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Impact of diets with vegetable oils on the growth, histological structure of internal organs, biochemical blood parameters, and proximate composition of pikeperch *Sander lucioperca* (L.)

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ABSTRACT

The aim of the study was to evaluate the impact feed supplemented with vegetable oils had on the growth, histological structure of the liver, intestines, and spleen, selected biochemical blood parameters, and proximate body composition of juvenile pikeperch (initial body weight 33 g). The fish were fed isoenergetic (21.0 MJ kg^{-1} feed) and isoprotein feeds (450 g protein kg^{-1} feed) supplemented with fish and vegetable oils: linseed and peanut — group LO/PO, fish and peanut oils — group FO/PO, fish and linseed oils — group FO/LO (oil supplements for each group were 40 and 100 g kg^{-1} feed, respectively). The fourth group of fish was fed feed with fish oil and soy oil — group FO/SO, and the addition of these oils was 100 and 40 g kg^{-1} feed, respectively. The fish were reared for 70 days in three replicates for each feed treatment. The replacement of fish oil with vegetable oils (group LO/PO) did not impact fish growth ($P>0.05$). Feeding fish peanut oil (groups LO/PO and FO/PO) resulted in a significant increase in lipid retention coefficient and in aspartate and alanine aminotransferase activity ($P<0.05$). Low hepatocyte vacuolization, increased supranuclear zone in intestinal enterocytes, and the highest ceruloplasmin activity in the blood were noted in group LO/PO ($P<0.05$). The amount of lipids in the fillets and/or whole fish was significantly higher in groups LO/PO and FO/PO. A high level of vegetable oils in the feed (groups LO/PO, FO/LO, FO/PO) was linked to increased viscerosomatic index ($P<0.05$). The feed with a high content of linseed oil (group FO/LO) caused a significant decrease in lipid content and an increase in protein in viscera. The quantity of n-6 polyunsaturated fatty acids (PUFA) in group LO/PO was significantly the highest in the whole fish, viscera, fillet, and liver. The n3/n6 index in fish fillets ranged from 3.24 (group LO/PO) to 6.79 (group FO/SO) ($P<0.05$). The quantity of eicosapentaenoic acid in fish liver and fillet was significantly the highest in group FO/SO ($P<0.05$). The contents of docosahexaenoic acid were similar in all groups ($P>0.05$).

1. Introduction

Pikeperch (*Sander lucioperca*) is a valuable species in aquaculture, and recently attempts have been undertaken to improve rearing methods (Fontaine et al., 2008; Zakęś, 2009). Pikeperch growth rates are high, and they utilize formulated feeds recommended for salmonids effectively when reared in recirculating systems (Molnár et al., 2006; Zakęś et al., 2003). Feeding pikeperch this type of feed increases the fat content of the whole body and fillet, in comparison with wild fish. The fatty acid (FA) profile, however, does not change significantly (Jankowska et al., 2003). Consequently, the higher absolute content of the valuable eicosapentaenoic (EPA, C20:5 n-3) and docosahexaenoic (DHA, C22:6 n-3) fatty acids in the meat of cultivated fish might be more attractive to the consumer (Jankowska et al., 2003).

The primary lipid source in formulated feeds is fish oil, which is rich in highly unsaturated fatty acids (HUFA), including EPA and DHA. The dynamic development of global aquaculture has led to increased production and consumption of formulated feeds. Vargas et al. (2008) report that the demand for fish oil among consumers (including feed manufacturers) has reached its sustainability limits. Concurrently, the production of vegetable oils has been increasing consistently in recent years, and is already a hundred times higher than that of fish oil (Turchini et al., 2009). Accordingly, vegetable oils are being used increasingly as more available and sustainable sources of energy in fish feeds.

Vegetable oils such as soy, rapeseed, sunflower, and linseed have been used in pikerperch studies conducted to date (Zakęś et al., 2004; Schulz et al., 2005; Molnár et al., 2006; Kowalska et al., 2008). These oils are characterized by higher levels of linolenic (ALA, C18:3 n-3) and linoleic (LA, C:18:2 n-6) fatty acids than those in fish oil, and by a lack of EPA and DHA (NRC, 1993). Replacing fish oil with vegetable oils can cause a decrease in the valuable EPA and DHA in the tissues of fish, and, consequently, in the state of fish health (Watanabe et al., 1989; Tacon,

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1992). This can also change the nutritional value of the fish, which is significant to the consumer (Turchini et al., 2009). However, it has been demonstrated that freshwater fish, including pikeperch, have an innate capacity to synthesize EPA and DHA from precursors with 18 carbon atoms (Sargent et al., 2002; Jankowska et al., 2003), which means that it is possible to use some vegetable oils to feed this species. However, changing the lipid source in feeds can impact both lipid metabolism and the state of fish health. When used as the primary lipid source in diets of European sea bass (*Dicentrarchus labrax*) and salmonids, soy, olive, and rapeseed oils resulted in histological changes in the liver and intestine (Olsen et al., 2000, 2003; Parpoura and Alexis, 2001; Caballero et al., 2002). This might be linked to excessive vegetable oils that are difficult to assimilate, a deficit of essential fatty acids (EFA), and/or the inhibition of HUFA synthesis (Metailler et al., 1981; Watanabe et al., 1989; McClelland et al., 1995; Parpoura and Alexis, 2001). Studies have confirmed that pathological changes in the alimentary tract increase the values of biochemical blood parameters such as aspartate (AST) and alanine (ALT) aminotransferase, alkaline phosphatase (ALP), ceruloplasmin (Cp), and bilirubin (Dunier et al., 1995; Lanari et al., 1999; Nivedita et al., 2002). Thus, the analysis of these indices could be used as a marker in diagnosing changes in fish resulting from inappropriately composed diets without the necessity of sacrificing the fish for analyses.

The aim of the study was to identify the impact of feed supplemented with vegetable oils (peanut, linseed, and soy with or without the addition of fish oil) on the growth, histological structure of the liver, intestines, and spleen, biochemical blood parameters, and proximate body composition of juvenile pikeperch.

2. Materials and methods

2.1. Rearing animals

The juvenile pikeperch were obtained from artificial spawning performed at the Inland Fisheries Institute in Olsztyn (Zakęś and Szczepkowski, 2004). Initially, the fish were fed mixed feed (*Artemia* sp. + formulated feed; first 3 weeks of rearing), followed by commercial trout feed (Perla, Skretting, Italy), which contained 620 g protein kg^{-1} feed, 110 g fat kg^{-1} feed, 100 g ash kg^{-1} feed, and had a digestible energy value of 18.5 MJ kg^{-1} feed. After the pikeperch had reached an average body weight of 33 g, the fish were stocked into 12 rearing tanks, each with a volume of 0.2 m^3, that were part of one of three recirculating systems. Initially, the stocking density was 9.9–10.0 kg m^{-3} (60 individuals per tank). The water temperature and oxygen content, total ammonia nitrogen (TAN = NH_3–N + NH_4^+–N), and water pH at the rearing tank outflow were as follows: 22.3 ± 0.1 °C, 5.7 ± 0.4 mg O_2 L^{-1}, 0.18 ± 0.09 mg TAN L^{-1}, 7.75–7.81. Water flow in the tanks was 4 L min^{-1}. The photoperiod applied was LD 24:0. Light intensity measured at the surface of the rearing tanks was 40–50 lx (Luchiari et al., 2006).

2.2. Feeding animals

The feed used to prepare the experimental diets was an Aller Safir base feed (Aller-Aqua, Golub-Dobrzyń, Poland) and a mix of fish oil (FO) (Peter Möller, Möller's Tran, Oslo, Norway) and/or peanut oil (PO) (F.LLI Ruata S.p.A, Goccia d'Oro, Italy), linseed oil (LO) (S.P.R.P. Gal, Poznań, Poland), soy oil (SO) (ZPT Olvit, Gdańsk, Poland). The base feed contained 450 g protein kg^{-1} feed, 40 g fat kg^{-1} feed, and 80 g ash kg^{-1} feed, with an energy value of 14.7 MJ kg^{-1} feed (granule size 3.0 mm). The dominant lipid source in the base feed was fish meal, while the source of protein was from fish meal and soy meal. The base feed was supplemented with oils using a vacuum pump (AGA Labor, Lublin, Poland). Three treatments of fish were fed base feed that had been supplemented with mixtures of two different oils: LO and PO (group LO/PO), FO and PO (group FO/PO), FO and LO (group FO/LO) in quantities of 40 and 100 g kg^{-1} feed, respectively. The fourth group of fish was fed feed in which fish oil was the main lipid source, and which had a chemical composition similar to that of the commercial feed used in the rearing of pikeperch. This feed comprised the base feed supplemented with FO and SO (group FO/SO) at quantities of 100 and 40 g kg^{-1} feed. The total lipid content of the feeds ranged from 183 to 187 g kg^{-1} feed. The contents of protein, fat, and carbohydrates in the experimental feeds were all within those recommended for juvenile pikeperch (Nyina-Wamwiza et al., 2005). The proximate composition and fatty acid profile of the feeds tested are presented in Table 1.

The fish were divided into four feeding treatment groups, each in three replicates ($n = 3$), and were reared for 10 weeks (70 days) and fed continually (19 h d^{-1}) with automatic band feeders (4305 FIAP, Fishtechnic GmbH, Germany). The daily feed ration was determined at weekly intervals and ranged from 1.2% stock biomass (first 3 weeks of rearing) to 0.8% stock biomass (last 3 weeks of rearing).

2.3. Experimental procedures

2.3.1. Calculations

On the first and last days of the experiment the fish were weighed ($W \pm 0.01$ g) and measured (total length, TL ± 0.1 cm), and samples were collected to determine the proximate composition of the bodies. The data collected served for calculating the following parameters:

$$\text{Specific growth rate (SGR, \% d}^{-1}) = 100 \times [(\ln W_f - \ln W_i) \times T^{-1}];$$

$$\text{Daily growth rate (DGR, g d}^{-1}) = (W_f - W_i) \times T^{-1};$$

$$\text{Condition factor (CF)} = 100 \times (W \times TL^{-3});$$

Table 1
Proximate (g kg^{-1} of wet weight) and selected fatty acid (FA) composition (g FA kg^{-1} of total FA) of the experimental diets.

	Diets[a]			
	FO/SO	LO/PO	FO/PO	FO/LO
Components				
Crude protein	450	450	450	450
Crude fat	184	183	187	183
NFE[b]	266	267	263	267
Crude ash	80	80	80	80
Gross energy (MJ kg^{-1} feed)	20.7	20.8	20.9	20.8
Fatty acid composition				
C14:0	46.9	16.0	30.2	36.1
C16:0	165.2	112.6	143.5	134.2
C18:1 cis 9	124.6	334.8	333.1	158.1
C18:2 n-6	38.3	130.3	123.4	97.3
C18:3 n-3	11.3	104.4	8.4	235.5
C20:4 n-6	11.9	4.3	4.3	4.2
C20:5 n-3	178.2	59.2	64.6	62.0
C22:6 n-3	94.9	48.1	57.1	49.4
Total saturated[c]	257.1	187.2	226.0	213.4
Total monoenes[d]	327.5	440.2	482.4	302.3
Total polyenes[e]	415.4	372.6	291.6	484.3
Total n-3[f]	310.1	220.2	139.7	356.7
Total n-6[g]	55.0	134.6	127.7	101.5
Total n-9[h]	164.9	369.4	382.2	195.6
n-3 HUFA[i]	298.8	115.8	131.3	121.2
n3/n6	5.6	1.64	1.09	3.51

[a] FO/SO-feed with fish and soy oils (rich in C14:0, C16:0, C20:5 n-3, C22:6 n-3), LO/PO-feed with linseed and peanut oils (rich in C18:1 cis 9, C18:2 n-6, C18:3 n-3), FO/PO-feed with fish and peanut oils (rich in C18:1 cis 9, C18:2 n-6), FO/LO-feed with fish and linseed oils (rich in C18:3 n-3).
[b] NFE—Nitrogen free extract, calculated as 1000 − (protein + lipid + ash + fiber) g kg^{-1}.
[c] Total saturated—C14:0, C15:0, C16:0, C18:0, C20:0, C22:0.
[d] Total monoenes—C14:1, C16:1, C17:1, C18:1 cis 9, C18:1 cis 11, C20:1 n-9, C21:1 n-7, C22:1 n-11, C22:1 n-9.
[e] Total polyenes—C16:4, C20:2, C21:5, C18:2 n-6, C20:3 n-6, C40:4 n-6, C22:5 n-6, C18:3 n-3, C20:3 n-3, C20:4 n-3, C20:5 n-3, C22:5 n-3, C22:6 n-3.
[f] Total n-3-C18:3 n-3, C20:3 n-3, C20:4 n-3, C20:5 n-3, C22:5 n-3, C22:6 n-3.
[g] Total n-6-C18:2 n-6, C20:3 n-6, C20:4 n-6, C22:5 n-6.
[h] Total n-9-C18:1 cis 9, C20:1 n-9, C22:1 n-9.
[i] n-3 HUFA-C20:3 n-3, C20:4 n-3, C20:5 n-3, C22:5 n-3, C22:6 n-3.