix A3), the ingredient digestibility of SEM, nor the digestible nutrient content (Table 4.9) of SEM. The toasting procedure of extruded solvent extracted canola meal for broilers degraded the lysine content, and decreased the amino acid apparent ileal digestibility coefficients and metabolizable energy (Newkirk et al., 2003b). The differences between the Newkirk et al.

(2003b) study and this study is they used a steam heat (100-110°C), for 60 minutes compared to 30 minutes at 100°C in the current study. The additional 30 minutes of heat may have increased a Maillard reaction reducing digestibility. The current study use of a dry heat resulted in no difference in the nutritional composition or digestibility of SEM with or without toasting. In the current study the meal turned to a darker brown colour, and started to clump together suggesting overheating that may have caused Maillard reaction. However the clumps could be easily broken apart. This is similar to the observations by researchers where toasted extruded solvent extracted canola meal which turned a brownish colour from the heating, evidence of some degree of Maillard reaction (Newkirk et al., 2003a,b).

Table 4.9. Effect of heat treatment on the expelled solvent extracted meal (SEM) on ingredient apparent digestibility and digestible nutrient content by rainbow trout.

		Test Ingredients	
Measure	Nutrient	SEM[1]	TSE[2]
Apparent Digestibility Coefficient	Dry Matter (%)	59.7 ± 0.35	57.7 ± 1.65
	Crude Protein (%)	87.2 ± 0.75	87.2 ± 1.53
	Gross Energy (%)	68.9 ± 1.24	65.8 ± 2.29
	Crude Fat (%)	66.7 ± 0.72	75.8 ± 3.68
Digestible Nutrient	Dry Matter (%)	55.1 ± 0.32	53.2 ± 1.52
	Protein (%)	35.2 ± 0.30	35.0 ± 0.61
	Energy (kcal/kg)	2992 ± 55	2809 ± 98
	Fat (%)	2.1 ± 0.02	2.7 ± 0.13

Mean ± sem; n = 3
No differences between ingredients ($p > 0.05$).
[1]SEM, extruded solvent extracted meal (low oil residue)
[2]TSE, toasted extruded solvent extracted meal

4.3.2.2 Effect of incubation on SEM

Commercial processing of oilseed meal uses moist heat, therefore a water soaking treatment was tested along with some enzyme and water incubation treatments. Water and mixed enzyme

treatments increased the digestible dry matter compared to untreated SEM (Table 4.10). No improvement was evident in the digestibility of diets (Appendix A4) and the ingredients (Table 4.10). However, there was a slight increase in growth using the PM, SM and MM treatments, and improvement in FCR for the same treatments (Appendix A5). The digestibility of nutrient in each of the incubated ingredients was similar. These results contrast with the increase in digestibility of protein and lipid of sal seed (*Shorea robusta*) meal fed to rohu (*Labeo rohita*) fingerling diets that had been soaked for 16 hours at room temperature (Mukhopadhyay and Ray, 1997). Soaking the seed increased digestibility of dry matter by about 30% at an inclusion of 20% of the diet. However, when included at 30% of the diet the dry matter digestibility was reduced approximately 6% (Mukhopadhyay and Ray, 1997). This shows that an anti-nutrient or a

Table 4.10. Effect of enzyme incubation on the expelled solvent extracted meal (SEM) on ingredient digestibility and digestible nutrient content.

		Test Ingredients				
Nutrient	Measure	SEM[1]	WM[2]	PM[3]	SM[4]	MM[5]
Dry Matter	ADC (%)	60.0±1.86	67.7±1.32	63.9±0.95	63.6±2.13	64.9±2.34
	DN (%)	55.6±1.72[b]	64.4±1.26[a]	60.3±0.90[ab]	60.4±2.02[ab]	61.5±2.22[a]
Protein	ADC (%)	84.6±1.73	86.2±0.58	84.2±1.21	85.1±1.05	83.6±1.98
	DN (%)	34.6±0.71	35.6±0.24	35.4±0.51	35.4±0.44	34.6±0.82
Energy	ADC (%)	69.0±1.54	75.1±0.79	71.5±1.51	72.0±2.31	70.7±1.88
	DN (kcal/kg)	3041±68	3333±35	3185±67	3207±103	3152±84
Fat	ADC (%)	78.8±1.44	87.2±0.36	81.0±1.44	79.9±1.36	87.6±2.08
	DN (%)	3.5±0.06[b]	4.6±0.02[a]	3.0±0.05[c]	3.7±0.06[b]	4.7±0.11[a]

Mean ± sem; n= 3
[a-c], shows significant differences within each nutrient between each ingredients ($p \leq 0.05$).
ADC, apparent digestibility coefficient
DN, digestible nutrient
[1]SEM, extruded solvent extracted meal
[2]WM, water incubated SEM
[3]PM, Bio-phytase incubated SEM
[4]SM, Superzyme™-OM incubated SEM
[5]MM, mixed enzyme incubated SEM

limiting nutrient is starting to create a negative effect on digestibility. They recommend an inclusion level of 20% soaked sal seed for use in rohu. Soaking reduced the tannin content from 3.4% to 0.7% (Mukhopadhyay and Ray, 1997). Rohu are a species of carp that are omnivores with a long gastro intestinal tract, while the species I studied were carnivores with short gastro intestinal tracts. This may explain the difference in digestibility by soaking of this ingredient by rohu as compared to our results presented here.

The similarity in the digestibility of each test ingredient, was a contrast to the differences in the digestible nutrient of the same ingredient. This would be due to a change in the test ingredient nutrient profile. For example, if the protein digestibility was similar for two products (50%), but one ingredient contained 80% crude protein (40% digestible crude protein) while the other contained 60% (30% digestible crude protein), then the higher protein ingredient will provide more 10% digestible protein. Water hydration for 24 hours would be the best treatment to improve digestible dry matter and fat content, as it produced the same increase in these nutrients compared to the more expensive enzyme treatment. However, to decrease the phytate content of the meal, adding a phytase enzyme would be required. The present results showed no improvement in the digestibility of SEM with phytase pre-treatment, which confirms the previous findings using phytase as a feed additive in Atlantic salmon (Sajjadi and Carter, 2004). They reported no change in the digestibilities of the diet when adding phytase, but they did see a positive response in growth and feed efficiency. Therefore, in future, growth and feed efficiency should be tested using the same enzyme treatments as used in this research, including Bio-phytase, Superzyme-OM, and the mixed enzyme treatments. Also fibre digestion should be

evaluated in the future as this may be the area of greatest change in the digestive capacity of the fish when water and enzymes incubations are used in feeds.

4.4 GENERAL DISCUSSION

The crude protein in all the camelina seed and meal by-products produced was highly digestible, ranging between 80 to 90 % for all species. The high protein digestibility of the HORM is similar to soybean meal (92.3%, Atlantic cod, Tibbetts et al., 2006; 83.5%, Atlantic salmon, and 90.2%, rainbow trout, Refstie et al., 2000), canola meal (88.1%, rainbow trout, Mwachireya et al., 1999; 76.0%, Atlantic cod, Tibbetts et al., 2006), and rapeseed meal (81.9%, Atlantic salmon, Aslaksen et al., 2007). Comparing the SEM to a solvent extracted rapeseed meal tested on rainbow trout showed the digestibility of the camelina solvent extracted meal was similar in protein digestibility (90.9%; Burel et al., 2000). The high level of methionine (0.6%; Table 4.3) means that this protein source could possibility be an alternative protein source when compared with other plant based protein sources such as canola (0.74%; Newkirk et al., 2003) and soybean meal (0.60%; NRC, 2011). If the trend of the high protein digestibility exists in the protein concentrates made, then these would be promising substitutes for other plant based protein concentrates, like soy protein concentrate (84%, Atlantic salmon; 87%, rainbow trout; Chowdhury et al., 2012).

The crude fat in the camelina oil was highly digestible by the Atlantic salmon (97.6 %) and rainbow trout (90.8 %), however was low for the Atlantic cod (53.0 %). The lower digestibility of lipid by the cod could be explained by the camelina oil being oxidized, oil quality was not tested for peroxidation. However, camelina oil has been reported as being highly stable to

oxidation due to the high level of total phenolics (400mg/kg fresh oil; Abramovič and Abram, 2005). The fresh oil in the study was stored for 3 weeks in the dark at 8°C before being tested. They tested the peroxidative values (PV) of camelina oil when stored in direct day light at ambient temperature (20 to 30°C), in the dark at ambient temperature, and in the dark at 8°C. The PV of camelina oil was found to be 10.6 meq O_2 /kg when stored in the dark at 8°C for 11 months, while storage at ambient temperature (20 to 30°C) increased this value reached above 20meq O_2 /kg in 1 month in direct light and 6.5 months in darkness (Abramovič and Abram, 2005). The upper Codex standard limit PV for unrefined oil is reported to be 15 meq O_2 /kg (FAO and World Health Organization (WHO), 2001). Since the camelina oil stored in the dark at 8°C for 11 months did not reach this Codex standard, the camelina oil used in this study stored at -20°C in the dark should not reach this level of peroxidation either. The camelina oil used in this study also had ethoxyquin added to prevent oxidation, and the cod trial was performed before the salmon and trout trials so if lipid oxidation was an issue it should be seen in these trials as well. However, it should be tested in the future.

When comparing the effect of water salinity between Atlantic salmon and rainbow trout, researchers found that there was no effect on the lipid or energy digestibility (Krogdahl et al., 2004). However, they did find an effect on the dry matter (2.5% higher in freshwater), starch (3.4% higher in freshwater), and protein (0.6% higher in freshwater) digestibility. This shows that salinity has more of an effect on the digestibility of starches than that of the lipids. They attributed the starch digestibility decrease in the fish in seawater to a change in the enzymes produced for digestion. The change in digestive enzymes may be due to physiological changes that allow the salt intake via drinking (osmoregulation) which the fish performs once transferred

to seawater (Krogdahl et al., 2004). However, this area of research into digestive enzymes at the freshwater to seawater transfer and their affect on digestibility is limited. This research shows that the water salinity could cause a change the production of digestive enzymes, but the low lipid digestibility in the Atlantic cod would be more of a species influence.

In Atlantic cod, the composition of the fatty acids in the flesh is higher in phospholipids compared to salmonid species which have flesh higher in triacylglycerols (Hixson et al., 2014). The higher phospholipids in the cod flesh could show a greater affinity to digest and absorb these types of lipids over the triacylglycerols. Camelina is high in triacylglycerols (49.9% of total lipid; Table 4.5) which may account for the lower digestibility of the camelina oil by the cod. The camelina oil has a high level of linoleic acid (Table 4.4; 18:2ω-6, 23.7% of FAME). Atlantic cod (juvenile) have a lower ability to lipase linoleic acid, shown by a increase in the linoleic content of triacylglycerol content of feed (2%) increasing to 10% in the fecal matter at the rectum (Lie et al., 1987). The residual triacylglycerol in the fecal matter within the rectum of the cod also contained 80% SFA and monoenoic fatty acids (Lie et al., 1987).

Atlantic cod (juveniles) showed the best apparent lipid digestibility for PUFA, MUFA and SFA when fed a diet with lipid content between 13.8 to 18.0 % of the diet (Hansen et al., 2008). This study fed the Atlantic cod a digestibility diet with a lipid content of 30.1% lipid, in order to stress the camelina oil in the diet of the fish. Once the lipid content started to rise from 18.0% of the diet to 26.8%, the digestibility of the MUFA and SFA lowered, this was not seen in the PUFA digestibility as the fish digested most of the PUFA provide even when fed the diet with 26.8%

lipid (Hansen et al., 2008), showing that Atlantic cod juveniles have an affinity to digest PUFA over the other fatty acids provided. Since camelina oil is high in PUFA (54.6 % of FAME; Table 4.5), it could be possible that the cod in my study fed the high level of lipid used the high amount of PUFA provided in the diet by the camelina oil as they did in the research using fish oil by Hansen et al. (2008). If the energy provided by the PUFA and the protein in the basal diet was sufficient for the growth and survival of the cod in my study, they would excrete the excess nutrients (MUFA and SFA) as they did in the study by Hansen et al. (2008). Fatty acid analysis of the fecal matter would be benefical to see if this is a case, and a re-run of the Atlantic cod digestibility trial (focused of the camelina oil) using graded levels of dietary lipid as in the Hansen et al. (2008) trial should be performed, accept instead of using fish oil, camelina oil should be used. The redesigned digestibility trial with the camelina oil would be able to determine if dietary lipid content was cause of the decrease in camelina lipid digestibility and show the effect of various classes of camelina lipid. The Atlantic cod juveniles fed graded levels of fish oil showed that total lipid digestibility decreased as the saturation of longer chained fatty acids increased in the total diet (Hansen et al., 2008). Camelina oil is high in the longer chained fatty acids (Table 4.4; 18:1ω-9, 14.4% of FAME; 18:2ω-6, 23.7%; 18:3ω-3, 28.3%; 20.1ω-9, 12.1%), therefore the total lipid digestibility of camelina oil could be lower due to this level of long chain fatty acid, as was found in the fish oil study (Hansen et al., 2008).

The high crude fat digestibility occurred in all of the by-products tested on the salmon, with digestibility ranging from 87 to 98 %. For the all three species, the camelina oil would be a suitable source of digestible fat and energy. These three species would then be able to use the SEM or the MM (SEM with the mixed enzyme water incubation) meal as a high protein and

energy source. However, PC70 or PC80, could be predicted based on crude protein content as the best potential protein and energy source, if processing can be up scaled. Overall, there is potential for camelina seed and its by-products in commercial fish diets. Based on the high digestible gross energy content of the high protein meals (SEM, 2992 to 3284 kcal/kg) and the camelina oil (6596 to 8524 kcal/kg; Table 4.8), these ingredients have potential use in feed formulation and partial replacement of fishery by-products in salmonid feeds.

Specific enzyme incubations reduced the anti-nutrients in the SEM, however this did not improve the palatability as there was no significant difference in the feed consumption data (Appendix A5). More research is needed on processing of camelina to improve digestibility of the total dry matter. Possible areas of interest would be removing carbohydrate, such as starch, and fibres. If the protein concentrate proves to be a viable product, it would be an important plant based protein source for the feed industry. Therefore, processes need to be explored to up-scale protein production, such as flash freezing during the acid precipitation process, to produce the PC80 with greater yield. Currently, phytate and glucosinolates can be removed with a water and phytase enzyme treatment. The future research should focus more on the removal of carbohydrate components, like starch and mucilage, which could be causing issues related to improving protein digestibility of camelina. A mixture of various enzymes has been shown to reduce the sticky portions of the camelina, however research as to the digestion of carbohydrates in camelina should be further evaluated.

CHAPTER 5 CONCLUSIONS

5.1 Conclusions

Camelina by-products with a wide range of digestible nutrient profiles were created. SEM provided the highest digestible crude protein for all three species. The camelina oil provided the highest digestible crude fat and digestible energy for all three species. The Atlantic salmon and rainbow trout had higher digestible crude fat values and digestible energy content ranging between 90 to 97% and between 8000 to 8500 kcal/kg, compared to the Atlantic cod with 52 to 53% and 6500 to 6600 kcal/kg, respectively. Toasting did not cause any significant changes to the SEM composition, or digestibility by rainbow trout, but did cause an increase in the dark colour of the meal. Water soaking eliminated the glucosinolate content of the SEM and is a potential process to apply to meals. The use of Bio-phytase and Superzyme -OM improved the digestible dry matter and digestible crude fat content of the SEM from 55% to between 65 to 61% and from 3.5% to between 4.6 to 4.7%, respectively.

The camelina protein concentrate could be produced and it had high crude protein content between 70 to 82%. Development of a scaled up process should be investigated. Digestibility should be determined for protein concentrate, as it has potential in carnivorous fish diets. ADC derived in this study can be applied to other sources of camelina products to calculate digestible nutrient contents for different species. The crude protein digestibility was high in all by-products for all species. Digestible nutrient content generated can be used for future diet formulations.

5.2 Recommendations

The enzymes Bio-Phytase® and Superzyme-OM® should be used as a pre-treatment before the SEM is offered for sale. Enzyme pre-treatment of the PC70 should be evaluated to reduce the glucosinolates present in this product. Water soaking and enzyme addition are processes that should be incorporated into commercial applications based on the reduced glucosinolate and phytate in the resulting meals from this study.

Camelina protein products created in the future should be created to contain a high protein content and low anti-nutrient content, while the camelina lipid should retain its high levels of omega 3 fatty acids. Certain lines are currently being developed through plant breeding to produce a seed with an improved amino acid and fatty acid profile, as well as reduced anti-nutrient levels. More camelina plant lines and by-products should be tested to improve the state of knowledge.

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APPENDIX A: DIET DIGESTIBILITY, WEIGHT AND FCR DATA

Table A.1. Diet digestibility of the basal diets and test diets including the four main *Camelina sativa* test ingredients tested on Atlantic cod (*Gadus morhua*), Atlantic salmon (*Salmo salar*), and rainbow trout (*Oncorhynchus mykiss*).

	Dry Matter ADC (%)			Crude Protein ADC (%)			Gross Energy ADC (%)			Crude Fat ADC (%)		
Trial	Cod	Salmon	Trout	Cod	Salmon	Trout	Cod	Salmon	Trout	Cod	Salmon	Trout
Diet												
Basal	64.3 ± 0.85	79.1 ± 0.36^{b}	80.4 ± 0.61^{a}	89.1 ± 0.36^{b}	91.8 ± 0.18^{a}	92.5 ± 0.29^{a}	74.1 ± 1.02	88.1 ± 0.15^{b}	90.7 ± 0.19^{a}	59.6 ± 3.14^{bc}	84.1 ± 1.58	93.4 ± 0.89^{a}
FFS~	62.4 ± 0.35	74.7 ± 0.44^{c}	69.4 ± 0.78^{c}	89.8 ± 0.10^{b}	90.6 ± 0.22^{b}	88.6 ± 0.80^{d}	73.0 ± 0.37	81.6 ± 0.66^{c}	76.5 ± 1.21^{c}	63.6 ± 1.98^{b}	86.1 ± 2.74	67.8 ± 0.33^{b}
Oil	63.6 ± 2.99	83.7 ± 1.38^{a}	80.6 ± 0.91^{a}	91.6 ± 0.56^{a}	92.5 ± 0.57^{a}	91.7 ± 0.26^{ab}	71.7 ± 2.10	90.5 ± 0.95^{a}	88.8 ± 0.94^{a}	53.6 ± 3.21^{c}	95.5 ± 1.55	91.1 ± 0.17^{a}
HORM^	59.6 ± 0.58	73.0 ± 0.24^{cd}	73.6 ± 0.41^{b}	89.8 ± 0.17^{b}	90.1 ± 0.35^{b}	90.6 ± 0.70^{bc}	73.3 ± 0.45	81.7 ± 0.22^{c}	81.3 ± 0.36^{b}	71.2 ± 0.35^{a}	88.5 ± 3.12	86.1 ± 0.45^{a}
SEM*	61.8 ± 0.08	71.1 ± 0.49^{d}	70.2 ± 0.17^{c}	89.4 ± 0.08^{b}	89.8 ± 0.18^{b}	90.0 ± 0.33^{cd}	74.5 ± 0.32	80.1 ± 0.24^{c}	80.2 ± 0.57^{b}	71.2 ± 0.07^{a}	90.6 ± 3.07	88.2 ± 0.14^{a}

Mean ± sem; n = 3

$^{a-d}$, shows significant differences between diets within each trial ($p \leq 0.05$).

ADC, apparent digestibility coefficient

~FFS, full fat seed (ground)

^HORM, expelled meal (high oil residue)

*SEM, expelled solvent extracted meal (low oil residue)

Table A.2. Weight gain and feed conversion ratio (FCR) of *Camelina sativa* test diets tested on Atlantic cod (*Gadus morhua*; 18 days), Atlantic salmon (*Salmo salar*; 14 days), and rainbow trout (*Oncorhynchus mykiss*; 12 days).

	Feed Consumption (g/tank)			Weight Gain (g/tank)			Feed Conversion Ratio		
Trial	Cod	Salmon	Trout	Cod	Salmon	Trout	Cod	Salmon	Trout
Test Diet									
Basal	383±17.8^{b}	390±38.5^{b}	934±26.1^{b}	324±11.4^{a}	205±31.1^{ab}	1006±11.4^{a}	1.18± 0.04^{b}	1.94± 0.13^{b}	0.91± 0.02^{b}
FFS~	472±49.2^{a}	310±14.0^{c}	635±23.3^{c}	366±67.9^{a}	131±16.4^{c}	417±65.7^{b}	1.33± 0.10^{b}	2.46± 0.36^{a}	1.63± 0.33^{a}
Oil	430±38.4^{a}	488±16.1^{a}	1037±45.6^{a}	337±21.6^{a}	261±69.2^{a}	1058±28.2^{a}	1.27± 0.04^{b}	2.23± 0.70^{ab}	0.98± 0.07^{b}
HORM^	299±20.9^{d}	296±18.2^{c}	467±54.0^{d}	239±28.1^{b}	108±10.6^{c}	294±63.6^{b}	1.27± 0.09^{b}	2.77± 0.16^{a}	1.80± 0.54^{a}
SEM*	343±18.5^{c}	350±22.6^{b}	593±43.2^{c}	181±31.6^{b}	146±28.7^{bc}	435±76.8^{b}	2.02± 0.34^{a}	2.54± 0.39^{a}	1.43± 0.19^{a}

Mean ± sem; n = 3

$^{a-d}$, shows significant differences between ingredients within each trial ($p \leq 0.05$).

~FFS, full fat seed (ground)

^HORM, expelled meal (high oil residue)

*SEM, expelled solvent extracted meal (low oil residue)

Table A.3. Diet digestibility of the basal diet and test diets showing the effect of toasting *Camelina sativa* expelled solvent extracted meal using rainbow trout (*Oncorhynchus mykiss*).

Diet	Apparent Digestibility Coefficient (%)		
	Basal	Expelled Solvent Extracted Meal	
		Non-Toasted	Toasted*
Nutrient			
Dry Matter (%)	80.4 ± 0.67[a]	70.2 ± 0.67[b]	69.2 ± 0.67[b]
Crude Protein (%)	92.5 ± 0.55[a]	90.0 ± 0.55[b]	90.2 ± 0.55[b]
Gross Energy (%)	90.7 ± 0.81[a]	80.2 ± 0.81[b]	79.5 ± 0.81[b]
Crude Fat (%)	93.4 ± 2.83[a]	85.2 ± 2.83[a]	87.4 ± 2.83[a]

Mean ± sem; n = 3

*Toasted solvent extracted meal underwent toasting at 100°C for 30 minutes in a drying oven.

Table A.4. Diet digestibility of the basal diet and test diets including enzyme treated expelled solvent extracted *Camelina sativa* meal ingredients tested on rainbow trout (*Oncorhynchus mykiss*).

	Dry Matter	Crude Protein	Gross Energy	Crude Fat
	ADC (%)	ADC (%)	ADC (%)	ADC (%)
Test Diet				
Basal	79.5 ± 0.40[a]	91.6 ± 0.18[a]	89.7 ± 0.27[a]	94.8 ± 0.51[a]
SEM*	69.7 ± 0.93[c]	88.5 ± 0.76[b]	79.8 ± 0.74[c]	89.0 ± 0.52[b]
WS^	73.5 ± 0.67[b]	89.2 ± 0.26[b]	82.7 ± 0.38[b]	91.7 ± 0.15[ab]
PM~	71.6 ± 0.48[bc]	88.3 ± 0.54[b]	80.9 ± 0.73[bc]	90.4 ± 0.46[b]
SM”	71.5 ± 1.07[bc]	88.7 ± 0.47[b]	81.2 ± 1.12[bc]	89.2 ± 0.51[b]
MM’	72.1 ± 1.18[bc]	88.0 ± 0.88[b]	80.6 ± 0.91[bc]	91.9 ± 0.85[ab]

Mean ± sem; n = 3
[a-c], shows significant differences between diets ($p \leq 0.05$).
ADC, apparent digestibility coefficient
*SEM, expelled solvent extracted meal (low oil residue)
^WS, water incubated SEM
~PM, phytase incubated SEM
“SM, superzyme incubated SEM
‘MM, mixed enzyme incubated SEM

Table A.5. Weight gain and feed conversion ratio (FCR) of the toasted and enzyme treated SEM *Camelina sativa* meal test diets tested on rainbow trout (*Oncorhynchus mykiss*).

Trial	Test Diet	Feed Consumption (g/tank)	Weight Gain (g/tank)	Feed Conversion Ratio
Toasted SEM (12 days)	Basal	916±26.1[a]	1006±11.4[a]	0.91±0.02[b]
	SEM*	593±43.2[b]	435±76.8[b]	1.43±0.19[a]
	TSE`	666±23.5[b]	455±47.9[b]	1.50±0.19[a]
Enzyme Incubation (8 days)	Basal	883±38.4	1038±17.5[a]	0.85± 0.04[c]
	SEM*	897±34.1	707±66.2[d]	1.30± 0.16[a]
	WS^	886±40.5	677±186.6[d]	1.60± 0.53[a]
	PM~	958±49.5	921±25.4[b]	1.04± 0.02[b]
	SM”	897±91.3	859±20.3[c]	1.04± 0.08[b]
	MM’	905±20.2	939±51.2[b]	0.97± 0.04[b]

Mean ± sem; n = 3
[a-d], shows significant differences between ingredients within each trial ($p \leq 0.05$).
*SEM, expelled solvent extracted meal (low oil residue)
`TSE, toasted expelled solvent extracted meal
^WS, water incubated SEM
~PM, phytase incubated SEM
“SM, superzyme incubated SEM
‘MM, mixed enzyme incubated SEM

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