Effect of dietary flaxseed oil level on the growth performance and fatty acid composition of fingerlings of rainbow trout, *Oncorhynchus mykiss*

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**Abstract**

This study evaluated the suitability of flaxseed oil as a source of supplemental dietary lipid for fingerlings of rainbow trout (*Oncorhynchus mykiss*). Triplicate groups of the 30 fingerlings held under identical culture conditions were fed twice daily by iso-nitrogenous, iso-calorific and iso-lipidic diets for 8 weeks. Experimental diets consisted of 30.2% protein, 18.6 kJ g⁻¹ energy and 16.5% lipid from fish oil (FO), flaxseed oil (FxO) and 1:1 blends of the oils (FFxO). Moisture, ash, protein, final body weight, specific growth rate, weight gain, feed conversion ratio, survival and hepatosomatic index were not affected by treatments but the percent of lipids was significantly highest in fish fed the flaxseed oil diet (FxOD). The condition factors of fingerlings reared on FxOD and fish and flaxseed oils diet (FFxOD) were significantly lower than those fed the fish oil diet (FOD). Protein efficiency ratio (PER) was significantly higher than those fed the FOD and FFxOD. Whole body fatty acid compositions mirrored those of diet treatments. The highest amounts of highly unsaturated fatty acids (HUFAs) were detected in fish fed 100% FO, which was significantly different from other treatments. In all treatments polyunsaturated fatty acids/saturated fatty acids (PUFAs/SFAs) and n-6/n-3 ratios were higher than 0.45 and lower than 4, respectively. Present results indicate the fingerlings can be reared on diets in which FO has been replaced with FxO, with no significant effects on fish performance.

**Keywords:** Body composition, Fatty acid, Fish oil replacement, Rainbow trout, Vegetable oil

**Introduction**

In the course of just a few decades, fish farming has developed into a highly productive and efficient industry to produce animal protein for human consumption. In addition to good growing conditions, a prerequisite for productivity and economic sustainability in fish farming can be a reliable supply of effective feeds. For various reasons, fish meal and fish oil have historically been the dominant raw materials in the production of fish feeds. Due to the development of more energy dense feed types as well as general growth of the aquaculture industry, a significant proportion of the total global fish oil is used for its feed preparation. A lipid requirement equal to 100% of the world’s total fish oil production is estimated by the year 2010 (New, 1999).

While marine oils are superior in their fatty acid composition they also contain a variety of toxic compounds including polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and dioxin-like polychlorinated biphenyls (DL-PCB), particularly the non-ortho and mono-ortho substituted PCBs (Jacobs *et al*., 2002a,b; Hites *et al*., 2004a,b). These compounds are suspected to be carcinogenic and immunosuppressive in humans (Birnbaum and Tuomisto, 2000; Baccarelli *et al*., 2002; Van Den Heuvel *et al*., 2002).

It is also well-known that lipid oxidation is one of the major concerns in fish-derived food products. Polyunsaturated fatty acids (PUFAs) are more easily oxidized than saturated fatty acids (SFAs), and therefore, food products enhaced with the PUFAs n-3 are also more prone to lipid oxidation. There is potential human health risks associated with increased consumption of oxidized PUFAs n-3 products (Fritsche and Johnston, 1990; Kubow, 1993). Another important factor to limit a more common use of
PUFAs n-3 enhanced food products is the development of off-flavors following lipid oxidation that may be offensive to consumers (Waagbo et al., 1993).

While it is obvious that a substitute must be found, replacing fish oil in diets has its own difficulties as most of the vegetable oils are relatively poor sources of n-3 fatty acids. Exceptions to this are flaxseed and canola oils which are rich in alpha linolenic acid (18:3n-3) (53% and 12%, respectively) (NRC, 1993). However, these oils are devoid of longer chain n-3 highly unsaturated fatty acids (HUFAs n-3) and their inclusion in trout diets results in a significant decrease in the tissue levels of eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) (Bell et al., 2002, 2003a). Moreover, enhancement of omega-3 fatty acid content in rainbow trout fillet was observed in farmed rainbow trout and brook trout as results of flaxseed oil inclusion in diet (Chen et al., 2006; Simmons et al., 2011).

Freshwater fish are capable of converting C18 PUFAs to the longer chain C20 and C22 PUFAs (Henderson and Tocher, 1987) which are the functionally essential to the longer chain C20 and C22 PUFAs (Henderson, 1999). However, these oils are devoid of longer chain n-3 highly unsaturated fatty acids (HUFAs n-3) and their inclusion in trout diets results in a significant decrease in the tissue levels of eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) (Bell et al., 2002, 2003a). Moreover, enhancement of omega-3 fatty acid content in rainbow trout fillet was observed in farmed rainbow trout and brook trout as results of flaxseed oil inclusion in diet (Chen et al., 2006; Simmons et al., 2011).

Several studies conducted on freshwater fish indicated that vegetable oils can successfully replace fish oil in the feed without affecting their survival and growth (Wonnacott et al., 2004; Subhadra et al., 2006). Caballero et al., (2002) reported that in rainbow trout (Oncorhynchus mykiss) up to 80–90% of vegetable oils e.g. soybean, rapeseed, olive, and palm oils can be used without compromising fish growth. It has also been reported that partial replacement of fish oil by vegetable oils such as rapeseed, soybean, flaxseed or palm oils in fish feeds had no negative impacts on growth and survival of Atlantic salmon (Salmo salar) (Rosenlund et al., 2001), brook char (Salvelinus fontinalis) (Guillou et al., 1995), gilthead sea bream (Sparus aurata), European sea bass (Dicentrarchus labrax) (Izquierdo et al., 2003) and rainbow trout (Greene and Selivonchick, 1990; Caballero et al., 2002).

The aim of the present study was to evaluate the effects of fish oil replacement with flaxseed oil (relatively easily obtained and low priced oil) on growth, feed conversion ratio and fillet fatty acid composition of fingerlings of rainbow trout.

Materials and methods

270 rainbow trout fingerlings with a mean initial body weight of 16.5 ± 0.5 g were purchased from Cheshmeh Dimah fish hatchery (Shahre kord, Chaharmahal and Bakhtiari, Iran) and used in this study. Prior to the start of the experiment the fish were acclimatized to the new environmental conditions and the commercial diet (SEF2 of Chineh feed production factory, Tehran, Iran) for a two week period within a semi re-circulating system.

Experimental diets

Three iso-nitrogenous, iso-calorific and iso-lipidic purified experimental diets were formulated from 100% fish oil (FO), 100% flaxseed oil (FxO) and 1:1 blends of the two oils (FFxO). The nutritional content and Fatty acid composition of the oils and experimental diets are presented in Tables 1 and 2, respectively. Diets were prepared and stored according to Abery et al., (2002) and De Silva et al., (2002).

Husbandry

This study was conducted indoors in a thermostatically controlled room. Fish were housed in nine 100 L fiberglass circular rearing tanks in a semi re-circulating system with an in-line oxygen generator and a physical and biological treatment plant (flow rate of 6 L min⁻¹). During experiment, fish were kept under a 12-h light:12-h dark cycle. The experiment was conducted at 13.6 ± 1.3°C, water quality parameters were measured every second day using Aquamerck test kits (Merck, Darmstadt, Germany).

Table 1 Ingredient (%), proximate composition (% wet weight) and energy (kJ g⁻¹) of the experimental diets

|          | FOD  | FxOD | FFxOD |
|----------|------|------|-------|
| Fish Meal| 58   | 58   | 58    |
| Soybean Meal| 20  | 20   | 20    |
| Wheat Meal| 8.6 | 8.6  | 8.6   |
| Fish Oil | 8    | 0    | 4     |
| Flaxseed Oil| 0  | 8    | 4     |
| Vitamin premix*| 2  | 2    | 2     |
| Mineral premix**| 1.5| 1.5  | 1.5   |
| Lysine    | 0.07 | 0.07 | 0.07  |
| Methionine| 0.13| 0.13 | 0.13  |
| Choline chloride| 0.2| 0.2  | 0.2   |
| Molasses  | 1    | 1    | 1     |
| Salt      | 0.5  | 0.5  | 0.5   |

Proximate composition

|          | Moisture | Ash | Crude protein | Crude lipid | Crude fiber |
|----------|----------|-----|---------------|-------------|-------------|
|          | 9.80     | 14.59 | 30.66        | 16.09       | 2.16        |
|          | 14.99    | 14.76 | 30.15        | 16.52       | 2.42        |
|          | 9.10     | 15.29 | 29.85        | 16.81       | 2.01        |
| NFE***   | 26.70    | 27.05 | 26.41        |             |             |

Energy****

|          | 18.56    | 18.71 | 18.57        |

Diet abbreviations, FOD: 100% fish oil; FxOD: 100% Flaxseed oil; FFxOD: 50% fish oil and 50% flaxseed oil.

*Contains (mg kg⁻¹ food): E (30), K (3), niacin (40), thiamine (2), riboflavin (7), pyridoxine (3), folacin (1.5), pantothenic acid (18), biotin (0.7) and cyanocobalamin (0.18).

**Contains (mg kg⁻¹ food): Mg (100), Zn (60), Fe (40), Cu (5), Co (0.1), I (1) and Antioxidant (100).

***NFE: nitrogen free extract, calculated by difference (100 – moisture – ash – crude protein – crude lipid – crude fibers).

****Calculated on the basis of 23.6, 39.5 and 17.2 kJ g⁻¹ of protein, fat and carbohydrate, respectively.
with a mean pH of 7.3 ± 0.2 and levels of ammonia and nitrate below 0.1 mg L$^{-1}$.

Experimental protocol
Two hundred and seventy individually weighed and measured rainbow trout (O. mykiss) fingerlings were randomly distributed into nine 100 L fiberglass tanks (30 fish per tank) and randomly assigned to one of the 3 different experimental diets (3 replicates for each experimental diet). Fish were fed twice daily at approximately 08.30 and 17.00 h to apparent satiation for a period of 56 days. At the end of the experiment a sample of 18 fish (2 fish per replicate) was taken and anesthetized in excess anesthetic (Benzocaine 0.5 mg L$^{-1}$) for both body composition and fatty acid profile analysis.

### Table 2 Fatty acid composition (percentage of total fatty acids) of the oils and experimental diets

| Fatty acid | Fish Oil | Flaxseed Oil | FOD | FxOD | FFxOD |
|-----------|----------|--------------|-----|------|-------|
| 14:0      | 0.06     | -            | 0.07| 0.04 | 0.05  |
| 15:0      | 0.32     | -            | 0.23| 0.09 | 0.17  |
| 16:0      | 20.73    | 6.79         | 22.71| 16.48| 19.80 |
| 17:0      | 0.72     | 0.11         | 0.72| 0.51 | 0.67  |
| 18:0      | 4.16     | 4.48         | 5.85| 6.04 | 6.20  |
| 19:0      | 2.89     | 1.23         | 2.94| 2.31 | 2.53  |
| 21:0      | 0.18     | -            | 0.25| 0.24 | 0.29  |
| 22:0      | 0.24     | 0.04         | 0.20| 0.13 | 0.15  |
| 23:0      | 0.21     | 0.57         | 0.22| 0.30 | 0.33  |
| 24:0      | 0.18     | -            | 0.14| 0.08 | 0.11  |
| SFAs      | 29.68    | 13.22        | 33.32| 26.22| 30.29 |
| 14:1      | 3.77     | 0.26         | 3.11| 1.37 | 2.26  |
| 15:1      | 0.80     | 0.07         | 0.71| 0.38 | 0.56  |
| 16:1n-7   | 5.24     | 0.34         | 4.92| 2.59 | 3.86  |
| 17:1      | 0.71     | 0.06         | 0.65| 0.27 | 0.41  |
| 18:1n-9   | 33.57    | 34.19        | 38.79| 41.75| 40.50 |
| 24:1n-9   | 0.44     | -            | 0.41| 0.14 | 0.26  |
| MUFA n-6  | 44.52    | 34.92        | 48.59| 46.50| 47.85 |
| 18:2n-6   | 0.37     | 0.38         | 0.42| 0.39 | 0.61  |
| 18:3n-6   | 0.05     | -            | 0.04| 0.05 | 0.05  |
| 20:2n-6   | 2.48     | 0.13         | 1.21| 0.24 | 0.68  |
| 20:3n-6   | 0.18     | -            | 0.20| 0.25 | 0.21  |
| 20:4n-6   | 0.02     | -            | 0.06| 0.03 | 0.05  |
| 22:2n-6   | 0.72     | -            | 0.65| 0.37 | 0.52  |
| 22:5n-6   | 0.36     | -            | 0.30| 0.18 | 0.21  |
| PUFA n-6  | 4.18     | 0.50         | 2.88| 1.51 | 2.32  |
| 18:3n-3   | 2.07     | 51.36        | 4.58| 21.93| 12.64 |
| 18:4n-3   | 0.32     | -            | 0.60| 0.71 | 0.84  |
| 20:3n-3   | 0.05     | -            | 0.61| 0.05 | 0.05  |
| 20:5n-3   | 5.90     | -            | 2.95| 0.73 | 1.69  |
| 22:5n-3   | 0.48     | -            | 0.36| 0.22 | 0.28  |
| 22:6n-3   | 12.82    | -            | 6.65| 2.16 | 4.05  |
| PUFA n-3  | 21.62    | 51.36        | 15.75| 25.80| 19.55 |
| HUFA n-3  | 18.72    | -            | 9.60| 2.89 | 5.74  |

- not detected.

See Table 1 for diet abbreviations.

Chemical analysis
Fishes allocated for flesh analysis were filleted (denuded of skin and bone) and stored at ~20°C until used for fillet proximate analysis. Fishes allocated for fatty acids analysis were stored at ~80°C. Proximate analysis was conducted using standard procedures (AOAC, 1990), percentage moisture (dried at 80°C to constant weight), protein (Kjeldahl nitrogen; N × 6.25) in an automated Kjeltech (Model 2300, Tecator, Sweden), total lipid by chloroform/methanol extraction (2:1 v/v) (Folch et al., 1957) as modified by Ways and Hanahan (1964) and ash by incineration in a muffle furnace (Model WIT, C & LTetlow, Australia) at 550°C for 18 h. Fatty acid analysis was carried out on each of the added dietary oils, experimental diets and fillet samples from each of the replicates. Fatty acid methyl esters (FAMEs) were prepared from aliquots of total lipids by acid catalyzed transmethylation with sulfuric acid in methanol overnight at 50°C (Christie, 1982). FAMEs were purified by TLC using hexane/diethyl ether/acetic acid (85:15:15 v/v/v) as solvent (Tocher and Harvie, 1988). Separation of FAMEs was carried out in a Gas Chromatograph system (Agilent Technologies, 6890 N, USA) equipped with a flame ionization detector (FID), and a cross-linked silica capillary column HP-88 (100 m, 250 μm ID, 0.2 μm film thickness), on-column injection and using helium as the carrier gas with a flow rate of 1.1 ml min$^{-1}$. The column was programmed for an initial temperature of 140°C held for 5 min, rising at a rate of 4°C min$^{-1}$ to the final temperature of 240°C and held for 10 min. Injector and detector temperatures were 230°C and 260°C, respectively. The flow rates of compressed air and hydrogen were 300 ml min$^{-1}$ and 30 ml min$^{-1}$, respectively. Identification and quantification of FAMEs were based on the comparison of the sample retention time with known standards (Sigma Chemicals, St. Louis, USA).

### Statistical analysis
Mean values and standard deviation for each parameter measured for all treatments were calculated first. The results were subjected to a one-way ANOVA to test the effect of the replacement of vegetable oil blends on fish performance. Data were analyzed using statistical packages SPSS v15 (SPSS Inc., Chicago, IL, USA). Differences between means were compared using Duncan’s multiple range test at significance of differences ($P<0.05$) among dietary treatments. Linear regression analyses were performed between dietary and fillet fatty acid concentrations.
Results

Growth
The mean final body weight (MFBW) of fingerlings of rainbow trout (O. mykiss) ranged from 56.6 ± 8.0 to 58.5 ± 14.6 for FxOD and fish and flaxseed oils diet (FFxOD) treatments, respectively. The differences between the MFBW of fish receiving different diets were not significant. Similarly, no significant differences were observed between survival rate, specific growth rate, weight gain, feed conversion ratio and hepatosomatic index. The condition factor of fish reared on FxOD and FFxOD were significantly (P < 0.05) lower than those fish fed the FOD. The protein efficiency ratio (PER) was highest in fish fed the FxOD and significantly (P < 0.05) higher than those fed with FOD and FFxOD (Table 3).

Fillet proximate composition
Results of the proximate analysis of fillet of fish receiving the different dietary treatments are tabulated in Table 4. No significant differences between percent moisture, ash and protein content of fish fed the experimental diets were observed, but the percent of lipid content was highest in fish fed the FxOD which was significantly (P < 0.05) higher than lipid content of fish fed on FFxOD.

Fillet fatty acid composition
The major fatty acid classes (SFAs, MUFAs and PUFAs) found in the highest concentration were palmitic, oleic, α-linolenic acids along with DHA, respectively (Table 5).

Table 3 Mean (±SD) of growth, feed utilisation and other body parameters of rainbow trout reared on the experimental diets

|                | FOD     | FxOD    | FFxOD   |
|----------------|---------|---------|---------|
| MIBW (g)       | 16.12 ± 0.27 | 16.30 ± 0.78 | 16.74 ± 0.33 |
| MFBW (g)       | 58.05 ± 6.98 | 56.56 ± 8.05 | 58.52 ± 14.58 |
| CF             | 1.22 ± 0.10 \(a\) | 1.15 ± 0.11 \(b\) | 1.12 ± 0.10 \(a\) |
| SGR            | 2.29 ± 0.02 | 2.22 ± 0.06 | 2.23 ± 0.08 |
| WG             | 260.09 ± 4.25 | 246.71 ± 10.78 | 249.54 ± 15.23 |
| FCR            | 0.90 ± 0.17 | 1.01 ± 0.05 | 0.99 ± 0.30 |
| SR             | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 |
| HIS            | 1.21 ± 0.13 | 1.14 ± 0.06 | 1.15 ± 0.06 |
| PER            | 1.92 ± 0.22 \(b\) | 3.12 ± 0.31 \(a\) | 2.37 ± 0.16 \(b\) |

Values in the same row with the same superscripts are not significantly different (P > 0.05).

Table 4 Fillet proximate compositions (mean ± SD) of rainbow trout reared on different diets, (% wet weight)

|                | FOD     | FxOD    | FFxOD   |
|----------------|---------|---------|---------|
| Moisture       | 78.44 ± 0.61 | 76.96 ± 0.20 | 76.48 ± 1.02 |
| Ash            | 1.34 ± 0.03 | 1.28 ± 0.05 | 1.36 ± 0.06 |
| Protein        | 15.29 ± 0.81 | 17.77 ± 0.16 | 18.55 ± 1.15 |
| Lipid          | 2.93 ± 0.20 | 3.46 ± 0.27 \(a\) | 3.48 ± 0.14 \(a\) |
| Moisture       | 78.44 ± 0.61 | 76.96 ± 0.20 | 76.48 ± 1.02 |
| Ash            | 1.34 ± 0.03 | 1.28 ± 0.05 | 1.36 ± 0.06 |
| Protein        | 15.29 ± 0.81 | 17.77 ± 0.16 | 18.55 ± 1.15 |
| Lipid          | 2.93 ± 0.20 | 3.46 ± 0.27 \(a\) | 3.48 ± 0.14 \(a\) |

See Table 1 for diet abbreviations.

The level of SFAs was observed in higher (P < 0.05) concentrations for fish fed the FOD compared to fish fed the FxOD and FFxOD. Levels of MUFAs ranged from 47.4 ± 0.5 (FxOD) to 53.0 ± 0.4 (FOD) and were observed to be significantly higher in fish fed the FOD. The fillet of fish fed the FOD and FxOD were particularly rich in oleic acid (44.8 ± 0.4%) and α-linolenic acid (19.3 ± 0.4%), respectively. DHA and arachidonic acid levels were found in higher concentrations in the fillet than in the diets. The highest level of EPA and DHA was observed in fish fed the FOD (P < 0.05). However, DHA was found in high concentrations within all of the dietary treatments, ranging from 5.7 ± 0.4% (FxOD) to 10.7 ± 0.4% (FOD). The level of n-3 fatty acids was higher in the fillet than the diet for each of the treatments, but the level of n-6 fatty acids was higher in the fillet than the diet only for FxOD and FFxOD, with n-6/n-3 ratios ranging from 0.12 ± 0.00 to 0.16 ± 0.02 in the fillet. The highest HUFAs n-3 concentrations (P < 0.05) were found in fish fed the FOD (12.8 ± 0.4%), while the lowest value was observed in fish fed the FxOD (6.6 ± 0.4%).

Regression analysis was used to identify dose response relationship between dietary and fillet fatty acids. As reported in Table 6, most of the fatty acid concentrations in the fillet were linearly correlated to the dietary fatty acid concentrations.

Discussion
The results of the present study suggest that flaxseed oil can be used to replace fish oil without adverse effects on growth performance of rainbow trout fingerlings, as reported in other studies (Montero et al., 2005; Bell et al., 2004; Izquierdo et al., 2005). This was evidenced by the weight gain and feed conversion ratio which ranged from 246.7 ± 10.8% to 260.1 ± 4.2% and 0.90 ± 0.17 to 1.01 ± 0.05, respectively, with no significant differences from fish fed all experimental diets.

In agreement with previous studies (Caballero et al., 2002; Martino et al., 2002; Glencross et al., 2003; Turchini et al., 2003b), considerable differences were evident in the fatty acid composition of fish fed different lipid sources. For example, there was a high increase in the levels of α-
As reported by other researchers, (Turchini et al., 2003a,b; Guillou et al., 1995; Guillou et al., 1995; Bell et al., 2003a, b; Torstensen et al., 2004; Chen et al., 2006; Chen et al., 2008; Simmons et al., 2011), a high correlation are also exist between the individual fatty acids as well as MUFAs and PUFAs of a diet and the fish fillet (Table 6). There was, however, a high correlation between the amount of SFAs in the diet and SFAs in the fillet, which was not in accordance with the findings of Turchini et al., (2003a,b) who postulated that SFAs were not used efficiently by Murray cod (Maccullochella peeli peeli) as an energy source and were subsequently deposited at an optimal level in preference to the other major fatty acid classes.

It is well known that freshwater fish have a dietary requirement for n-3 and n-6 fatty acids, predominantly in the form of α-linolenic and linoleic acids (Kanazawa et al., 1979, 1980; Guillou et al., 1995; Martino et al., 2002; Izquierdo et al., 2003; Tocher, 2003). In comparison to marine fish species, freshwater fish are also generally better adapted to desaturate and elongate these base fatty acids to higher homologs (Guillou et al., 1995; Tocher, 2003). This study observed α-linolenic acid in lower concentrations in the muscle than in the diets. It is therefore suspected that a high degree of metabolism of this fatty acid for β-oxidation and/or desaturation and elongation is taking place in fingerlings of rainbow trout (O. mykiss). This is further bolstered by the presence of n-3 desaturation and elongation enzyme products in the form of 18:4n-3 and 20:3n-3 in fish fed FxOD and FFxOD. These fatty acids were found in much lower concentrations in the diets. Likewise, fish fed the FxOD and FFxOD contained n-6 desaturation and elongation intermediates (18:3n-6 and 20:3n-6) and indicate an elongation and desaturation of linoleic acid via Δ6 desaturase. However, further desaturation of 20:3n-6 to 20:4n-6 and 20:3n-3 to EPA and ultimately DHA was shrouded by high concentrations of these fatty acids within the fillet of initial fish samples. The Department of Health of England (Committee on Medical Aspects of Food Policy 1994) recommends

### Table 5 Fillet fatty acid composition (percentage of total fatty acids) of rainbow trout reared on the different diets (mean ± SD)

| Initial | FOD | FxOD | FFxOD |
|---------|-----|------|-------|
| 140     | 0.03| 0.04 | 0.01  |
| 150     | 0.09| 0.16 | 0.01  |
| 160     | 10.11| 17.45| 0.29 |
| 170     | 0.14| 0.50 | 0.01  |
| 180     | 2.90| 4.46 | 0.10 |
| 190     | 3.07| 3.72 | 0.45 |
| 210     | 0.21| 0.19 | 0.06 |
| 220     | 0.77| 0.55 | 0.06 |
| 230     | 0.72| 0.28 | 0.02 |
| 240     | 0.41| 0.41 | 0.03 |
| SFAs    | 18.44| 27.74| 0.53 |
| 14.1    | 1.42| 2.25 | 0.08 |
| 15.1    | 0.17| 0.52 | 0.02 |
| 16.1n-7 | 1.77| 4.49 | 0.09 |
| 17.1    | 0.18| 0.48 | 0.03 |
| 18.1n-9 | 61.25| 48.85| 0.45 |
| 24.1n-9 | 0.10| 0.40 | 0.02 |
| MUFAs   | 64.88| 52.99| 0.38 |
| 18n-6   | 0.71| 0.41 | 0.05 |
| 18n-3   | 0.05| 0.06 | 0.00 |
| 20n-6   | 0.88| 0.89 | 0.06 |
| 20n-3   | 0.13| 0.09 | 0.01 |
| 20n-6   | 0.86| 0.15 | 0.03 |
| 22n-6   | 0.43| 0.77 | 0.07 |
| 22n-5   | 0.33| 0.24 | 0.03 |
| PUFAs   | 3.40| 2.60 | 0.24 |
| 18n-3   | 4.27| 1.94 | 0.07 |
| 18n-4   | 2.61| 1.04 | 0.04 |
| 20n-3   | 0.53| 0.30 | 0.03 |
| 20n-5   | 0.87| 2.08 | 0.10 |
| 22n-3   | 0.25| 0.59 | 0.05 |
| 22n-6   | 4.76| 10.73| 0.40 |
| PUFAs   | 13.28| 16.67| 0.52 |
| PUFAs   | 5.63| 12.80| 0.45 |
| PUFAs   | 16.68| 19.27| 0.66 |
| PUFAs   | 0.90| 0.69 | 0.04 |
| n-6/n-3 | 0.26| 0.16 | 0.02 |

See Table 1 for diet abbreviations.

### Table 6 Correlation between dietary fatty acid concentrations and fatty acid concentrations in fillet of rainbow trout fed the experimental diets for 8 weeks

| Fatty acids | Correlation coefficient (r) | Slope |
|-------------|----------------------------|-------|
| 16:0        | 0.974                      | 0.719 |
| 18:0        | 0.451                      | 0.201 |
| SFAs        | 0.957                      | 0.757 |
| 18:1n-9     | 0.861                      | −0.513|
| MUFAS       | 0.997                      | 2.637 |
| 18:2n-6     | 0.537                      | −0.274|
| 20:4n-6     | 0.945                      | −0.357|
| PUFAs n-6   | 0.986                      | −0.431|
| 18:3n-3     | 0.999                      | 1.001 |
| 20:5n-3     | 0.999                      | 0.521 |
| 22:6n-3     | 0.984                      | 1.098 |
| PUFAs n-3   | 0.978                      | 1.035 |
| PUFAs       | 0.982                      | 1.287 |

Values in the same row with the same superscripts are not significantly different (P > 0.05).
a minimum PUFAs/SFAs ratio of 0.45, and a maximum n-6/n-3 of 4.0. Table 5 shows that our fish in all treatments met the PUFAs/SFAs and n-6/n-3 ratios. Despite the decrease in EPA and DHA in fillet from fish fed FFxOD, the trout fillets contained a relatively rich source of these fatty acids (584 mg of EPA plus DHA) with a 200 g serving portion of the fillets from fish fed FFxOD. This meets the intake of 500 mg day\(^{-1}\) of EPA plus DHA recommended by the International Society for the Study of Fatty Acids and Lipids (Simopoulos et al., 1999).

**Conclusion**

Present study showed the substitution of fish oil with flaxseed oil in the rainbow trout (O. mykiss) diet have been possible without any negative effects on the growth and feed conversion ratio. However, the reflection of the dietary oil source on the fillet fatty acid composition of the fish could be a potential drawback for vegetable oil substitution from a human nutritional point of view, given the decreases in levels of EPA and DHA in fish fed the vegetable oil diets. Further investigation into the benefits of other vegetable oils or indeed a blend of various vegetable oils is required in order to reduce usage of traditionally used fish oils, while simultaneously avoiding a reduction in the human health protective properties found within fish flesh.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

All As a MSc student carried out most experimentat work. NMS: As a supervisor of MSc give the advise for experimental work and paper preparation. EE: Acted as a co-supervisor and helped with MSc work and paper preparation. AK: Helped and advised for fatty acid analysis. MR: Helped with experimental work. All authors read and approved the final manuscript.

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**References**

Abery NW, Gunasekera RM, De Silva SS (2002) Growth and nutrient utilization of Murray cod (Maccullochella peeli peeli) fingerlings fed diets with varying levels of soybean meal and blood meal. Aquac Res 33:279–289

AOAC (1990) In: Helrich K (ed) Official Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, Arlington, VA, USA

Baccarelli A, Mocarelli P, Patterson DG, Bonzini M, Pesatori AD, Caporaso N et al (2002) Immunologic effects of diet: new results from seveous and comparison with other studies. Environ Health Persp 110:1169–1173

Bell JG, Henderson RJ, Tocher DR, McGhee F, Dick JR, Porter A et al (2002) Substituting fish oil with crude palm oil in the diet of Atlantic salmon (Salmo salar) affects tissue fatty acid compositions and hepatic fatty acid metabolism. J Nutr 132:222–230

Bell JG, Tocher DR, Henderson RJ, Dick JR, Crampton VO (2003a) Altered fatty acid compositions in Atlantic salmon (Salmo salar) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. J Nutr 133:2793–2801

Bell JG, McGhee F, Campbell PJ, Sargent JR (2003b) Rapsseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (Salmo salar): changes in flesh fatty acid composition and effectiveness of subsequent fish oil “wash out”. Aquaculture 218:515–528

Bell JG, Henderson RJ, Tocher DR, Sargent JR (2004) Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid compositions in Atlantic salmon (Salmo salar), using a fish oil finishing diet. Lipids 39:223–232

Birnbaum LS, Tuomisto J (2000) Non-carcinogenic effects of TCDD in animals. Food Addit Contam 17:275–288

Caballero MJ, Obach A, Rosenlund G, Montero D, Gisvold M, Izquierdo MS (2002) Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout (Oncorhynchus mykiss). Aquaculture 214:253–271

Chen YC, Nguyen J, Semmens K, Beamer S, Jaczynski J (2006) Enhancement of omega-3 fatty acid content in rainbow trout (Oncorhynchus mykiss) fillets. J Food Science 71(1):C383–C389

Chen YC, Nguyen J, Semmens K, Beamer S, Jaczynski J (2008) Chemical changes in omega-3-enhanced farmed rainbow trout (Oncorhynchus mykiss) fillets during abusive-temperature storage. Food Control 19(6):599–608

Christie WW (1982) Lipid analysis, 2nd edn. Pergamon Press, Oxford

Committee on Medical Aspects of Food Policy (1994) Nutritional Aspects of Cardiovascular Disease. Department of Health Report on Health and Social Subjects, No. 46, HMSO, London

De Silva S, Gunasekera R, Collins R, Ingram B (2002) Performance of juvenile Murray cod (Maccullochella peeli peeli), fed with diets of different protein to energy ratio. Aquacult Nutr 8:79–85

Folch JM, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509

Fritsche KL, Johnston PV (1990) Effect of dietary a-linolenic acid on growth, metastasis, fatty acid profile and prostaglandin production of two marine mammay adenocarcinomas. J Nutr 120:1601–1609

Glencross B, Hawkins W, Curnow J (2003) Evaluation of canola oils as alternative lipid resources in diets for juvenile red sea bream (Pleurus auratus). Aquacult Nutr 9:305–315

Greene DHS, Selvonnchick DP (1990) Effects of dietary vegetable, animal and marine lipids on muscle lipid and haematology of rainbow trout (Oncorhynchus mykiss). Aquaculture 89:165–182

Guillou A, Soucy P, Khalil M, Adambounou L (1995) Effects of dietary vegetable and marine lipid on growth, muscle fatty acid composition and organoleptic quality of flesh of brook char (Salvelinus fontinalis). Aquaculture 136:351–362

Henderson RJ, Tocher DR (1987) The lipid composition and biochemistry of freshwater fish. Prog Lipid Res 6:281–347

Hites RA, Foran JA, Carpenter DO, Hamilton MC, Knuth BA, Schwager SJ (2004a) Global assessment of organic contaminants in farmed salmon. Science 303:226–229

Hites RA, Foran JA, Schwager SJ, Knuth BA, Hamilton MC, Carpenter DO (2004b) Global assessment of polybrominated diphenyl ethers in farmed and wild salmon. Environ Sci Technol 38:4945–4949

Izquierdo MS, Obach A, Arantzamendi L, Montero D, Robaina L, Rosenlund G (2005) Dietary lipid sources for sea bream and sea bass: growth performance, tissue composition and flesh quality. Aquacult Nutr 9:397–407

Izquierdo MS, Montero D, Robaina L, Caballero MJ, Rosenlund G, Gine R (2005) Alterations in fillet fatty acid profile and flesh quality in gilthead sea bream (Sparus aurata) fed vegetable oils for a long term period, recovery of fatty acid profiles by fish oil feeding. Aquaculture 250:431–444

Jacobs MN, Ferrario J, Byrne C (2002a) Investigation of polychlorinated dibenzo-p-dioxins dibenzo-p-dioxins and selected coplanar biphenyls in Scottish farmed Atlantic salmon (Salmo salar). Chemosphere 47:183–191

Jacobs MN, Covaci A, Schepens P (2002b) Investigation of selected persistent organic pollutants in farmed Atlantic salmon (Salmo salar), salmon aquaculture feed, and fish oil components of the feed. Environ Sci Technol 36:2797–2805

Kanazawa A, Teshima S, Ono K (1979) Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of...
linolenic acid to highly unsaturated fatty acids. Comp Biochem Phys 63B:295–298
Kanazawa A, Teshima SI, Sakamoto M, Awal M (1980) Requirements of Tilapia zilli for essential fatty acids. B Jpn Soc Sci Fish 46:1353–1356
Kubow S (1993) Lipid oxidation products in foods and atherogenesis. Nutr Rev 51:33–40
Lauritzen L, Hansen HS, Jorgensen MH, Michaelsen KE (2001) The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res 40:1–94
Martina RC, Cyrino JEP, Pontz L, Trugo LC (2002) Performance and fatty acid composition of surubim (Pseudopleuronectes coruscans) fed diets with animal and plant lipids. Aquaculture 209:233–246
Montero D, Robaina L, Caballero MJ, Gine’s R, Izquierdo MS (2005) Growth, feed utilization and flesh quality of European sea bass (Dicentrarchus labrax) fed diets containing vegetable oils: A time-course study on the effect of a re-feeding period with a 100% fish oil diet. Aquaculture 248:121–134
New M (1999) Global aquaculture: current trends of challenges for the 21st century. World Aquac 30:8–13
NRC (National Research Council) (1993) Nutrient Requirement of Fish. National Academy Press, Washington, DC
Rosenlund G, Obach A, Sandberg MG, Standal H, Tveit K (2001) Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon (Salmo salar). Aquac Res 32:323–328
Simmons CA, Turk P, Beamer S, Jaczynski J, Semmens K, Matak KE (2011) The effect of a flaxseed oil-enhanced diet on the product quality of farmed brooktrout (Salvelinusfontinalis) fillets. J Food Science 76(8):192–197
Simopoulos AP, Leaf A, Salem N (1999) Workshop on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. International Society for the Study of Fatty Acids and Lipids. J Am Coll Nutr 18(5):487–489
Subhadra B, Lochmann R, Rawles S, Chen RG (2006) Effect of dietary lipid source on the growth, tissue composition and hematological parameters of largemouth bass (Micropterus salmoides). Aquaculture 255:210–222
Tocher DR, Harvie DG (1988) Fatty acid compositions of the major phosphoglycerides from fish neural tissues: (n-3) and (n-6) polyunsaturated fatty acids in rainbow trout (Salmo gairdneri) and cod (Gadus morhua) brains and retina. Fish Physiol Biochem 5:229–239
Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fish Sci 11:107–184
Torstensen L, Frøyland L, Lie Ø (2004) Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil, effects on Atlantic salmon (Salmo salar) tissue and lipoprotein composition and lipogenic enzyme activities. Aquacult Nutr 10:175–192
Turchini GM, Gunasekera RM, De Silva SS (2003a) Effect of crude oil extracts from trout offal as a replacement for fish oil in the diets of the Australian native fish Murray cod (Maccullochella peeli peeli). Aquac Res 34:697–708
Turchini GM, Mentasti T, Frøyland L, Orban E, Caprino F, Moretti VM et al (2003b) Effects of alternative dietary lipid sources on performance, tissue chemical composition, mitochondrial fatty acid oxidation capabilities and sensory characteristics in brown trout (Salmo trutta). Aquaculture 222:251–267
Van Den Heuvel RL, Kopper G, Staessen JA, Hond ED, Verheyen G, Nawrot TS et al (2002) Immunologic biomarkers in relation to exposure markers of PCBs and dioxins in Flemish adolescents (Belgium). Environ Health Persp 110:595–600
Waaqbae R, Sandnes K, Torissen OJ, Sandvin A, Lie Ø (1993) Chemical and sensory evaluation of fillets from Atlantic salmon (Salmo salar) fed three different levels of n-3 polyunsaturated fatty acids at two levels of vitamin E. Food Chem 46:361–366
Ways P, Hanahan DJ (1964) Characterization and quantification of red cell lipids in normal man. J Lipid Res 5:318–328
Wonnacott EJ, Lane RL, Kohler CC (2004) Influence of dietary replacement of menhaden oil with canola oil on fatty acid composition of sunshine bass. N Am J Aquacult 66:243–250

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