Influence of replacing fish oil with canola oil on nutritive value of Rainbow Trout

Ali Masiha[1], Eisa Ebrahimi[1], Nasrollah Mahboobi Soofiani[1], Mahdi Kadivar[2], Mohammad Reza Karimi[1]

[1]Division of Fisheries, Department of Natural Resources, [2]Department of Food science, College of Agriculture, Isfahan University of Technology. masiha.ali@gmail.com

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 [24].

While marine oils are superior in their fatty acids 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 [14, 15, 17, 18]. These compounds are suspected to be carcinogenic and immunosuppressive in humans [2, 6, 32]. 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 enhanced 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 [10, 21].

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) [25]. 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) [3, 4].

Freshwater fish are capable of converting C18 PUFAs to the longer chain C20 and C22 PUFAs [13] which are the functionally essential fatty acids in vertebrates [22].

The aim of the present study was to evaluate the effects of fish oil replacement with canola oil on nutritive value of fish for human consumption.

Materials and methods

Rainbow trout fingerlings with a mean initial body weight of 16.5±0.5 g were purchased from Cheshmeh Dimeh fish hatchery (Shahre kord, Chaharmahal and Bakhtiari, Iran) and used in this study.

Three iso-nitrogenous, iso-calorific and iso-lipidic purified experimental diets were formulated from 100% fish oil (FO), 100% canola oil (CO) and 1:1 blends of the oils (FCO). Diets were prepared and stored according to Abery *et al.*, (2002) [1] and De Silva *et al.*, (2002) [9].

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) with a mean pH of 7.3±0.2 and levels of ammonia and nitrate below 0.1 mg L^{-1}.

270 individually weighed and measured fingerlings were randomly distributed into the tanks (30 fish per tank) and randomly assigned to one of the 3 different experimental diets (3 replicates for each experimental diets). 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 fillet fatty acid profile analysis.

Fish allocated for fatty acid analysis were filleted (denuded of skin and bone) and stored at −80°C until analyzed for fatty acids. Fatty acid analysis was carried out on each of the added dietary oils, experimental diets, fillet sample from initial and each of the replicates at the end of experiment. 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 [8]. FAMEs were purified by TLC using hexane/diethyl ether/acetic acid (85:15:15 v/v/v) as solvent [27]. Separation of FAMEs was carried out in a Gas Chromatograph system (Agilent Technologies, 6890N, 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).

Mean values and standard deviation for each fatty acid were calculated first. The results were subjected to a one-way ANOVA to test the effect of the replacement of vegetable oil on fatty acid profile. 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

Fatty acids composition of the oils, experimental diets, fillet sample from initial and each of the replicates at the end of experiment and correlation between dietary fatty acids concentrations and fatty acids concentrations in fillet are presented in Table 1.

Table 1. Fatty acids composition (percentage of total fatty acids) of the oils, experimental diets and rainbow trout reared on the experimental diets and correlation between dietary fatty acids concentrations and fatty acids concentrations in fillet of rainbow trout fed the experimental diets for 8 weeks.

	Oils		Experimental diets			Initial	Rainbow Trout reared on the experimental diets (mean±SD)			correlation between dietary fatty acids concentrations and fatty acids concentrations in fillet	
	Fish Oil	Canola Oil	FOD	COD	FCOD		FOD	COD	FCOD	Correlation coefficient (*r*)	Slope
16:0	20.73	5.77	22.71	14.33	17.41	10.11	17.45±0.29^{a}	12.85±0.28^{c}	14.13±0.39^{b}	0.995	0.556
18:0	4.16	1.27	5.85	4.46	5.08	2.90	4.46±0.10^{a}	3.78±0.09^{b}	3.90±0.16^{b}	0.953	0.492
SFAs (others)	4.79	4.76	4.76	4.38	4.63	5.43	5.83	6.03	5.53	-	-
SFAs	29.68	11.80	33.32	23.17	27.12	18.44	27.74±0.53^{a}	22.66±0.29^{c}	23.56±0.45^{b}	0.974	0.516
16:1n-7	5.24	0.34	4.92	2.54	3.61	1.77	4.49±0.09^{a}	2.46±0.20^{c}	3.20±0.13^{b}	-	-
18:1n-9	33.57	77.33	38.79	59.87	49.63	61.25	44.85±0.45^{c}	58.82±0.56^{a}	53.91±0.33^{b}	0.988	0.664
MUFAs (others)	5.71	0.24	4.88	2.16	3.37	1.89	3.65	2.00	2.62	-	-
MUFAs	44.52	77.91	48.59	64.57	56.61	64.88	52.99±0.38^{c}	63.28±0.24^{a}	59.73±0.17^{b}	0.985	0.644
18:2n-6	0.37	1.01	0.42	0.70	0.47	0.71	0.41±0.05^{c}	0.78±0.06^{a}	0.54±0.03^{b}	0.985	1.274
18:3n-6	0.05	0.38	0.04	0.21	0.14	0.05	0.06±0.00^{c}	0.17±0.01^{a}	0.11±0.01^{b}	-	-
20:2n-6	2.48	0.14	1.21	0.29	0.82	0.88	0.89±0.06^{a}	0.46±0.05^{c}	0.60±0.06^{b}	-	-
20:3n-6	0.18	nd	0.20	0.22	0.28	0.13	0.09±0.01^{b}	0.12±0.01^{a}	0.12±0.02^{a}	-	-
20:4n-6	0.02	nd	0.06	0.04	0.04	0.86	0.13±0.03	0.17±0.05	0.17±0.01	0.971	-1.750
PUFAs n-6 (others)	1.08	nd	0.95	0.56	0.90	0.76	1.01	0.93	0.98	-	-
PUFAs n-6	4.18	1.53	2.88	2.03	2.65	3.40	2.60±0.24	2.63±0.17	2.52±0.13	0.502	-0.065
18:3n-3	2.07	7.05	4.58	4.87	3.95	4.27	1.94±0.07^{c}	3.23±0.25^{a}	2.76±0.03^{b}	0.157	0.218
18:4n-3	0.32	1.71	0.60	1.25	0.87	2.61	1.04±0.04^{b}	1.75±0.32^{a}	1.48±0.14^{a}	-	-
20:3n-3	0.05	nd	0.61	0.04	0.07	0.53	0.30±0.03^{b}	0.62±0.07^{a}	0.40±0.05^{b}	-	-
20:5n-3	5.90	nd	2.95	0.96	2.29	0.87	2.08±0.10^{a}	0.59±0.04^{c}	1.17±0.11^{b}	0.949	0.704
22:6n-3	12.82	nd	6.65	2.91	5.82	4.76	10.73±0.40^{a}	5.04±0.19^{c}	8.00±0.32^{b}	0.959	1.390
PUFAs n-3 (others)	0.48	nd	0.36	0.21	0.32	0.25	0.59±0.05^{a}	0.21±0.02^{c}	0.38±0.02^{b}	-	-
PUFAs n-3	21.62	8.76	15.75	10.24	13.32	13.28	16.67±0.52^{a}	11.43±0.04^{c}	14.19±0.45^{b}	0.999	0.949
HUFAs n-3	18.72	nd	9.60	3.87	8.11	5.63	12.80±0.45^{a}	5.63±0.22^{c}	9.18±0.43^{b}	-	-
PUFAs	-	-	-	-	-	16.68	19.27±0.66^{a}	14.06±0.17^{c}	16.71±0.58^{b}	0.996	0.811
PUFAs/SFAs	-	-	-	-	-	0.90	0.69±0.04^{a}	0.62±0.02^{b}	0.71±0.04^{a}	-	-
n-6/n-3	-	-	-	-	-	0.26	0.16±0.02^{b}	0.23±0.02^{a}	0.18±0.01^{b}	-	-

nd: not detected, FOD: fish oil diet, COD: canola oil diet, FCOD: fish and canola oils diet.
Values in the same row with the same superscripts are not significantly different (P>0.05).

The fish oil diet (FOD) contained the highest level of SFAs (33.3%) predominantly in the form of palmitic (16:0) and stearic acids (18:0) which accounted for (22.7%) and (5.9%), respectively. Monounsaturated fatty acids (MUFAs) concentrations were highest in the canola oil diet (COD) (64.6%), represented mainly as oleic acid (18:1n-9, 59.9%). The FOD was richest in PUFAs (n-6+n-3) (18.6%) with α-linolenic acid (18:3n-3, 4.6%) and linoleic acid (18:2n-6, 0.4%) as the principal fatty acids. The highest levels of EPA and DHA were in the FOD, with 3%

and 6.7%, respectively. Fatty acids of the n-3 and n-6 series were observed in highest concentration in the FOD, which accounted for (15.8%) and (2.9%), respectively. Levels of HUFAs n-3 were found in highest concentrations in the FOD with 9.6%.

Individual fatty acids found in the highest concentration across the major fatty acid classes (SFAs, MUFAs and PUFAs) were palmitic, oleic, α-linolenic acids along with DHA, respectively. The level of SFAs was observed in higher (P<0.05) concentrations for fish fed with FOD compared to fish fed with COD and FCOD. Levels of MUFAs ranged from 53±0.4 (FOD) to 63.3±0.2 (COD) and were observed to be significantly higher in fish fed the COD. The fillet of fish fed with the COD were particularly rich with oleic acid (58.8±0.9%) and α-linolenic acid (3.2±0.3%). 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 across all of the dietary treatments, ranging from 5±0.2% (COD) to 10.7±0.4% (FOD). Level of n-3 fatty acids were higher in the fillet than the diet for each of the treatments, but level of n-6 fatty acids were higher in the fillet than the diet only for treatment 2 (COD), with n-6/n-3 ratios ranging from 0.16±0.02 to 0.23±0.02 in the fillet. The highest HUFAs n-3 concentrations (P<0.05) were found in fish fed the FOD (12.8±0.5%), while the lowest amount was observed in fish fed the COD (5.6±0.2%).

Regression analysis was used to identify dose response relationship between dietary and fillet fatty acids. As reported in Table 1, most of the individual dietary fatty acids were linearly correlated to their concentrations in fillet of rainbow trout.

Discussion and Conclusion

The results of the present study suggest that canola oil can be used to replace fish oil without adverse effects on nutritional value of fish for human consumption. In agreement with previous studies [7, 11, 23, 31], considerable differences were evident in the fatty acid composition of rainbow trout fed different lipid sources. There was a prominent increase in the levels of linoleic and α-linolenic acids in all treatments where fish were fed either with COD and/or FCOD. As reported previously in other experiments [5, 12, 29, 30, 31], high correlations for individual fatty

acids as well as MUFAs and PUFAs were observed between the diets and fillets of fingerlings of rainbow trout. There was, however, high correlation between the amount of SFAs in the diet and SFAs in the fillet, which was not in according of the findings of Turchini *et al.* (2003a,b) who postulated that SFAs were not used efficiently by Murray cod *Maccullochella peelii peelii* (Mitchell) 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 [12, 16, 19, 20, 23, 28]. In comparison to marine fish species, freshwater fish are also generally better adapted to desaturate and elongate these base fatty acids to higher homologs [12, 28]. 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. 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 COD and FCOD. These fatty acids were found in much lower concentrations in the diets. Likewise, fish fed the COD and FCOD 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, the 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 (HMSO, 1994) recommends a minimum PUFAs/SFAs ratio of 0.45, and a maximum n-6/n-3 of 4.0. Table 1 shows that the fingerlings of all treatments met the PUFAs/SFAs and n-6/n-3 ratios. Despite the decrease in EPA and DHA in fillet from fish fed FCOD, the trout fillets contained a relatively rich source of these fatty acids with a 200 g serving of the fillets from fish fed FCOD supplying 704 mg of EPA plus DHA. 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 [26].

The results of this study showed that, the substitution of fish oil with canola oil in rainbow trout diet have been possible without any negative effects on nutritional value of fish for human consumption. However, the reflection of the dietary oil source on the fillet fatty acid composition of

the fingerlings of rainbow trout 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.

References

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

Baccarelli A, Mocarelli P, Patterson DG, Bonzini M, Pesatori AD, Caporaso N et al (2002) Immunologic effects of dioxin: new results from seveso 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) Rapeseed 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.

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.

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

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

Fritsche KL, Johnston PV (1990) Effect of dietary a-linolenic acid on growth, metastasis, fatty acid profile and prostaglandin production of two marine mammary 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 (*Pagrus auratus*). *Aquacult Nutr* 9:305–315.

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 charr (*Salvelinus fontinalis*). *Aquaculture* 136:351–362.

Henderson RJ, Tocher DR (1987) The lipid composition and biochemistry of freshwater fish. *Prog Lipid Res* 26:281–347.

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 (2003) Dietary lipid sources for sea bream and sea bass: growth performance, tissue composition and flesh quality. *Aquacult Nutr* 9:397–407.

Jacobs MN, Ferrario J, Byrne C (2002a) Investigation of polychlorinated dibenzo-p-dioxins dibenzo-p-furans 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 persistant 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 SI, 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 KF (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.

Martino RC, Cyrino JEP, Portz L, Trugo LC (2002) Performance and fatty acid composition of surubim (*Pseudoplatystoma coruscans*) fed diets with animal and plant lipids. *Aquaculture* 209:233–246.

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.

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.

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 retinas. *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 O (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 peelii peelii*). *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* 225:251–267.

Van Den Heuvel RL, Koppen 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.