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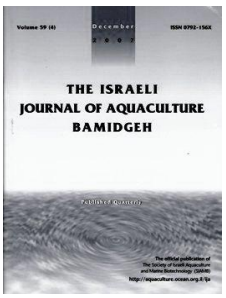

Effect of Dietary Fat Replacement on Body Composition of Intensively Reared Hybrids of Pikeperch and Volga Pikeperch

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Abstract

Juvenile hybrids (9.43±0.09 g) of pikeperch (*Sander lucioperca* ♀) and Volga pikeperch (*S. volgensis* ♂) were reared in a recirculation system for 54 days. Fish were fed one of four experimental diets in which the principal source of lipid was fish oil, rapeseed oil, sunflower oil, or soy oil (5%), added to a basal diet (fat content 5.9%) and fed at a ratio of 2% of the body weight. The specific growth rate was moderate (0.47-0.66%/day) and did not differ between groups. Body composition traits (dry matter, crude protein, crude ash, crude fat, fillet fat content) were not affected by the different diets. However, the accumulation of oleic acid (C18:1 n-9) was significantly highest in the rapeseed group, and sunflower oil resulted in the highest proportion of linoleic acid (C18:2 n-6). Among the n-3 polyunsaturated fatty acids, the α-linolenic acid (C18:3 n-3) content in the fillet of fish fed the diet containing rapeseed oil was significantly higher than in the fillet of animals fed the diets with fish or sunflower oil and the eicosapentaenoic acid (C20:5 n-3) content in the sunflower group was significantly lower than in the fish or rapeseed groups. Thus, some of the fatty acid muscle contents were influenced by their concentration in the feed.

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Introduction

Inter-specific hybrid fishes have been produced for aquaculture and stocking programs to increase growth rate and combine the desirable traits of two species into a single group. Pike perch (*Sander lucioperca*) has been chosen for diversification of European inland aquaculture (Hamza et al., 2008). A close relative from the same genus is the Volga pikeperch (*S. volgensis*). Although the growth of the Volga pikeperch is slower than that of the pikeperch (Specziár, 2005), it is less sensitive to stress during seining, grading, and transport. Thus, an inter-specific hybrid could be a more stress-tolerant genotype than pikeperch. Hybridization of *S. lucioperca and S. volgensis* is rare in nature (Müller et al., 2010) due to their different spawning times and strategies (Specziár et al., 2009). However, out-of-season, hormonally-induced gamete production enables the crossing of female pikeperch with male Volga pikeperch (Müller et al., 2006). Pikeperch (Schulz et al., 2005; Molnár et al., 2006) and Volga pikeperch (Bercsényi et al., 2001; Szabó et al., 2006) can be reared in recirculation systems and fed artificial diets (Müller et al., 2011).

The feed requirements and body compositions of pikeperch (Nyina-Wamwiza et al., 2005; Schulz et al., 2005; Molnár et al., 2006; Kamaszewski et al., 2010) and Volga pikeperch (Szabó et al., 2006) are known. Fish oil can be totally or partially replaced in diets for pikeperch (Schulz et al., 2005; Molnár et al., 2006; Kowalska et al., 2010). Vegetable oils, although suitable for fish, can significantly influence characteristics such as fatty acid composition and product quality. The influence of dietary fat on the fatty acid composition of new species or hybrids should be determined. In this experiment, the effect of fat source (animal vs. vegetable) on production traits, whole body and fillet fat content, and composition of the inter-specific hybrid of the two *Sander* species was evaluated.

Materials and Methods

Experimental fish. Hybrids were produced by artificial propagation (*S. lucioperca* ♀ × *S. volgensis* ♂) as described by Müller et al. (2006). Siblings were grown in one 400-l aquarium and fed live *Paramecium*, *Artemia*, and *Chironomus* for two months until they reached 30-40 mm in body length. The hybrids were gradually weaned to formulated feed (Nutra 2.0, extruded, crumbled trout) with *Chironomus* as a live food supplement for 2 weeks, then only Nutra 2.0 for 3 weeks, and Classic Marine 1st P marine grower pellets thereafter. The initial body weight and standard length were 9.43±0.09 g and 104±3.0 mm (means±SD, n = 72), respectively, and the mean condition factor was 0.68±0.02.

Culture facilities. The rearing unit contained thirty 70-l (33 x 30 x 60 cm) aerated plastic tanks integrated into a 2450-l recirculation system attached to a simple biofilter unit and a settling tank from which water was pumped back to the fish tanks. Water was replaced a daily rate of about 10% of the total volume. The water flow rate was adjusted to 1.5 l/min and the temperature was 18.4±1.1°C. During the experiment, the photoperiod was 12 light hours with a low intensity of 7-10 lux at the water surface and 12 hours of total darkness. The stocking density was 0.8 g/l.

Feeding. Four experimental diets with the same crude fat and crude protein contents were prepared (Table 1). These were supplemented with 60 g/kg fish oil, rapeseed oil, sunflower oil, or soybean oil and pelleted to 3 mm. The composition of the diets was analyzed according to procedures of the Hungarian Standard (MSZ). Crude protein was determined by the Kjeldahl method after acid digestion (MSZ 6830/4) while crude fat was measured according to the Soxhlet method (MSZ 6830/19). Seventy-two hybrids were randomly distributed into 12 tanks in the recirculation system, and each tank was stocked with six fish. The diets were randomly allocated in triplicate and offered from automatic belt feeders (FIAP GmbH, Germany) for 10 h/day (08:00 to 18:00), at a ratio of 20 g/kg of the fish body weight. The feeding trial lasted 54 days.

Measurements. At the beginning and end of the experiment, standard body length (±1 mm) and body weight (±0.01 g) of the fish were measured individually. Growth was quantified as specific growth rate (SGR) = [ln(final wt) - ln(initial wt)]/days x 100 and

Table 1. Chemical and fatty acid composition of the experimental diets.

	Diet				
	Basal	*Fish*	*Rape-seed*	*Sun-flower*	*Soy-bean*
Chemical composition (g/kg)					
Dry matter	891.9	898.4	897.8	894.4	898.2
Crude protein	419.4	399.0	399.4	406.3	409.8
Crude fat	59.3	107.0	102.3	106.9	107.9
Crude fiber	20.4	19.0	19.9	20.8	18.1
Crude ash	93.0	87.7	90.0	90.4	89.4
Fatty acid (% total fatty acids by wt)					
C10:0	0.2	0.2	0.1	0	0.1
C12:0	0.5	0.6	0.3	0.3	0.3
C14:0	24.4	44.5	15.5	13.9	14.2
C14:1	0.2	0.3	0.1	0.1	0.1
C15:0	2.3	3.2	1.5	1.3	1.3
C16:0	166.6	172.3	112.9	111.3	130.8
C16:1 n-7	37.4	59.6	23.2	20.8	21.5
C17:0	3.2	3.9	2.2	1.9	2.2
C17:1	5.6	10.5	3.3	2.9	3.1
C18:0	50.4	44.3	33.8	43.7	46.8
C18:1 n-9c	156.6	122.7	333.9	178.1	195.1
C18:1 n-7	24.6	27.5	29.4	14.8	19.6
C18:2 n-6t	1.1	1.5	0.7	0.8	0.6
C18:2 n-6c	222.2	115.3	200.5	427.5	352.0
C18:3 n-6	1.0	2.1	0.6	0.6	0.6
C18:3 n-3	22.2	15.6	47.4	13.6	47.0
C20:0	2.6	2.4	4.4	2.8	3.9
C20:1 n-9	20.7	16.0	22.6	11.2	12.0
C20:2	3.3	4.5	2.4	1.7	2.0
C20:3 n-3	1.3	1.8	0.9	0.7	0.7
C20:3 n-6	1.7	1.8	1.1	0.9	1.0
C20:4 n-6	9.2	11.4	5.7	5.0	4.9
C20:5 n-3	97.0	182.2	63.3	56.6	56.2
C22:0	2.9	2.3	3.4	5.5	4.4
C22:1 n-9	3.2	2.6	3.2	1.6	1.9
C22:5 n-3	13.7	19.5	8.5	7.9	7.6
C22:6 n-3	114.5	123.4	70.7	66.5	61.9
C24:0	3.1	1.9	2.4	2.9	2.4
C24:1	8.3	6.1	5.9	5.0	5.8
Σ saturated	256.2	206.3	183.6	176.6	275.5
Σ monoenoic	256.6	259.1	234.5	421.5	245.2
Σ n-3	23.4	47.7	14.3	48.3	17.4
Σ n-6	226.1	354.2	429.8	202.9	120.7
n-6/n-3	9.65	7.42	30.09	4.20	6.94
Σ PUFA	487.1	534.4	581.8	401.8	479.1
Unsaturation index*	206.53	182.58	188.35	178.51	234.67

* Unsaturation index = (1 x Σ monoenoic) + (2 x Σ dienoic) + (3 x Σ trienoic)...

condition factor (CF) was calculated as W/L^3 x 100 where W means live weight and L means standard length.

Sampling and chemical analysis. At the end of the trial three fish from each treatment were dissected and approximately 3 g fillet samples were taken for analysis of fatty acid composition. Samples were immediately frozen to -70°C until analyzed. The remaining part of the body was homogenized and subjected to chemical body analysis. Dry matter (MSZ ISO 1442:2000), ash (MSZ ISO 936:2000), and crude protein (MSZ ISO 937:2002) of the fillet were determined. Total lipid content of muscle tissue was extracted according to Folch et al. (1957). Lipid extracts were converted to fatty acid methyl esters using BF3-methanol. Fatty acid methyl esters were separated and analyzed by gas chromatography in a Trace 2000 chromatograph (Thermo Finnigan Italia, SpA, Rodano, Italy) equipped with an Omegavax 320 capillary column (30 m length × 0.32 mm I.D., 0.25 µm film; Supelco, Bellefonte, USA). Individual fatty acids were identified using a standard mixture of fatty acid methyl esters (PUFA-2, catalogue no. 4-7015-U; Supelco, Bellefonte, USA).

Statistical analysis. Statistical analyses were carried out with SPSS® for Windows (Version 10, 1999). Analysis of variance (one-way ANOVA) was used to test the main effects of the treatments. Treatment means were compared using alpha = 0.05 for significance in Tukey's *post hoc* test. Data are given as means±SD. ANOVA was used on aquarium growth means.

Results

The specific growth rate of the hybrid fingerlings ranged 0.47-0.66%/day (Table 2). Differences between groups in final body weight and length were not significant. The condition factor and chemical composition of the fish body were similar in all treatments but the fatty acid composition of the fillets was influenced by the experimental diet. The proportion of oleic acid (C18:1 n-9) in the rapeseed group was significantly higher than in the other groups. Sunflower oil resulted in the highest proportion of linoleic acid (C18:2 n-6) in the fillet lipids. Among n-3 polyunsaturated fatty acids, the α-linolenic acid (C18:3 n-3) content in the fillet of fish fed the diet containing rapeseed oil was significantly higher than in the fillet of animals fed diets supplemented with fish or sunflower oil and the eicosapentaenoic acid (EPA; C20:5 n-3) content in the sunflower group was significantly lower than in the fish or rapeseed groups. The experimental diets did not have a significant effect on the total amount of saturated, monounsaturated, or

polyunsaturated fatty acids of the fillet, and the unsaturation index was similar in all groups, but the diets containing sunflower or soybean oil increased the total amount of n-6 polyunsaturated fatty acids.

Table 2. Growth, condition factor, total body chemical composition, and fillet fatty acid composition of hybrid pikeperch fed diets containing oils as lipid sources (means±SD, n = 3).

	Diet				*Significance*
	Fish oil	*Rapeseed oil*	*Sunflower oil*	*Soybean oil*	*(p)*
Final wt (g)	18	12.3±1.5	13.6±2.1	12.8±1.4	12.2±1.0
Final length (mm)	18	103±6.6	107±6.4	104±6.6	103±7.8
SGR (%/day)	3	0.47±0.22	0.66±0.28	0.57±0.20	0.48±0.15
Final condition factor	3	1.10±0.05	1.10±0.02	1.13±0.06	1.09±0.05
Chemical composition of body (g/kg)					
Dry matter	279.1±6.1	291.3±3.1	286.6±2.4	278.3±9.9	NS
Crude protein	181.8±7.3	178.3±1.4	179.7±1.7	174.0±2.9	NS
Crude ash	45.8±5.5	48.6±6.3	48.3±5.2	45.4±5.0	NS
Crude fat	63.6±6.0	70.1±3.9	71.6±6.9	68.0±5.6	NS
Fillet fat	19.0±1.8	18.7±2.6	19.8±0.8	19.5±3.1	NS
Fatty acid (% total fatty acids by wt)					
C14:0	36.6±1.4	32.4±5.6	32.8±1.5	32.9±4.2	NS
C16:0	204.0±11.7	195.1±9.8	197.5±0.8	208.5±17.8	NS
C16:1 n-7	60.7±6.5	48.1±3.5	50.0±0.6	51.4±8.5	NS
C18:0	34.7±1.9	35.6±2.4	35.6±0.8	37.9±9.0	NS
C18:1 n-9c	176.1±6.2^{a}	208.2±19.8^{b}	181.7±2.7^{a}	177.4±13.9^{a}	0.043
C18:1 n-7	20.8±0.7^{b}	19.9±1.0^{ab}	18.5±0.7^{a}	19.4±0.4^{ab}	0.017
C18:2 n-6t	112.9±1.0^{a}	109.1±7.0^{a}	154.4±4.8^{b}	133.6±11.2^{c}	0.001
C18:3 n-3	5.5±1.1^{a}	10.9±3.4^{b}	5.7±0.8^{a}	8.2±1.5^{ab}	0.033
C20:1 n-9	1.8±0.1	1.1±0.3	1.8±0.8	2.7±1.3	NS
C20:4 n-6	13.3±1.9	11.0±2.5	12.1±1.1	12.2±0.4	NS
C20:5 n-3	69.7±1.6^{a}	69.2±4.4^{a}	57.3±1.4^{b}	62.4±4.7^{ab}	0.012
C22:4 n-6	3.0±2.9	1.2±0.3	2.8±0.5	0.7±1.2	NS
C22:5 n-3	14.0±5.5	13.5±5.0	12.2±4.8	12.5±4.3	NS
C22:6 n-3	169.7±29.2	192.0±15.4	165.8±28.2	179.4±12.6	NS
Σ saturated	275.3±14.8	263.2±12.7	265.9±2.2	279.3±22.6	NS
Σ monoenoic	259.5±5.8	277.5±18.4	252.1±3.3	250.9±23.9	NS
Σ n-3	259.1±30.2	285.7±9.0	241.0±32.0	262.6±14.8	NS
Σ n-6	129.2±4.3^{a}	121.3±4.5^{a}	169.3±3.8^{b}	146.5±10.7^{c}	0.001
n-3/n-6	2.00±0.23^{bc}	2.35±0.14^{c}	1.42±0.21^{a}	1.80±0.22^{ab}	0.005
Σ PUFA	388.3±31.4	407.0±7.0	410.4±28.3	409.1±6.7	NS
Unsaturation index*	200.49±18.73	214.34±4.60	198.03±18.56	204.59±5.93	NS

NS = not significant (p>0.05)

* Unsaturation index = (1 x Σ monoenoic) + (2 x Σ dienoic) + (3 x Σ trienoic)...

Discussion

The composition of the experimental diets in the present study meets suggested requirements for other percid species (Xu and Kestemont, 2002; Nyina-Wamwiza et al., 2005). The minimal differences between groups showed no effect of the vegetable oil supplements. SGR was similar (Kowalska et al., 2010) or lower than reported for other percids fed vegetable oil supplementation (Xu and Kestemont, 2002; Schulz et al., 2005).

The fatty acid composition of diets with the same total fat content may affect the whole body composition of fish. Replacement of fish oil with rapeseed oil in diets for Atlantic salmon affected lipid and protein muscle contents (Bell et al., 2001). In the present work, the percent lipid in the hybrid muscle was almost identical in all groups, similar to pikeperch where the dietary fat source (6 and 12% fish oil and linseed oil) did not affect the whole body chemical composition (Molnár et al., 2006).

Fatty acid composition of muscle tissue lipids of marine and freshwater fish can be influenced by the fatty acid composition of the dietary lipids (Jankowska et al., 2003; Torstensen et al., 2004), as confirmed in the present study. Palmitic acid (C16:0) was the major saturated fatty acid in both the diets and the muscle tissue lipids, as in experiments with pikeperch and Volga pikeperch using similar diets (Molnár et al., 2006; Szabó, 2009). The oleic acid (C18:1 n-9) content of the fillet also reflected the dietary fatty acid profile. Rapeseed oil was the richest source of oleic acid and fish fed this diet accumulated the highest amount of this fatty acid in the muscle tissue lipids. However, the proportion of total monounsaturated fatty acids in the fillet was not influenced by the dietary treatment.

Compared to fish oil, supplementation of vegetable oils in the present study increased the percent of linoleic acid (C18:2 n-6) in the diet. Linoleic acid was the main fatty acid in the sunflower and soy oil diets, resulting in a significant increase of linoleic acid in the muscle tissue lipids of fish in these groups. However, the difference in dietary linoleic acid concentrations greatly exceeded the difference in muscle tissue concentrations. There was no difference in percent arachidonic acid (C20:4 n-6) in the fillets, indicating that the conversion of linoleic acid to arachidonic acid by Δ6-desaturase was negligible. Likewise, linoleic acid was not converted to arachidonic acid in the liver of pike (Henderson et al., 1995). There is a competitive interaction between n-6 and n-3 PUFA in hepatic elongation and desaturation pathways. As demonstrated *in vitro*, Δ6-desaturase n-3 PUFA more readily than n-6 PUFA (Seiliez et al., 2001). However, desaturation enzymes do not specifically favor n-3 over n-6 fatty acids in perch lipid metabolism, and are greatly influenced by the fatty acid content of the diet (Xu and Kestemont, 2002).

Docosahexaenoic acid (DHA; C22:6 n-3) was the highest n-3 PUFA in the hybrid muscle, as observed in coho salmon *Oncorhynchus kisutch*, rainbow trout *Salmo gairdneri* (Nettleton, 2001), and pikeperch *S. lucioperca* (Jankowska et al., 2003). The other important long-chain n-3 fatty acid, EPA, was present in lower amounts. Although replacement of fish oil reduced the dietary DHA nearly twofold and EPA threefold, the muscle tissue contents failed to reflect these dietary differences. The EPA concentrations in the diet and in the fillet were similar, while DHA accumulated in the fillet. Metabolic conversion of α-linolenic acid to longer chain n-3 PUFA may be more efficient in freshwater than marine fish (Tocher et al., 2001). The ability of trout liver hepatocytes to synthesize DHA from α-linolenic acid and EPA may be considerably enhanced by elimination of long-chain n-3 PUFA from the fish diet (Buzzi et al., 1996). The higher α-linolenic acid and lower EPA and DHA concentrations in the rapeseed and soy oil diets than in the fishmeal diet led to utilization of this fatty acid for effective metabolic production of DHA. Our results suggest that pikeperch-Volga pikeperch hybrids are able to convert linolenic acid to 20:5 n-3 and 22:6 n-3, similar to other percids (Xu et al., 2001; Jankowska et al., 2003).

The n-3/n-6 ratio of the fillet fatty acids was 2.0 in the present study but 4.4-6.1 in pikeperch (Jankowska et al., 2003; Molnár et al., 2006) and Volga pikeperch (Szabó, 2009) fed similar diets based on fish oil. The much lower final body weight of the hybrid fish in the present study may explain this difference.

In conclusion, among the vegetable oils investigated in the present experiment, only rapeseed oil met all the important criteria for replacing fish oil. It did not affect the production traits or whole body composition of the experimental fish. Dietary supplementation of rapeseed oil prevented excessive deposition of linoleic acid and enhanced conversion of α-linolenic acid to EPA and DHA in the fillet. The n-3/n-6 fatty acid ratio in the fillet of the hybrids fed the fish and rapeseed oil diets were similar. Thus, rapeseed oil is an effective substitute for fish oil in diets for the pikeperch × Volga pikeperch hybrid.

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