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Replacing fish oil in aquaculture diets using a mixture of canola and algae oil

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Summary

Four experiments were performed using Nile tilapia and rainbow trout to test the effect of extrusion on the digestibility of algae oil in algae biomass and to determine the effect of adding this extruded algae biomass to canola oil-based diets on the fatty acid composition of the two fish species. An algal biomass production containing ~20% EPA as a percentage of total lipid was used in these experiments. In the extrusion/digestibility experiments, algae biomass was coextruded in a 50:50 mixture with wheat flour. The extrusion experiment was set up as a 3x3 factorial design of specified temperatures (110, 130 and 150°C) and moisture contents (16.9, 21.9 and 26.9 %) while maintaining other extrusion parameters constant to generate 9 extrudates containing algae biomass. The digestibility of these extrudates was then measured in Nile tilapia and rainbow trout. In Nile tilapia, the digestibility of 16:0 and 18:2n-6 were significantly higher for the extrudates extruded at 130 °C. However, there were no significant differences between the extrusion parameters for EPA digestibility. Based on the improvement in digestibility for 16:0 and 18:2n-6, all subsequent experiments used a mix of the 3 extrudates produced at 130 °C. In rainbow trout, the digestibility of fatty acids was higher for the extrudates produced at the 130 and 150 °C temperatures and lowest for those produced at 110 °C. The digestibility of EPA was close to 100% at the 2 higher temperatures and this supports the use of the 130 °C temperature chosen for the subsequent growth trials.

The 130 °C extrudates were used to formulate diets that contained the following percentages of canola and algae oil; 15+0, 14+1, 13+2, 12+3 and 11+4. A diet containing 15% fish oil was included as a positive control. Nile tilapia were fed these diets for 70 days and then their whole body fatty acid composition was measured. The EPA

concentrations of the fish fed the 15+0 canola oil diet were significantly lower than for the fish fed fish oil. However, the fish fed the 11+4 canola/algae diets had EPA levels that were equal to those fed fish oil. DHA levels in the tilapia did not increase when algae oil was added to diets suggesting that the synthesis of DHA from EPA was not significant. In the rainbow trout growth trial, fish were fed for 78 days and then the whole body fatty acid composition of the fish was determined. The result was similar to the results seen with the tilapia experiment. Fish fed the 15+0 diet had EPA and DHA levels significantly lower than fish fed fish oil. However, fish fed the 12+3, 13+2 and 14+1 diets had EPA concentrations in tissue that were not significantly different that trout fed fish oil. As with the tilapia, DHA levels were not increased by feeding algae oil.

The overall conclusions are:

1) the digestibility of algal biomass can be increased to nearly 100% using extrusion
2) the addition of an EPA-rich algae oil to canola oil-based diets can significantly increase the concentration of EPA in rainbow trout and Nile tilapia
3) to increase the levels of DHA in fish fed diet containing algae oil and algae species that produces an oil rich in DHA will be required

Introduction

The health benefits of consuming highly unsaturated omega-3 fatty acids (HUFA) are well established (Review: Van Horn et al., 2008). The most important of these omega-3 fatty acids are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The primary source of these lipids in the human diet are cold water fish, such as salmon, trout, sardines, mackerel and tuna.

The fatty acid composition of farmed fish is a reflection of dietary fatty acid composition (Robin et al., 2003) and presently, HUFAs present in farmed salmonids are derived from dietary fish oil. While fish oil is an excellent source of omega-3 HUFAs, the use of this product in aquafeeds presents several problems. The total production of fish oil is stagnant at from 0.8-1.5 million tonnes per year (Tacon and Mettian, 2008). In 2008, aquaculture used approximately 835,000 tonnes of the world's fish oil and it is predicted that 88% of the total fish oil production will be used in aquafeeds in 2012 (Tacon and Mettian, 2008). The result of stagnant supply and increasing demand has been a 130% increase in fish oil prices from 2005-2008 (Naylor, 2009). Further, it is questionable whether the high usage of fish oil by aquaculture is a sustainable or even an appropriate use for this valuable source of HUFA given that it takes 2.0-2.3 kg of fish meal to produce enough oil to produce 1 kg of salmon (Ellingsen et al., 2009; Norwegian Seafood Federation 2009). Taken together, the above observations indicate that the replacement of fish oil in aquaculture diets in general and salmonid diets in particular are of central importance to the sustainability of the industry.

Alpha linolenic acid (ALA) is a shorter chain omega-3 fatty acid found in vegetable oils including canola and is a precursor for EPA and DHA. However, while fish can convert ALA to EPA and DHA, this conversion is inefficient and only 2-5% of ALA is converted to longer chain n-fatty acids (Goyens et al., 2005; Hussein et al., 2005). When fish including rainbow trout and Nile tilapia, are fed canola oil-based diets, the level of EPA and DHA in the fish fillets is lowered to unacceptable levels. Very little research has been done on this species. Justi et al., (2003) fed linseed oil to Nile tilapia and reported that EPA and DHA concentrations in fish flesh were 0.2 and 1.9% of total fatty acids. This compares to levels in fish oil fed fish of 1.5 and 6.8%. I am not aware of any other papers on the replacement of fish oil with vegetable oils in Nile tilapia indicating that this area needs attention.

One solution to this problem is to add EPA and DHA to canola oil, thus creating a viable replacement for fish oil. Algae oil contains approximately 30% EPA + DHA. The addition of algae oil to a vegetable oil high in ALA produces a product with properties similar to fish oil. Canola oil meets this requirement and is the vegetable oil of choice by the aquafeed industry due to its high omega-3 fatty acid content and excellent storage and handling properties. The addition of algae oil to canola oil will increase its value in the large and growing aquafeed industry. This research will also be applicable to the use of other omega-3-rich vegetable oils produced in Saskatchewan, including flax and camelina oils.

However, a major hurdle to this strategy is the low digestibility of algae oils in fish. Algae oil is produced by drying the entire algal mass. The oil in the product is entrapped in the carbohydrate and protein portions of the algae. This has the advantage of protecting

the oil from oxidation. However, this lowers the digestibility of the valuable lipids in the product. Previous work in my lab measured the digestibility of a dried algae product containing 55% oil. The digestibility of the oil was only 61%. This increases the cost of using algae oil significantly.

The obvious approach to increasing lipid digestibility in the product is through the use of extrusion. Extrusion provides heat, shear and expansion to manufactured aquafeeds and has the effect of increasing the digestibility of nutrients. Therefore, the purpose of this proposed research is to determine the optimal extrusion parameters required to maximize the digestibility of the algae oil/canola oil product and measure the effect of feeding this product on the fatty acid composition of rainbow trout and Nile tilapia. The lipid metabolism of rainbow trout in cold water and Nile tilapia in warm water are markedly different so that both of these animal models were used to evaluate the use of the algae/canola oil blend as a fish oil replacement.

Project Objectives

Overall Objective: To develop methods to improve the fatty acid composition of aquaculture fish fed algae-vegetable oil blends instead of fish oils.

Specific Objectives:

1) Determine the optimum extrusion parameters to maximize the digestibility of a algae and canola oil blend in commercial type diets fed to Nile tilapia and rainbow trout.

2) Using Nile tilapia in warm water and rainbow trout in fresh water, determine the effect of varying dietary inclusion rates of algae oil product on: i) the level of EPA and DHA in

fillets and ii) fatty acid retention of the fish determined using the whole body fatty acid balance method.

Milestone 1: The effect of extrusion parameters on the digestibility of algae and canola oils in tilapia diets

Materials and Methods

Extrusion of algae

Algal biomass was obtained from Aurora Algae Inc. (Hayward, CA). Its chemical composition is as follows: 16% moisture, 17% ash, 42 % crude protein, 12% fat of which 30% are long chain PUFA and 4.5% fibre. Prior to extrusion, the algae biomass was mixed for 15 minutes with wheat flour at a ratio of 1:1 and 0.15 g/kg of Ethoxyquin (Santoquin) to aid in the prevention of lipid oxidation. Following mixing, 9 x 20 kg batches of the algae biomass-wheat flour mixture were separately extruded (Saskatchewan Food Industry Development Centre; Saskatoon, SK) following the temperature and moisture parameters as outlined in Table 1.

The extrusion experiment was set up as a 3x3 factorial design of specified temperatures (110, 130 and 150°C) and moisture contents (16.9, 21.9 and 26.9 %) while maintaining other extrusion parameters constant to generate 9 extrudates containing algae biomass. The moisture contents of the feeds were originally to have been 15, 20 and 25%. However, exact control of the moisture content is difficult and these values were as close as we could get to the originally planned ones.

Measurement of digestibility

After each extrudate cooled, bulk density, expansion index and extrudate diameter and puff were measured. The nine extrudates were then separately ground using a hammer mill fitted with a 1mm screen and individually combined with the remaining dietary ingredients of each experimental diet. The diets were mixed in a Legacy Hobart Floor Mixer (Hobart Corporation, Troy, OH) for 15 minutes and then cold pelleted in a 3 mm 4822 Hobart Food Grinder (Hobart Corporation, Troy, OH). Following pelleting, the diets were dried in a forced air oven (55 °C, 12 hours), chopped and screened to obtain a uniform pellet size, approximately 10 mm in length.

The digestibility of the 9 extrudates was determined using the indirect method using Celite (Celite 545, Celite Corporation, World Minerals Co., Lompoc, CA) as previously described (Randall and Drew, 2010). The experimental diets were formulated by replacing 30% of a basal diet with the experimental ingredient.

The fish were adapted to the experimental diets for six days prior to collection. Three tanks of fish were randomly assigned to each diet for a total of 30 tanks (Reference diet and 9 extrudate diets). After the adaptation period feces were collected using a settling column located at the bottom of each tank. The feces were then centrifuged (3000 RPM for 10 min.) and frozen. Collections were carried out until each tank had filled a 40 dram vial, at which time the samples were freeze dried.

Fish management

Nile tilapia (*Oreochromis niloticus*) were purchased from Current Prairie Fisherman Inc. (Nobleford, AB) and maintained in 150 L tanks that were part of a semi-closed recirculation system filtered biologically at the Prairie Aquaculture Research

Centre (PARC; Saskatoon, SK). The fish were acclimated to their respective diets for 14 days and fed to satiety twice daily. Feed consumption for each experimental unit was recorded on a daily basis. Water temperature was maintained at 27 ± 1°C. Dissolved oxygen, pH and temperature were observed and recorded daily. Chlorine, nitrate, nitrite and ammonia were monitored on a weekly basis. Photoperiod was a 14 h light/10 h dark cycle. Animal protocols were approved by the University of Saskatchewan Committee on Animal Care and Supply, and followed principles established by the Canadian Council on Animal Care (2005).

Sampling and analysis

Faecal samples were collected from each tank via a settling column for a period of 83 days and combined for each experimental diet. The faecal samples were then freeze-dried prior to proximate analysis. A sample of each experimental diet and all faecal samples were ground using a ZM 100 Retsch Mill (Retsch GmbH, Haan, Germany) fitted with a 1 mm screen. Diets and faecal samples were sent to Central Testing Labs Inc. (Winnpeg, MB) and analysed in duplicate using the following methods: moisture (AOAC, 1990; method no. 934.01), energy (oxygen bomb calorimetry; Parr Adiabatic Calorimeter, Model 1200), ash (AOAC, 1990; method no. 924.05), gross energy was determined using a 1281 Bomb Calorimeter (Parr Adiabatic Calorimeter, Model 1200) and protein was determined using a Leco protein N^{-1} analyser (Model FP-528, Leco Corporation, St. Joseph, MI). The nitrogen content of samples was obtained using a combustion nitrogen analyser (Leco FP-528, AOAC 1995, method no. 990.03). Crude protein (CP) was estimated by multiplying nitrogen content by 6.25.

Prior to fish allocation and the start of the trial, 5 fish were randomly selected, euthanized by sharp blow to the head and weighted. The whole fish were then uniformly ground using a food processor (Moulinex DPA2, France), freeze-dried and analysed for fatty acid composition (Lipid Analytical Laboratories; Guelph, ON). At the end of the 56-day growth period, two fish per tank were euthanized, weighted and uniformly ground as described above and freeze-dried. All diets, faeces and fish samples were sent to Lipid Analytical Laboratories (Guelph, ON) for determination of fatty acid composition (Bligh and Dyer, 1959) using gas chromatography (Agilent Technologies, Mississauga, ON).

Results

Physical properties of wheat/algae extrudates

The physical properties of the 9 extrudates is shown in Table 2. Since only one extrudate was made at each temperature and moisture combination, no statistics are possible. Bulk densities of the extrudates varied markedly. The 110/16.9 and 110/21.9 extrudates had bulk densities of 68.8 and 80.4 g/l which were much lower than the density of the 110/26.9 extrudate with a bulk density of 239.4. A similar pattern was seen at 130 and 150 °C where the highest moisture extrudate had a bulk density markedly higher than for the 2 lower moisture extrudates. No large differences in expansion indices or extrudate diameter were observed between any of the extrudates.

Digestibility of Algal extrudates

Although only a single replicate of the extrudates was produced, each of these extrudates were fed to 3 tanks of fish and therefore, it was possible to perform statisitical analyses on the digestibility data. Given that digestibility is of much greater importance than the

physical properties of the extrudates, this study provides reliable information on the most relevant aspects of the project.

The fatty acid composition of the extrudates is shown in Table 5. The algal extrudates contained from 18.7-23.0% of EPA as % of total fatty acids. The extrudates were also high in 16:0, 16:1 and 18:2n-6. However the algae product was devoid of many fatty acids and, since this was the case, the digestibilty values for those fatty acids is not reported.

The digestibilities of individual fatty acids are shown in Table 7. There are many coefficients above 1.00 and below 0. This is due to the extremely low concentrations of these fatty acids in the diets and the confounding affect of endogenous losses from the intestinal tract. The digestibilities of the fatty acids in the highest concentrations in the diets are the values with the highest accuracy.

The digestibility of 16:0 ranged from 0.64-1.00. There were no significant differences in digestibility values. However, the extrudates manufactured at 130 °C all had digestibilities of 1.00 suggesting this is the optimal temperature for the manufacture of algal/wheat extrudates. The digestibilities of 18:n-6 in the 9 extrudates did not differ significantly, however, as with 16:0, the extrudates manufactured at 130 °C had the highes digestibilies ranging from 0.94-1.00. A similar trend was seen with 18:3n-3 where no significant differences were observed by the extrudates manufactured at 130 °C had digestibilities from 0.93-1.00. Finally the digestibility of EPA (20:5n-3) ranged from 0.83-0.98 with no significant differences observed between the extruder treatments.

Table 1. Temperature (°C) and moisture content (%) of wheat/algae extrudates

	Moisture Content (%)		
Extrusion Temperature (°C)	**16.9**	**21.9**	**26.9**
110	1	2	3
130	4	5	6
150	7	8	9

Table 2. Physical properties of algae-containing extrudates

Parameter	Extrudate								
	1	2	3	4	5	6	7	8	9
Bulk density (g/L)	68.8 ± 1.0	80.4 ± 0.5	239.4 ± 4.0	177.1 ± 2	179.9 ± 1	235.2 ± 0.8	177.1 ± 1	180.9 ± 0.6	223.6 ± 0.9
Expansion index, major axis	3.4 ± 0.1	3.6 ± 0.3	3.2 ± 0.1	3.2 ± 0.2	3.6 ± 0.2	3.4 ± 0.1	3.2 ± 0.3	3.4 ± 0.1	3.4 ± 0.1
Expansion index, minor axis	3.2 ± 0.2	3.4 ± 0.1	3.1 ± 0.2	3.0 ± 0.1	3.4 ± 0.1	3.2 ± 0.1	2.9 ± 0.3	3.0 ± 0.2	3.0 ± 0.2
Extrudate diameter, major axis (mm)	16.4 ± 0.2	17.5 ± 0.9	15.7 ± 0.3	15.5 ± 0.6	17.6 ± 0.6	16.4 ± 0.3	15.9 ± 0.7	16.4 ± 0.4	16.4 ± 0.4
Extrudate puff diameter, minor axis (mm)	15.4 ± 0.6	16.9 ± 0.4	15.1 ± 0.5	14.6 ± 0.4	16.8 ± 0.4	15.7 ± 0.6	14.0 ± 0.7	14.6 ± 0.7	14.6 ± 0.7

Table 4. Fatty acid compositions of the extrudates (% of total fat)

	Extrudate								
Fatty acid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
14:00	3.8	3.7	3.6	3.7	3.8	3.7	3.8	3.8	3.7
16:00	22.1	21.0	20.8	22.6	21.0	20.7	23.2	21.5	20.7
18:00	1.2	1.1	1.1	1.2	1.2	1.2	1.4	1.2	1.2
20:00	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1
22:00	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0
14:1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
16:1	24.2	24.0	24.0	24.3	23.7	23.7	24.7	24.0	23.6
18:1	5.6	5.3	5.2	5.8	5.5	5.2	6.0	5.6	5.3
20:1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3
22:1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
18:2n6	15.7	15.6	15.3	15.9	15.8	15.9	15.9	15.8	15.6
18:3n6	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
20:2n6	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0
20:3n6	0.5	0.5	0.6	0.6	0.5	0.6	0.5	0.5	0.6
20:4n6	4.2	4.4	4.5	4.1	4.3	4.4	3.9	4.2	4.5
22:2n6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:4n6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:5n6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:3n3	1.0	1.0	1.0	1.0	1.1	1.1	1.0	1.1	1.0
18:4n3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:3n3	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
20:4n3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
20:5n3	20.6	22.2	23.0	19.9	21.9	22.5	18.7	21.3	22.7
22:5n3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:6n3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 5 The total tract digestibilities of fatty acids in the 9 extrudate products in Nile tilapia. Only fatty acids that were present in the algae product are included in this table.

Fatty acid	1	2	3	4	5	6	7	8	9	SEM	*P*-Value
14:0	0.83	0.90	0.95	0.94	0.97	0.88	0.64	0.84	0.93	0.03	0.091
16:0	0.64	0.93	1.00	1.00	1.00	0.83	0.84	0.66	1.00	0.09	0.149
18:0	-0.54	1.15	1.91	1.71	2.13	0.55	-3.77	-0.44	1.66	0.47	0.126
20:0	0.92	2.98	4.98	2.73	4.97	3.11	-4.02	0.77	4.70	0.68	0.082
22:0	-0.07[a]	2.15[a]	9.55[a]	1.96[a]	2.09[a]	0.86[a]	-4.00[a]	-1.33[a]	68.94[b]	4.98	0.017
24:0	-81.99	0.17	0.00	-0.36	0.68	-0.08	-10.70	-2.43	-2.04	6.69	0.118
14:1	0.40	0.93	0.96	0.77	0.91	0.77	-0.20	0.62	0.87	0.09	0.140
16:1	0.87	0.94	0.97	0.96	0.98	0.91	0.67	0.87	0.95	0.03	0.145
18:1	-4.03	0.39	1.94	1.42	2.55	-1.77	-15.14	-3.92	1.27	1.42	0.152
20:1	-1.39	0.89	1.64	1.68	1.89	-0.24	-6.29	-1.85	1.25	0.70	0.201
18:2n6	0.86	0.84	0.99	0.94	1.00	1.00	0.53	0.29	0.93	0.19	0.132
18:3n6	0.89	0.97	0.98	0.98	0.98	0.85	0.71	0.89	0.97	0.02	0.200
20:3n6	0.93	0.96	0.98	1.00	1.00	0.92	0.72	0.90	0.99	0.02	0.092
20:4n6	0.89	0.94	0.96	0.96	0.97	0.89	0.72	0.89	0.95	0.02	0.119
18:3n3	1.00	0.62	1.00	0.93	1.00	0.92	0.48	0.51	0.93	0.78	0.123
20:5n3 (EPA)	0.94	0.97	0.98	0.97	0.98	0.94	0.83	0.94	0.97	0.01	0.122

[abcd] Means in the same row with different superscripts are significantly different ($P < 0.05$); See Table 3 for diet abbreviations; SEM=Standard error of the mean; nd = not detected

Milestone 2: The effect of the inclusion rate of algae oil to a canola oil-based diet on the fatty acid composition and fatty acid metabolism of Nile tilapia

Materials and Methods

Fish Management

Nile tilapia were housed at the Prairie Aquaculture Research Centre at the University of Saskatchewan. The fish were housed in a recirculating aquaculture system, which was filtered biologically. Water temperature was maintained at 27 ± 1°C. Daily, dissolved oxygen, pH and temperature were monitored. Chlorine, nitrate, nitrite and ammonia were monitored on a weekly basis. Photoperiod was a 14 h light/10 h dark cycle. The fish were maintained in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993; CCAC, 2005).

Before the experiment, 6 fish were selected at random from the whole population to determine initial whole body fatty acids of the fish. Then the fish were randomly allocated to 18 x 360 L tanks with 17 fish per tank (~155g mean start weight). Treatments were randomly assigned to tanks with three replicates per treatment. During the 70-day experiment, the fish were fed twice daily to apparent satiation and feed consumption was recorded weekly. The tanks of fish were weighed on d 0 and 70. After the last day of feeding, 3 fish from each tank were randomly collected, killed and stored at -80 °C before chemical analysis. The 3 fish from each tank were pooled and homogenized for fatty acid analysis of fish tissue.

Experimental Design

The experiment used a completely randomized design with 6 treatments and 3 experimental units (tanks) per treatment. The diets were formulated to contain 386.2g/kg

digestible crude protein and 17.58 MJ/kg DE and met all other nutrient requirements (Table 6). A diet containing 15% fish oil (FO) was fed as a positive control. Five diets were formulated to contain mixtures of canola oil and algae oil; 15% canola oil + 0 % algae oil, 14% canola oil + 1 % algae oil, 13% canola oil + 2% algae oil, 12% canola oil + 3% algae oil and 11% canola oil + 4 % algae oil. The algae oil was added using a mixture of the 3 extrudates extruded at a temperature of 130 °C during Milestone 1. A mixture was used because all three extrudates had similar digestibilities and to provide a larger source of ingredient for subsequent experiments. The fatty acid compositions of the diets are shown in Table 7.

Analytical Methods

All analytical methods used were the same as described in Milestone 1.

Statistical analysis

The experiment was analyzed as a fully randomized design using the General Linear Model procedure of SPSS (Version 19, SPSS Inc., Chicago, IL, USA). Differences between dietary treatments were separated using the Tukey's test and were considered significantly different when $P < 0.05$.

Table 6. Composition of the diets used in growth trial for Nile tilapia (g/kg).

	Diets					
		Canola+Algae oil diets				
Ingredient	**Fish oil**	**15+0**	**14+1**	**13+2**	**12+3**	**11+4**
Blood meal	20.0	20.0	20.0	20.0	20.0	20.0
Poultry meal	200.0	200.0	200.0	200.0	200.0	200.0
Soy protein concentrate	214.0	214.0	214.0	214.0	214.0	214.0
Corn gluten meal	185.3	185.3	276.2	230.4	188.0	162.4
Wheat flour	200.0	200.0	154.9	109.8	64.7	1.1
Dicalcium phosphate	18.5	18.5	18.5	20.1	18.5	18.5
DL-methionine	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin mineral premix	6.7	6.7	6.7	6.7	6.7	6.7
Vit C	0.1	0.1	0.1	0.1	0.1	0.1
Choline Cl	4.0	4.0	4.0	4.0	4.0	4.0
Extrudate	0.0	0.0	90.2	180.4	270.6	360.8
Canola oil	0.0	150.0	14.0	13.0	12.0	11.0
Fish oil	150.0	0.0	0.0	0.0	0.0	0.0
Total	1000	1000	1000	1000	1000	1000

Table 7. Fatty acid composition of the diets used in the 70-day growth experiment using Nile Tilapia (mg/g DM basis)

	Diet					
		Canola+Algae oil diets				
	Fish oil	**15+0**	**14+1**	**13+2**	**12+3**	**11+4**
C14:0	11.9	0.5	2.3	3.8	4.2	5.0
C16:0	34.2	15.6	23.9	26.9	28.9	32.8
C18:0	9.1	5.1	4.1	4.1	3.3	3.4
C20:0	0.2	0.2	0.2	0.2	0.2	0.2
C22:0	0.1	0.0	0.1	0.1	0.1	0.1
C16:1	15	2.8	14.5	23.0	25.6	33.2
C18:1	30.3	97.8	70.6	61.9	49.5	37.8
C20:1	5.4	2.2	1.7	1.5	1.3	1.1
C22:1	0.6	0.4	0.2	0.2	0.2	0.1
C18:3N3 (ALA)	2.2	19.0	15.3	11.9	9.1	7.5
C18:4N3	3.8	0.1	0.1	0.1	0.1	0.1
C20:4N3	0.1	0.0	0.1	0.1	0.1	0.1
C20:5N3 (EPA)	24.3	0.5	12.6	19.5	22.6	27.2
C22:5N3	3.1	0.1	0.1	0.1	0.1	0.1
C22:6N3 (DHA)	14.6	0.3	0.2	0.2	0.2	0.1
C18:2N6	10.9	34.4	34.4	35.8	31.6	32.0
C18:3N6	0.4	0.1	0.3	0.3	0.4	0.4
C20:4N6	1.6	0.2	2.4	3.8	4.7	5.5
C22:4N6	1.3	0.1	1.1	1.0	0.9	1.0
C22:5N6	0.5	0.0	0.4	0.4	0.5	0.4

Results

Growth

The growth performance of the fish during the 70 day study is shown in Table 8. There were no significant differences in growth performance with the exception of Feed:Gain ratio. The fish fed the 12+3 diet had significantly lower feed:gain ratios than fish fed the Canola oil, 14+1, 13+2 and 11+4 diets. The fish fed the Fish oil diet were not significantly different from either of the two groups ($P > 0.05$).

Fatty acid composition of fish

The fatty acid composition of the fish initially and after the 70-day trial are shown in Table 9. The fatty acid composition of the fish at the end of the experiment reflected the composition of the diets they were fed and thus, significant differences between the fish oil diet fed fish and the canola oil-based diets were present for C18:0, C22:n-3 and DHA.

Of most interest is the content of EPA in the fish fed the Canola oil+Algae oil diets. The algae oil used in the experiment contained approximately 21% EPA as a percent of total fat. It also contained no DHA at all. Levels of EPA in the Canola oil+Algae oil diets ranged from 0.5 mg/g for the 15+0 diet to 27.2 mg/g for the 11+4 diet. The Fish oil diet contained 24.3 mg/g EPA. Thus, the addition of 4% algae oil to the diet resulted in EPA levels similar to those found in fish oil. Furthermore, the EPA composition of the fish fed the 11+4 diet was not significantly different than the fish fed the Fish oil diet. The DHA composition of the fish fed the Canola oil+Algae oil diets did not differ significantly ($P > 0.05$). This indicates that there was little conversion of EPA to DHA during the growth trial.

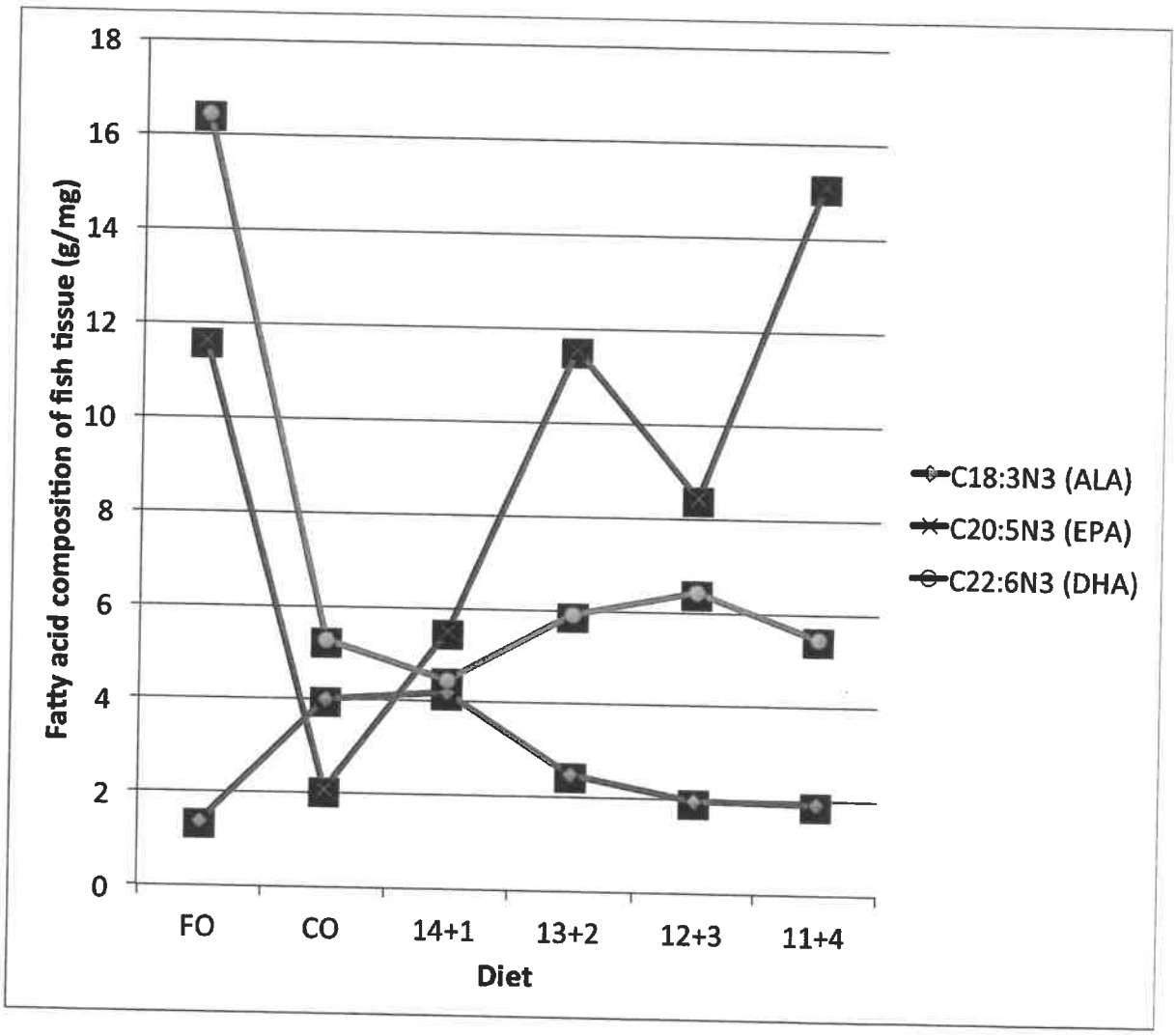
Table 8 The growth performance of Nile tilapia during the 70 day growth trial.

		Canola+Algae oil diets					
Parameter	**Fish oil**	**15+0**	**14+1**	**13+2**	**12+3**	**11+4**	***P*-value**
Initial Wt. (g)	151	157	159	141	154	148	.28
SGR	1.67	1.67	1.72	1.66	1.73	1.71	.41
Final Wt (g)	503	475	498	465	484	462	.07
Feed intake (g)	444	431	498	440	390	447	.12
Feed:Gain (g/g)	1.26ab	1.36bc	1.47c	1.36bc	1.18a	1.43c	.02

Table 9. Fatty acid concentrations in Nile tilapia tissue initially and after a 70-day growth experiment.

		Canola+Algae oil diets						
Fatty Acid	**Initial**	**FO**	**15+0**	**14+1**	**13+2**	**12+3**	**11+4**	***P*-value**
C14:0	3.9	7.1b	1.7a	2.6a	2.6a	3.3a	4.2a	<0.01
C16:0	18.5	25.5b	14.4a	22.0b	17.3ab	22.6b	26.6b	<0.01
C18:0	4.2	6.9b	4.3a	3.3a	3.0a	3.1a	3.4a	<0.01
C20:0	0.1	0.2a	0.6ab	0.9b	0.0a	0.0a	0.0	0.01
C22:0	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.22
C16:1	6.3	10.7	4.3	11.8	12.3	16.0	23.5	0.11
C18:1	19.8	27.6a	49.1bc	50.2c	41.8bc	30.4a	35.1ab	<0.01
C20:1	1.2	1.5	2.2	2.2	2.0	2.0	2.4	0.18
C22:1	0.2	0.2	0.3	0.3	0.4	0.4	0.3	0.44
C18:3N3 (ALA)	1.7	1.4a	4.0b	4.2b	2.5ab	2.0ab	1.9a	<0.01
C18:4N3	0.7	1.8	1.5	1.9	1.2	1.8	1.7	0.21
C20:4N3	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.37
C20:5N3 (EPA)	5.4	11.6bc	2.1a	5.5a	11.5bc	8.4ab	15.1c	<0.01
C22:5N3	1.9	3.5b	0.9a	0.8a	0.9a	0.9a	1.0a	<0.01
C22:6N3 (DHA)	10.6	16.4b	5.3a	4.5a	5.9a	6.4a	5.5a	<0.01
C18:2N6	7.9	9.8a	14.7b	12.9b	18.0b	16.8b	12.9	<0.01
C18:3N6	0.2	0.3	1.0	0.6	0.6	0.2	0.2	0.15
C20:4N6	0.3	0.4	0.7	0.4	0.6	0.6	0.5	0.24
C22:4N6	0.5	1.0	0.3	1.1	0.9	0.8	1.0	0.06
C22:5N6	23.8	37.9b	14.3a	31.6b	30.8b	34.6b	38.9b	<0.01

Figure 1. Concentration of ALA, EPA and DHA in fish tissue of Nile tilapia fed canola oil/algae diets during an 71 day growth experiment.



Milestone 3) The effect of extrusion parameters on the digestibility of algae and canola oils in rainbow trout diets

Diet formulation

This experiment used the extrudates produced during Milestone 1. The conditions, experimental procedures, experimental design and statistical analyses for Milestone 3 were the same as for Milestone 1 with the exception of fish species and management, which, are described below.

Fish management

Rainbow trout (female triploid) were housed at the Prairie Aquaculture Research Centre at the University of Saskatchewan. The fish were housed in a recirculating aquaculture system, which was filtered biologically. Water temperature was maintained at 14 ± 1°C. Daily, dissolved oxygen, pH and temperature were monitored. Chlorine, nitrate, nitrite and ammonia were monitored on a weekly basis. Photoperiod was a 14 h light/10 h dark cycle. The fish were maintained in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 2005).

Before the experiment, 6 fish were selected at random from the whole population to determine initial whole body fatty acids of the fish. Then the fish were randomly allocated to 12 x 360 L tanks with 17 fish per tank (~70g mean start weight). Treatments were randomly assigned to tanks with three replicates per treatment. During the 12-week experiment, the fish were fed twice daily to apparent satiation and feed consumption was recorded weekly. The tanks of fish were weighed on d 0 and 84. After the last day of

feeding, 3 fish from each tank were randomly collected, killed and stored at -80 °C before chemical analysis. The 3 fish from each tank were pooled and homogenized.

Results

Digestibility of Algal extrudates

The digestibilities of individual fatty acids are shown in Table 7. Like the Nile tilapia digestibility experiment in Milestone 1, there are many coefficients above 1.00 and below 0. This is due to the extremely low concentrations of these fatty acids in the diets and the confounding affect of endogenous losses from the intestinal tract. The digestibilities of the fatty acids in the highest concentrations in the diets are the values with the highest accuracy.

The variation on the analysis was lower in this experiment and this resulted in many more significant differences between the means than seen in the results from Milestone 1. The digestibility of EPA was high for all the extrusion conditions. The digestibility of extrudate 3 (110 °C 26.9% moisture) was significanlty lower than the other 8 extrudates. The 3 extrudates produced at 130 °C had the highest overall digestibility and this was similar to the results seen in Milestone 1. The digestibility of the extrudates in rainbow trout were generally similar. To our knowledge, there are no published comparisons of fatty acid digestibility using the same ingredient batch in rainbow trout and Nile tilapia.

Table 10. The total tract digestibilities of fatty acids in the 9 extrudate products in rainbow trout. Only fatty acids that were present in the algae product are included in this table.

	Algae extrudate product										
Fatty Acid	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**SEM**	***P*-Value**
C14:0	0.45^{a}	0.63^{c}	0.42^{a}	0.53^{b}	0.59^{bc}	0.86^{d}	0.89^{d}	0.84^{d}	0.86^{d}	0.02	< 0.001
C16:0	0.38^{a}	0.64^{b}	0.38^{a}	0.51^{ab}	0.53^{b}	0.79^{cd}	0.84^{d}	0.72^{cd}	0.70^{cd}	0.021	< 0.001
C18:0	0.01^{abc}	0.44^{bc}	-0.44^{ab}	0.10^{abc}	0.03^{abc}	0.56^{bc}	0.81^{c}	-0.33^{ab}	-0.64^{a}	0.009	< 0.001
C20:0	-0.72	0.03	-1.79	-1.00	-0.86	-0.02	0.51	-0.48	-0.69	0.006	0.064
C22:0	0.38^{a}	0.64^{b}	0.38^{a}	0.51^{ab}	0.53^{b}	0.79^{cd}	0.84^{d}	0.72^{cd}	0.70^{cd}	0.021	< 0.001
C16:1	0.79	0.92	0.54	0.96	0.94	0.91	0.94	0.84	0.91	0.004	0.027
C18:1	0.93	0.96	0.92	0.98	0.96	0.96	0.99	0.9	0.91	0.004	0.001
C20:1	0.84	0.94	0.88	0.97	0.96	0.94	0.93	0.92	0.94	0.004	< 0.001
C18:2N6	0.92	0.92	0.91	0.91	0.9	0.92	0.95	0.94	0.91	0.91	0.003
C18:3N6	0.78	0.87	-	0.87	0.92	0.89	0.77	0.73	0.87	0.023	0.688
C20:4N6	0.76	0.94	0.9	0.97	0.96	0.94	0.94	0.93	0.94	0.006	< 0.001
C18:3N3	0.79b	0.92c	0.54a	0.96c	0.94c	0.91c	0.94c	0.84b	0.91c	0.003	0.004
C20:3N6	0.76^{a}	0.94^{b}	0.90^{b}	0.93^{b}	0.94^{b}	0.97^{b}	0.96^{b}	0.93^{b}	0.94^{b}	0.011	< 0.001
C20:4N3	0.88	0.93	0.79	0.97	0.95	0.91	0.93	0.88	0.91	0.007	0.563
C20:5N3 (EPA)	0.80^{b}	0.99^{b}	0.62^{a}	0.89^{b}	1.14^{b}	1.11^{b}	1.05^{b}	0.95^{b}	0.96^{b}	0.003	0.002
C22:5N3	0.76^{b}	0.89^{b}	-2.68^{a}	0.73^{b}	1.02^{b}	1.07^{b}	1.04^{b}	0.94^{b}	0.94^{b}	0.004	< 0.001

Milestone 4 The effect of the inclusion rate of algae oil to a canola oil-based diet on the fatty acid composition of rainbow trout

Materials and Methods

The same experimental design and fish management and feeding was used in Milestone 4 as Milestone 2 with the exception of the species of fish involved.

Fish Management

Rainbow trout were housed at the Prairie Aquaculture Research Centre at the University of Saskatchewan. The fish were housed in a recirculating aquaculture system, which was filtered biologically. Water temperature was maintained at 14 ± 1°C. Daily, dissolved oxygen, pH and temperature were monitored. Chlorine, nitrate, nitrite and ammonia were monitored on a weekly basis. Photoperiod was a 14 h light/10 h dark cycle. The fish were maintained in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993; CCAC, 2005).

Before the experiment, 6 fish were selected at random from the whole population to determine initial whole body fatty acids of the fish. Then the fish were randomly allocated to 18 x 360 L tanks with 15 fish per tank (~95g mean start weight). Treatments were randomly assigned to tanks with three replicates per treatment. During the 78-day experiment, the fish were fed twice daily to apparent satiation and feed consumption was recorded weekly. The tanks of fish were weighed on d 0 and 78. After the last day of feeding, 3 fish from each tank were randomly collected, killed and stored at -80 °C before chemical analysis. The 3 fish from each tank were pooled and homogenized for fatty acid analysis.

Results

There were no significant differences in the growth rates of fish due to diet ($P > 0.05$; Table 11). The fatty acid composition of rainbow trout after the 78 day experiment showed a pattern similar to that seen in the experiment done with Nile tilapia (Table 12; Figure 2). The concentration of EPA in trout increased with increasing inclusion rate of algae in the diet. Furthermore, the concentrations of EPA in the fish fed the 12+3, 13+2 or 14+1 diets were not significantly different from those in fish fed the fish oil diet. However, the addition of algae to canola oil had no effect on the DHA content of the fish. Again, this suggests that there is limited conversion of EPA to DHA under this dietary regime.

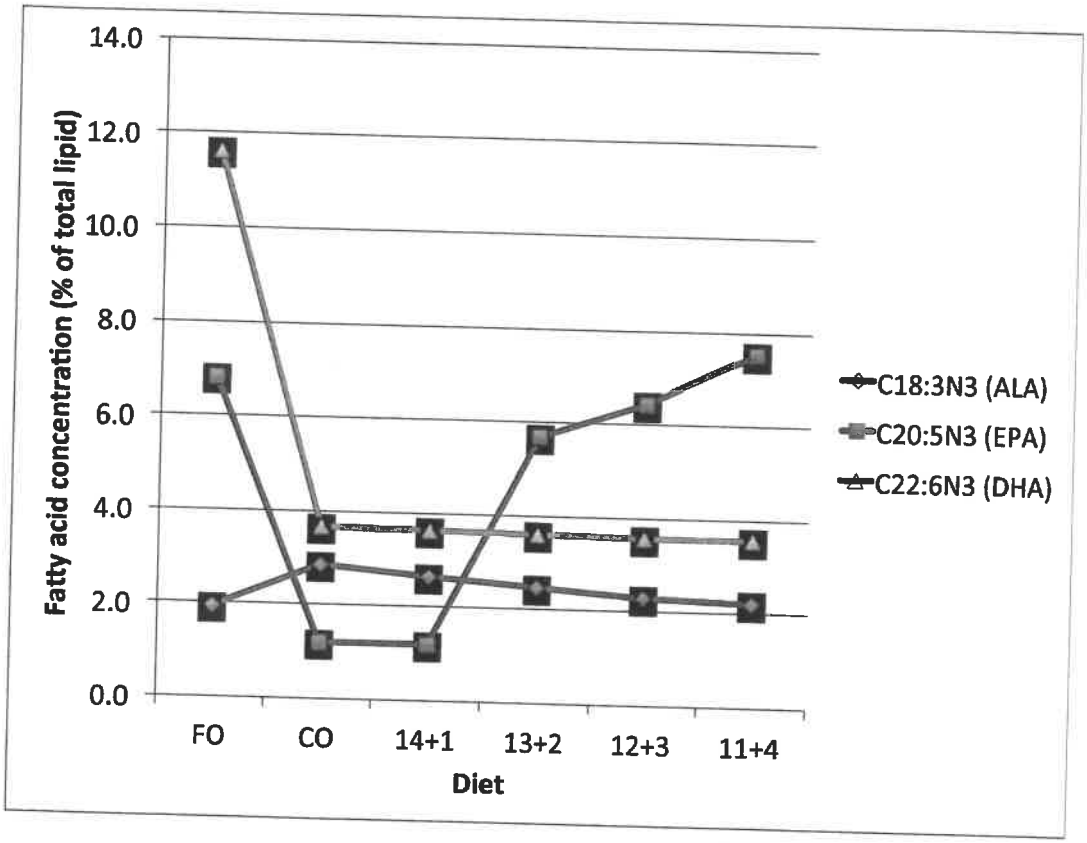
Table 11 The growth performance of rainbow trout during the 78 day growth trial.

Parameter	Fish oil	15+0	14+1	13+2	12+3	11+4	*P*-value
Initial Wt. (g)	90	87	97	95	100	96	0.07
Specific growth rate (%/d)	1.21	1.27	1.19	1.10	1.22	1.30	0.06
Final Wt (g)	233	236	247	224	259	266	0.10
Feed intake (g)	175	200	193	185	197	219	0.09
Feed:Gain (g/g)	1.17	1.24	1.18	1.34	1.16	1.18	0.22

Table 12. Fatty acid concentrations (% of total lipid) in rainbow trout tissue initially and after a 78-day growth experiment.

		Diet						
Fatty Acid	**Initial**	**FO**	**CO**	**14+1**	**13+2**	**12+3**	**11+4**	***P*-value**
C14:0	5.17	5.3b	1.6a	2.2ab	2.6ab	2.8ab	3.0ab	<0.01
C16:0	18.92	17.7c	12.5a	14.8ab	15.7bc	16.2bc	17.3bc	0.02
C18:0	4.49	4.4b	3.4a	3.1a	3.1a	2.9a	2.9	<0.01
C20:0	0.14	0.2	0.1	0.0	0.3	0.3	0.4	0.16
C22:0	0.11	0.1	0.1	0.1	0.1	0.1	0.1	0.63
C16:1	7.54	7.6ab	3.4a	7.4ab	10.4bc	11.3bc	13.9c	<0.01
C18:1	3.35	3.6a	33.3b	21.4ab	17.5ab	12.1ab	6.9a	<0.01
C20:1	1.35	.8a	1.4b	1.5b	1.5b	1.6b	1.6b	<0.01
C22:1	0.57	0.3a	.01a	1.3b	1.3b	1.4b	1.3b	<0.01
C18:3N3 (ALA)	1.25	1.9a	2.9b	2.7b	2.5ab	2.3ab	2.2ab	0.03
C18:4N3	1.00	1.5b	0.4a	0.3a	0.3a	0.3a	0.3a	<0.01
C20:4N3	0.05	0.1	0.2	0.1	0.0	0.0	0.1	0.48
C20:5N3 (EPA)	6.88	6.8b	1.2a	1.2a	5.7b	6.4b	7.5b	<0.01
C22:5N3	2.43	2.2	0.5	0.5	0.5	0.5	0.5	0.71
C22:6N3 (DHA)	12.71	11.6b	3.7a	3.6a	3.6a	3.6a	3.6a	<0.01
C18:2N6	7.50	6.3a	7.9b	7.9b	8.0b	7.7b	7.7b	0.02
C18:3N6	0.15	0.2	0.2	0.2	0.2	0.2	0.2	0.81
C20:4N6	1.14	0.2	0.4	0.4	0.3	0.9	0.4	0.13
C22:4N6	0.66	0.6	0.1	0.5	0.5	0.4	0.5	0.80
C22:5N6	0.31	0.3a	10.2b	-	-	-	-	<0.01

Figure 2. Concentration of ALA, EPA and DHA in fish tissue of rainbow trout fed canola oil/algae diets during an 71 day growth experiment.



Overall Discussion

Since all diets contained equal amounts of digestible crude protein and digestible energy, the growth response of fish to different oil sources was not expected to be significantly different. This result is in accordance with previous studies, which have shown that fish oil could be partially or completely replaced by dried algae or algae derived oil in diets for Atlantic salmon parr (Carter et al., 2003; Miller et al., 2007) and for sea bream larvae (Ganuza et al., 2008) without compromising fish growth.

The present study indicated that the final whole body fatty acid composition of fish generally reflected the fatty acid composition of diets. This finding has been well documented for many species, such as Murray cod (Francis et al., 2006), gilthead sea bream (Menoyo et al., 2004), Atlantic salmon (Torstensen et al., 2004) and rainbow trout (Thanuthong et al., 2011). In addition, the fatty acid composition in fish was also affected by *in vivo* fatty acid metabolism, including β-oxidation, biosynthesis of longer and/or more unsaturated fatty acids (Thanuthong et al., 2011). The DHA concentration in final whole fish body was lower in fish fed all experimental diets expect for the FO-fed fish, compared with DHA level in initial fish samples. Furthermore, the fish fed CO diets with added algae oil did not have significantly different tissue concentrations of DHA than the fish fed only CO. The algae oil used in the experiment did not contain DHA so this result is not unexpected. However, it indicates that the conversion of EPA to DHA was not significant. Mozaffarian *et al.,* (2005) reported that high tissue levels of EPA inhibited the expression of Δ6 desaturase, the enzyme responsible for the conversion of EPA to DHA. This negative feedback mechanism might be partly responsible for the lack of conversion of EPA to DHA seen in these studies.

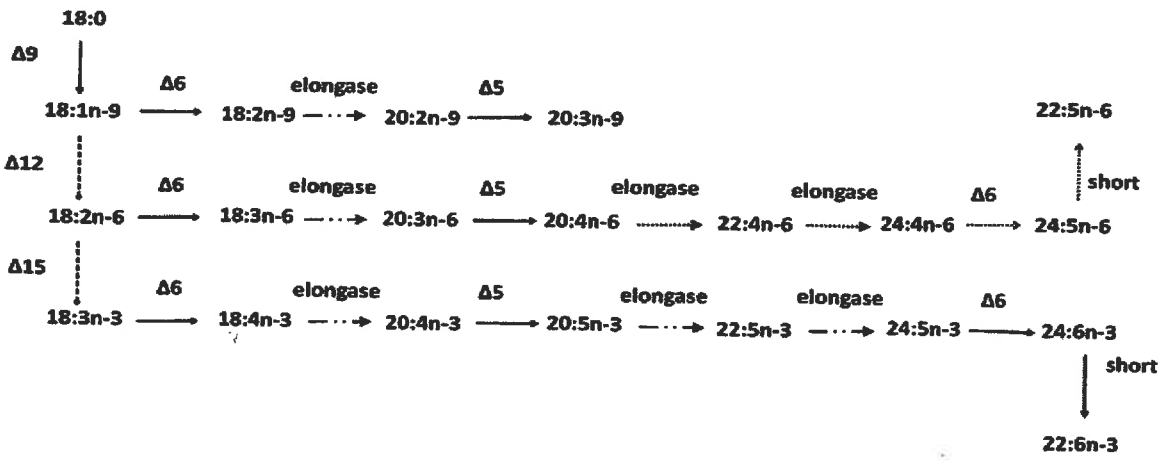
The tissue concentration of EPA increased with increasing inclusion of algae oil in the diet. This agrees with the findings of Stubhaug et al. (2007). Stubhaug et al. (2007) reported that EPA is readily β-oxidized when present in high dietary content. Similar results were found by previous research reporting that EPA is selectively catabolized over DHA for energy production and/or a possible chain elongation to DHA (Hansen et al., 2008; Torstensen et al., 2004). It is possible in the present experiment than EPA was used for β-oxidation rather than the biosynthesis of DHA. This might explain the low levels of tissue DHA seen in the fish fed diets containing algae oil. This indicates that an algae oil containing DHA might be required to increase the concentration of DHA in Nile tilapia.

In conclusion, feeding an EPA rich algae oil at a concentration of 4% of the diet resulted in EPA levels in Nile tilapia equal to those found in fish fed fish oil. However, feeding this EPA-rich algae oil diet did not result in higher levels of DHA. It has been noted in many papers that the rate-limiting step in the production of EPA and DHA from ALA is the conversion of ALA (18:3n-3) to 18:4n-3 by Δ6 desaturase (Figure 3). Based on this observation, we assumed that the conversion of EPA to DHA would be sufficient to significantly increase the levels of DHA in the tissues of the fish. However, this was not the case in these studies. Thus, we cannot assume that feeding any single long chain fatty acids past the Δ6 desaturase bottleneck will result in adequate levels of fatty acids farther down the biosynthetic pathway.

In support of this, in a recent experiment in my lab, we fed oil from a genetically modified flax variety that contained high levels of 18:4n-3. Given that 18:4n-3 is past the Δ6 desaturase bottleneck, feeding this oil should have resulted in increased levels of EPA and DHA compared to conventional flax. However, when it was fed to rainbow trout, the concentration of EPA and DHA in fish tissues was slightly but not significantly higher than in fish fed conventional flax that contained only ALA (Qi Wu, MSc thesis in preparation).

The major conclusion from this study and the work with 18:4n-3 is that to get high concentrations of EPA and DHA in fish tissues, you have to provide both of them in the diet. There are many varieties of algae and DHA-rich varieties are available. Thus, this is not a major hurdle to the concept of feeding a mixture of canola and algae oil to replace fish oil in aquafeeds. Commercial application of this technology requires only that feed companies know that they must identify algae oils with both EPA and DHA to make an effective replacement for fish oil.

Figure 3. Biosynthesis of LC-PUFAs in fish from C18 fatty acids (After Tocher, 2003).



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Expense Statement

A financial statement will be sent by research services.