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Preliminary Study on the Partial Substitution of Fish Oil with Amaranth Oil in diets for Rainbow trout (*Oncorhynchus mykiss*) Fingerlings: Effects on Body Composition and Fatty Acids Contents

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Abstract

The effect of the substitution of fish oil with amaranth oil in fish feed on body composition was studied in rainbow trout *Oncorhynchus mykiss*. Two experimental feeds, containing 7.2% and 5.0% of amaranth oil were used. The experiment was conducted in triplicate for each treatment (n=50). Initial body wet weight of experimental fish was 7.4±0.1 g. Feed was offered as 2.5% of fish biomass of respective group for 30 days. Chemical body composition and fatty acids composition were determined. No significant differences were found between the experimental groups and the control one. The highest specific growth rate (3.75%/day) was found in fish fed with feed containing the highest level of amaranth oil. The content of crude protein and crude fat in fish flesh was also highest in this group, 16.3% and 10.5% of body weight respectively. Fatty acids composition of fish fat was similar, however, higher content of amaranth oil in feed resulted in visible decrease of eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA) in fish fat.

Our results indicate that amaranth oil can be a substitution of fish oil in feeds for rainbow trout, however, the study period of the experiment was relatively short and the fish number in experimental groups was rather limited. Hence, the results of the present trial might be considered as a preliminary study.

Keywords: *Amaranthus cruentus*, *Oncorhynchus mykiss*, fat composition, vegetable oil, fish feed.

Introduction

The supply of fish meal and oil has shown downward trend for many years due to overfishing. This triggered the need for research on the substitution, even partial, of fish components by vegetable products in feeds for fish (Bell *et al.*, 2002; Bell and Sargent, 2003; Figueiredo- Silva *et al.*, 2005; Lin and Shiau, 2007; Piedecausa *et al.*, 2007). Moreover, both vegetable proteins and oils are usually less expensive compared to fish ones. Many authors studied this problem in rainbow trout (Higgs *et al.*, 1995; Mwachireya *et al.*, 1999; Sargent and Tacon, 1999; Caballero *et al.*, 2002; New and Wijkstrom, 2002 and many others).

Great nutritional value of amaranth (*Amaranthus cruentus*) results from beneficial chemical composition of its seeds and leafs. The main advantage of this plant is high protein content seed, rich in exogenous amino acids (sulphur rich amino acids and lysine above all) (Gorinstein *et al.*, 2001). Another important amaranth advantage is high fat content when compared to other corn plants (6.5÷10.9% of grain) (Mlakar *et al.*, 2010).

Unsaturated fatty acids (UFA) are the main amaranth fat components. Linoleic, oleic, linolenic and arachidonic ones show the highest concentrations. Saturated fatty acids (SFA), mainly palmitic and stearic, are present in lower amounts. According to Prahasha and Pala (1992) the sum of UFA in amaranth oil ranges between 67 and 80%. Important component of lipid fraction of amaranth seeds are tocopherols, tocotrienols and phytosterols, present in concentrations from 0.22 to 0.36 mg g^{-1} (Mendonça *et al.*, 2009).

The aim of the present study was the assessment of the effect of feeds containing amaranth oil on biochemical composition of fish body, with special emphasis to fatty acid content. The influence of the substitution on fish growth was also studied.

Materials and Methods

Animals, Feeds and Experimental Procedures

Rainbow trout with initial mean body weight of 7.4 (±0.1) g were used in the experiment. Before the experiment, 20 randomly chosen specimens (initial

control group, IC) were caught, immediately anaesthetized with MS-222 (300 mg dm^{-3}), killed by brain destruction with sharp scissors, measured and frozen in -21°C for further analysis.

Fish were fed with 3 experimental diets containing different levels of lipid components: amaranth oil, fish oil and soybean oil. Experimental feeds were modifications of the commercial feed Saphir, which was supplied by Aller Aqua Polska as an intermediate product pellets before oil supplementation (base feed). Oils were added in the laboratory in proportions different for each experimental feed (Table 1) according to following procedure: sample weight of base feed was placed in the incubator and heated to 40°C, sample weight of oils mixture was added slowly and gently mixed. Then, feed "rested" in room temperature until oils were completely absorbed and then stored in refrigerator. New batch of each experimental feed was prepared every week of experiment.

The experiment was done in triplicates. Trout (n=450) were randomly placed in flow through tanks (350 L of volume). Depending on the group, fish were fed with experimental feeds (EF1 or EF2) or control feed (CF). Feed was offered with 2.5% of fish biomass of respective group. Daily dose of feed was divided into four portions and fish were fed manually each 4 hours between 8:00 and 20:00. Fish were sampled after 30 days of feeding. Body length and weight were measured. Specific growth rate (SGR) was calculated as follow:

$$SGR = 100(\ln W_2 - \ln W_1) / t$$

Where, W_1: mean initial body weight (g), W_2: mean final body weight (g), t: number of days of feeding. Feed conversion ratio (FCR) was calculated according to the formula:

$$FCR = F/G$$

Where, F: food consumption, G: realized weight gain.

Experimental tanks were supplied with laboratory tap water (0.5 L min^{-1}) and mechanically aerated. Water parameters were monitored every day morning before feeding. All experimental tanks were cleaned twice a day. Mean (±SD) water temperature was 11.3±1.2°C and pH 7.48±0.1. Oxygen saturation was above 80% throughout the period of the experiment.

Results were analysed with Statistica 9.0 software. Normality of data distribution was tested by Shapiro-Wilk test and variance homogeneity by Leven's test. Differences between means were analysed using ANOVA and Tuckey's test at significance level $P<0.05$.

Table 1. Composition of experimental feeds (%)

Component	Experimental feed		Control feed
	EF 1	EF2	
Fish meal	43.0	43.0	43.0
Soy flour	22.0	22.0	22.0
Wheat flour	16.0	16.0	16.0
Amaranth oil	7.2	5.0	0.0
Soybean oil	5.0	5.0	5.0
Fish oil	2.8	5.0	10.0
Rape oil cake	4.0	4.0	4.0
Gross energy (kJ)	16,813	16,836	16,884

Analysis of the Chemical Body Composition

All sampled fish were anaesthetized with MS-222 (300 mg dm^{-3}) and killed by medulla destruction with sharp scissors, grounded and analysed.

The contents of the basic chemical components (dry matter, crude protein, crude fat, crude ash) in feed and muscles of the experimental fish were determined with the standard methods (AOAC, 1996). Dry matter was determined by drying in an oven at 105°C for 24 h. Total protein was determined by Kjeldahl's method and crude fat by Soxhlet's method. Lipids were extracted from the sample in anhydrous ethyl ether, crude ash by combustion at 550°C in a muffle furnace for 24 h.

The fatty acid composition of fish tissue, experimental feeds and amaranth oil was determined by gas chromatography. Before analysis, the methylation of fatty acids in a sample was done by Peisker method; 1.5 cm^3 of the mixture of methanol-chlorophorm-sulfuric acid (100:100:1) was added to 50 mg of a fat sample and heated in boiling water bath (Christie, 1993; Jankowska *et al.*, 2005).

Results

Fish Growth

Mean final weight of fish was 18.9, 22.8 and 21.8 g and SGR was 3.12, 3.75 and 3.62% day^{-1} in CF, EF1 and EF2 groups respectively. FCR was 0.87, 0.71 and 0.78 in CF, EF1 and EF2 groups respectively. Results were not significantly different ($P>0.05$).

Amaranth Oil Composition

Diunsaturated fatty acids had the highest (48.02%) share in amaranth oil (Table 2). The share of mono unsaturated fatty acids (MUFA) was 26.6% and saturated ones was 24.43% including 19.46% of palmitic acid (C16:0). The percentage of tri unsaturated fatty acids was as low as 0.88%.

Feeds Composition

The contents of main components in all experimental feeds were similar. Crude protein content ranged between 46.2 and 46.9%, crude fat

19.7 and 20.0%, dry matter 92.9–93.8%, crude ash 7.47–7.52 and crude fibre 1.49–1.96 %.

The share of saturated fatty acids was also similar in all experimental feeds and ranged between 21.8 and 23.0%. C16:0 was dominant and its highest content was found in EF1 feed.

The highest content of MUFA was determined in control diet group (39.89%) and oleic acid (C18:1) prevailed in this feed (Table 3). The highest share of PUFA was found in EF1 feed (43.7%). In this feed linoleic acid (C18:2) content was as high as 31.4%, being 2 fold when compared to control feed. The lowest content of n-3 PUFA was determined in EF1 feed (11.4%) and the highest in CF feed (20.6%). Opposite relationship was found for n-6 PUFA where EF1 content was 31.9% and CF 17.2%. C20:5n-3, docosapenatenoic acid (DPA; C22:5; n-3) and C22:6n-3 were determined in CF feed as 6.7, 0.58 and 7.56 % respectively.

Table 2. Fatty acids composition of amaranth oil used for experimental feeds supplementation

Fatty acid	% of total FA
C16:0	19.46
C18:0	3.85
C18:1	26.32
C18:2	48.02
C18:3	0.88
C20:0	0.78
others put together	0.69

Table 3. Fatty acids content in the control and experimental feeds (%). CF – commercial feed; EF1, EF2 – feeds supplemented with amaranth oil

Fatty acid	CF	EF1	EF2
C14:0	3.98	2.31	2.66
C16:0	14.03	14.03	15.06
C18:0	2.81	2.81	3.16
Other SFA	1.02	1.02	1.03
Total SFA	21.84	21.84	21.91
C16:1	7.07	7.07	1.61
C18:1	27.92	27.92	26.95
C20:1 n-9	1.71	1.71	1.60
C22:1 n-11	1.44	1.46	1.44
Other MUFA	1.75	1.22	1.20
Total MUFA	39.89	33.25	34.00
C18:2 n-6	16.26	31.43	26.67
C18:3 n-3	3.41	3.05	3.24
C18:4	1.69	0.87	1.25
C20:5 n-3	6.74	3.00	4.36
C22:6 n-3	7.61	3.91	5.25
Other PUFA	3.70	2.15	3.19
Total PUFA	38.29	43.75	42.71
n-3/n6 ratio	0.91	3.16	2.08

Fish Tissue Composition

Figure 1 summarises results achieved for fish tissue composition. Crude protein level was between 15.6 and 16.3% for IC and EF1 groups respectively. The lowest crude lipid content (9.1%) was found in fish before experimental feeding (IC) while the highest level of lipid content (10.5%) was found in fish tissues in the EF1 treatment group. Crude ash ranged between 1.8 to 2.0% in CF and EF2 groups , respectively.

Fish fat composition analysis (Table 4) showed that MUFA were the most prevalent fatty acids in all experimental groups, however, MUFA highest content (43.1 %) was found in IC fish. The highest content of SFA was determined in EF1 fish (22.35%). These fish tissues had also the highest content of PUFA (37.5%) also. The highest n-3 FA level was found in fish of the IC group (23.65%)". The share of C18:2n-3 in EF1 fish (17.77%) was 2 fold of this in IC group. The highest n-3/n-6 ratio was found in IC group (Table 4).

The highest content of C20:5n-3, C22:5n-3 and C22:6n-3 was found in IC fish, 5.54, 1.52 and 13.16% respectively. Visibly lower level of these FA was found in all experimental fish when compared to IC fish (Figure 2).

Discussion

The substitution of fish oil with amaranth oil in diets for rainbow trout did not show any negative effect on fish growth rate. SGR found in the experimental fish were similar to those reported by Okumuş and Mazlum (2002) for respective time

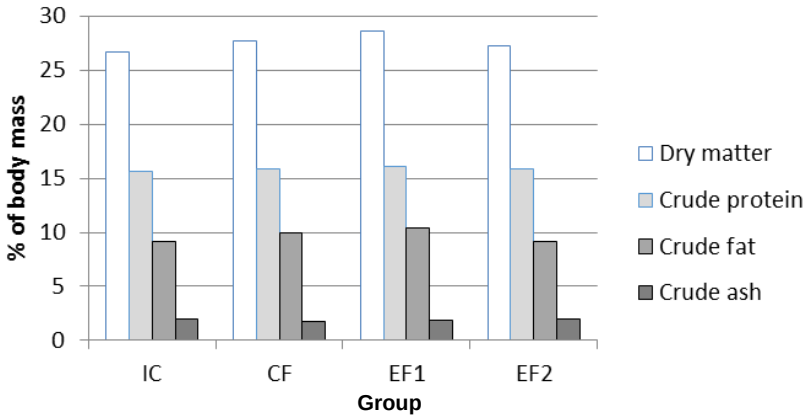

Figure 1. The composition of the body of experimental fish.

Table 4. Fatty acids content in fish meat (%)

Fatty acid	IC	CF	EF1	EF2
C14:04	4.9	3.95	3.51	4.01
C16:0	13.29	14.14	15.03	14.72
C18:0	2.48	2.92	3.08	2.82
Other SFA	0.12	0.76	0.73	0.79
Total SFA	21.49	21.75	22.35	22.34
C16:1	6.52	6.64	4.97	5.52
C18:1	25.24	27.25	28.08	27.15
C20:1 n-9	5.02	2.95	3.28	3.26
C22:1 n-11	3.88	2.33	2.31	3.05
Other MFA	2.40	2.27	1.71	2.16
Total MUFA	43.06	41.44	40.15	41.14
C18:2 n-6	7.20	11.66	17.77	15.57
C18:3 n-3	2.16	2.37	2.07	2.14
C18:4	1.99	1.49	1.20	1.36
C20:4 n-3	1.10	0.88	0.71	0.76
C20:5 n-3	5.54	4.57	3.10	3.52
C22:5 n-3	1.52	1.19	0.84	0.91
C22:6 n-3	13.16	11.93	9.09	9.46
Other PUFA	2.80	2.07	2.33	2.27
Total PUFA	35.47	36.79	37.50	36.52
n-3/n-6 ratio	3.26	1.80	0.89	1.08

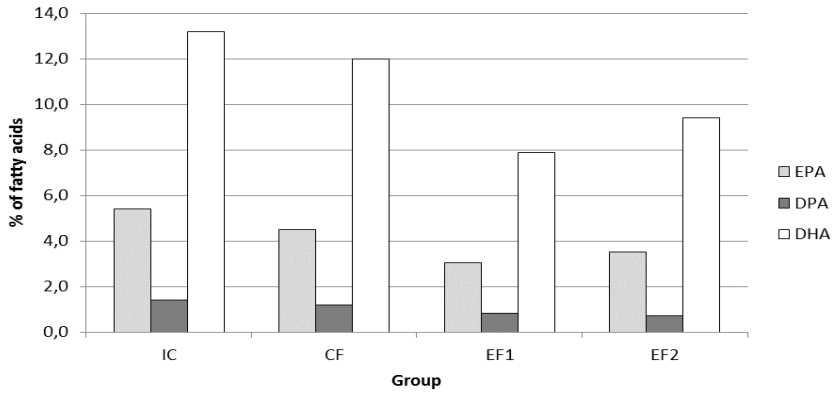

Figure 2. Eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) content in the fat of the experimental fish.

period.

PUFA are among biologically active nutrients remarkably influencing human health. PUFA, belonging n-6 and n-3 families, known also as indispensable fatty acids, includes: C18:2n-6, C18:3n-3 (α linolenic acid), C20:4n-6 (arachidonic acid), C20:5n-3 and C22:6n-3. C18:2n-6 and C18:3n-3 FA are not synthesized *de novo* in humans and most of animals and thus have to be provided in food (Lall *et al.*, 2002). FA are known as components influencing structure and permeability of cell membranes. Beside structural function, PUFA play important role in many biochemical processes (Hornstra, 2000; Sargent *et al.*, 2002; Simopoulos, 2002; Uauy and Dangour, 2006).

PUFA are synthesized only in plants (mainly n-6 ones) and marine algae (n-3). Oils contained in corns or fruits of oleaginous plants (sunflower, soybean, rape, olives) are good source of C18:2n-6 (Bell and Sargent, 2003; Schulz *et al*., 2005). Green plants oils contain much more. There is almost no long-chain PUFA belonging to n-3 family in plants oils. Marine algae are able to transform C18:2n-6 to C18:3n-3 and synthesize long-chain n-3 PUFA (Zhukova and Aizdaicher, 1995). Amaranth oil is a rich source of these FA.

Biochemical composition of fish fat is strongly determined by food (Haard, 1992; Shearer, 1994; Steffens and Wirth, 2005). In culture conditions, body composition reflects the type and amount of feed. In nature, the trophy of water body and the season of the year influence tissue composition through food availability (Woźniak and Gomułka, 2008).

The meat of wild fish contains less dry matter, fat and minerals in compare to cultured ones (Shearer, 1994; Haard, 1992). Crude fat is the most variable component in fish dependent on the species, body weight, season of the year and food type (Koskela *et*

al., 1998). The addition of amaranth oil to the experimental feeds did not effect on the level of crude protein and crude fat in experimental trout body (Figure 1). Vranić *et al.* (2011) reported, in their review, that crude protein ranged between 13.7 and 21.2% and crude fat between 2.94 and 9.07% for rainbow trout of different origin. In our experiment, the content of crude protein was similar to mean values given by these authors, but the fat level was close to the upper limit. However, Valente *et al.* (2001) reported similar values to our results for both crude fat and crude protein for rainbow trout fingerlings fed with commercial trout feed.

The highest content of C18:2n-6 in feed supplemented with the highest dose of amaranth oil (EF1) is worth to be noted. This feed contained also the highest content of PUFA and linoleic acid. The PUFA content ranged between 33.47 and 43.75% in experimental fish. Similar results was reported by Bieniarz *et al.* (2000) in freshwater predatory fish like rainbow trout (32.38–36.86%), European catfish (23.36–36.45%) and pike (29.79–44.07%). PUFA level found in these species (30-40%) was similar to many marine fish (Osman *et al.*, 2001).

The content of C20:5n-3 and C22:6n-3 has a great influence on nutritional value of fish meat. There is no of these fatty acids in the meat of other animals (Lall *et al.*, 2002). Similar content of C20:5n-3 (4.36–5.24%) and higher levels of DHA (12.07–16.61%) were found in farm trout when compared to our results (Bieniarz *et al.*, 2000). Much more higher EPA (10.3%) and similar DHA (11.52%) levels were found in cultured Atlantic salmon. Nutritional value of fish meat is characterized also by the n-3/n-6 ratio. In marine fish the ratio ranged between 4.7 and 14.4 while in freshwater fish it is between 0.5 to 3.8 (Kołakowska *et al.*, 2000). In the present study, n-3/n-6 ratios were found between 0.89 to 3.26. Similar value was reported by Bieniarz *et al.* (2000) in trout and European catfish, 2.48 and 2.39, respectively. Jankowska *et al.* (2003) found n-3/n-6 ratio as high as 3.25 and 4.4 in cultured and wild pikeperch respectively. Simopolus (1991) reported higher levels of 10.0 and 2.2 for wild and cultured trout, respectively.

Conclusions

Our results indicate that amaranth oil can be a substitution of fish oil in feeds for rainbow trout. Nutritional value of experimental trout meat (in means of fat composition and FA ratio) was not affected. Moreover, experimental feeds containing amaranth oil resulted in slightly higher growth rate. Our results seems to be very promising, however, one has to take into account that the experiment was relatively short and the fish number in experimental groups was rather limited. Thus our results should be considered as a preliminary study.

Long term studies on substitution of fish oil by amaranth oil in trout diets in terms of their effects on fatty acid composition or fish growth performance are encouraged.

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