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Influence of different dietary 18:3n-3/18:2n-6 ratio on growth performance, fatty acid composition and hepatic ultrastructure in Eurasian perch, *Perca fluviatilis* ☆

Gersande Blanchard *, Judith G. Makombu, Patrick Kestemont

University of Namur (FUNDP), Unit of Research in Organismal Biology, 61 rue de Bruxelles, 5000 Namur—Belgium

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ABSTRACT

It has been shown that numerous fish species require n-3 polyunsaturated fatty acids (PUFA) for normal growth, while the growth-enhancing effect of dietary n-6 PUFA differ substantially among species. The present study aimed to evaluate the effect of different n-3 to n-6 ratios, underlying the effect of linoleic (LA) and linolenic (LnA) levels, on growth performance, tissue fatty acid composition and hepatic ultrastructure of Eurasian perch. Four diets were formulated using 3 different lipid sources (cod liver, safflower and linseed oils) in variable proportions in order to prepare diets with different LnA/LA ratios: 0.43; 0.03; 3.03 and 0.6 for CO, SO, LO and SLO diets, respectively. Significant results confirm the statement that an increase proportion of 18:2n-6 and 18:3n-3 in the diet of Eurasian perch, or a partial substitution of fish oil by vegetable oil, does not appear to compromise growth performance of juvenile perch. Modification of lipid utilisation by a modulation of desaturation and elongation activity in liver tissue of perch is discussed. Analyses of tissue FA profiles indicate that a minimum level of EPA and DHA has to be incorporated into the diets to comply with the EFA requirement of this species and that the overabundance of 18:2n-6 observed in SO fed fish, may cause an apparent deficiency in 18:3n-3. Consequently, the quantity of 18:2n-6 and 18:3n-3 in the SLO diet (0.64 LnA/LAratio) seems to be more suitable than in the SO diet (0.03 LnA/LA ratio) to promote ArA biosynthesis. However fish with a tissue FA profile the most similar to that of fish fed cod liver oil were fish fed the LO diet (3.03 LnA/LA ratio), suggesting the higher activity of Δ6 desaturase rather than Δ5 desaturase.

1. Introduction

It is now well established that fish, like all vertebrates, cannot synthesize *de novo* polyunsaturated fatty acids (PUFA) and consequently that essential fatty acids (EFA) must be provided in the diet. Two common signs of EFA deficiency have been reported in several freshwater and marine species: poor growth and low feed conversion. Other specific signs, such as a shock syndrome in rainbow trout deprived of dietary PUFA for several months, fin erosion, fin rot of both the tail and dorsal fin, rot or erosion of the lower jaw, increase of hepatic index, pale and swollen liver were also reported (Sargent et al., 2002).

In addition, feeding rainbow trout with n-3 PUFA far in excess of their requirement has been shown to result in poor growth and low feed conversion (Watanabe, 1982). The competition between n-3 and n-6 fatty acids as substrates for the same desaturase enzymes involved in lipid metabolism has been reported, suggesting the importance of a dietary ratio of n-3/n-6 PUFA (Sargent et al., 1989). Evaluation of both n-3 and n-6 PUFA effects on lipid metabolism are difficult because the effect of n-3 PUFA changes with the level of n-6 PUFA (and vice versa). One of the main indications of n-3/n-6 EFA imbalance is an increase of lipid deposition in the liver (Robaina et al., 1998). Feeding a diet containing a balanced n-3/n-6 fatty acid ratio to gilthead seabream juveniles improved the utilization of liver lipids and consequently reduced liver histological alterations (Robaina et al., 1998). Caballero et al. (2002) reported the importance of the final balance of dietary polyunsaturated/saturated fatty acids, in order to obtain the best feed conversion ratio (FCR) and demonstrated the importance of DHA. In juvenile turbot, Bell et al. (1995) demonstrated that feeding a well-balanced DHA/EPA ratio was essential to promote good growth and development in juvenile turbot. Ng et al. (2003) reported the importance of a balanced n-3/n-6 PUFA ratio in diets for African catfish. It has been shown that numerous fish species require n-3 PUFA for normal growth, while the growth-enhancing effect of dietary n-6 PUFA differs substantially among species. But all species probably need a small amount of n-6 PUFA in order to form eicosanoids. Indeed, the presence of ArA in the diet is essential to avoid phospholipid ArA depletion that may induce prostaglandin changes and consequently influence a wide range of physiological processes important for growth and general health of fish (Tocher, 2003). The excess of one

☆ Diet LnA/LA ratio on perch juveniles.

* Corresponding author. University of Namur (FUNDP), Unit of Research in Organismal Biology, 61 rue de Bruxelles, 5000 Namur, Belgium. Tel.: +32 81 724287; fax: +32 81 724362.
E-mail addresses: gersande.blanchard@fundp.ac.be, gersande.blanchard@yahoo.fr (G. Blanchard).

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Table 1
Formulation (g/kg) and proximate composition (% wet weight±S.D.) of the test diets (CO, SO, LO and SLO) provided to juvenile Eurasian perch (*Perca fluviatilis*)

Ingredients	CO	SO	LO	SLO
	CO	CO/SO (2/1)	CO/LO (2/1)	CO/SO/LO (1/1/1)
Fish meal[a]	505	505	505	505
Corn starch	150	150	150	150
Cod liver oil	190	127	127	63
Safflower oil	0	63	0	63
Linseed oil	0	0	63	63
Betaine, glycine, proline, alanine (1/1/1/1)	10	10	10	10
Carboxymethylcellulose	20	20	20	20
Vitamin mixture[b]	5	5	5	5
Mineral mixture[b]	20	20	20	20
Cellulose	100	100	100	100
BHT & BHA[c]	0.2	0.2	0.2	0.2
Astaxanthin[d]	1	1	1	1
Crude protein (% wet wt.)	46.09±0.83	46.40±0.50	46.85±0.60	46.48±0.58
Crude fat (% wet wt.)	18.61±0.2	18.24±1.1	18.17±0.1	18.36±0.2
Ash (% wet wt.)	4.67±0.19	4.87±0.08	5.52±0.37	5.88±0.08
Dry weight (% wet wt.)	87.68±0.05	85.99±0.22	86.63±0.20	88.73±0.14

[a] Fish meal is codfish powder provided by Snick Ingredients (Brugge, Belgium).
[b] Vitamin and mineral mixture were obtained from INVE Aquaculture (Dendermonde, Belgium). Composition of vitamin mixture according to Griffin et al. (1994). Composition of mineral mixture (g kg^{-1} of mixture): $CaHPO_4$ $2H_2O$, 727.7775; $MgSO_4$ $7H_2O$, 127.5000; NaCl, 60.000; KCl, 50.000; $FeSO_4$ $7H_2O$, 25.000; $ZnSO_4$ $7H_2O$, 5.5000, $MnSO_4$ $4H_2O$, 2.5375, $CuSO_4$ $5H_2O$, 0.7850; $CoSO_4$ $7H_2O$, 0.4775; $CaIO_3$ $6H_2O$, 0.2950; $CrCl_3$ $6H_2O$, 0.1275.
[c] 0.1 g.kg^{-1} BHT (2,6-Di-tert.-butyl-4 methylphenol 99%) and 0.1 g.kg^{-1} BHA (Butylated hydroxyanisole) provided by Sigma-Aldrich (Steinheim-Germany).
[d] 1 g Lucantin ® Pink containing 11.2% astaxanthin, provided by BASF Aktiengesellschaft (Ludwigshafen, Deutschland).

PUFA may cause an apparent deficiency in another PUFA (Bell, 1998). The interactive effects of dietary linoleic (LA) and linolenic (LnA) acids have also been observed by other researchers, who also suggest the importance of the dietary n-3 and n-6 PUFA balance (Glencross et al., 2002). However, in addition to growth performance, it is important to evaluate the effect of n-3/n-6 PUFA balance on fat deposition, FA composition of tissues and liver histology. Histological studies concerning the effect of different n-3/n-6 PUFA ratios are limited but can provide information on diet quality and utilization, and can also be used as an indicator of the nutritional status of the fish (Caballero et al., 2004).

In Eurasian perch, a freshwater species of increasing interest in European inland aquaculture (Fontaine et al., 1993; Kestemont and Dabrowski, 1996), relatively few studies have been conducted on the nutritional requirement of essential fatty acids. Xu and Kestemont (2002), in a study dedicated to the influence of dietary fats (16% of either olive oil, safflower oil, linseed oil or cod liver oil as the only lipid source), demonstrated the bioconversion of n-3 and n-6 FA in Eurasian perch, and the importance of the dietary n-3/n-6 FA ratio in the lipid. However, there is a lack of information about the EFA requirement of this species. Moreover, the effect of different diet n-3/n-6 FA ratios on hepatic ultrastructure have not been reported. The present study aimed to evaluate the effect of different n-3/n-6 FA ratios, including the effect of LA and LnA levels, on growth performance, tissues fatty acid composition and hepatic ultrastructure of Eurasian perch.

2. Materials and methods

2.1. Facilities, fish and diets

The Eurasian perch, *Perca fluviatilis*, used in this experiment were obtained from a commercial intensive rearing system (Lucas Perch, France). A total of 360 juveniles were transferred to the facilities of the University of Namur and randomly distributed into 12 tanks (30 fish per tank). A 2 week adaptation period to the new diet formulation and rearing conditions was applied before the start of the experiment. The experiment was then conducted for 76 days with fish of 43.3±0.3 g initial body weight at constant temperature (22.9±0.6 °C) in an indoor recirculating system (120-l rectangular tanks, 12 L:12D) with a flow-rate of approximately 4-l min^{-1} in each tank. The water was continuously purified by a series of filtration treatments including mechanical and bio-filter systems followed by UV treatment. The oxygen concentration was monitored each morning and maintained at 7.5±0.4 mg l^{-1}. Concentrations of NH_4^+ and NO_2^- were determined twice a week (0.11±0.08 mg l^{-1} and 0.19±0.11 mg l^{-1}, respectively). During the experiment, fish were fed by hand, 3 times a day (0900, 1300 and 1700 h), except on Sunday. Fish were considered as satiated when they did not exhibit any feeding behaviour after 2 distributions of pellets.

Four dietary treatments were fed in triplicate during the experiment. The four isoenergetic and isonitrogenous diets (17.2 MJ kg^{-1}, 45% crude protein, 19% total lipid) were formulated using 3 lipid sources (cod liver, safflower and linseed oils) in variable proportions in order to prepare diets with different LnA/LA ratios: 0.43; 0.03; 3.03 and 0.64 in the CO, SO, LO and SLO diets, respectively (Tables 1 and 2). Diet CO, prepared with 100% cod liver oil and considered as the reference diet, was characterized by a high content of eicosapentaenoïc (EPA) and docosahexaenoïc (DHA) acids. SO and LO diets were prepared with 2/3 cod liver oil and respectively, 1/3 safflower oil (Sigma S8281) and linseed oil (Sigma 430021),which were characterized by high contents of linoleic (18:2 n-6; 75%) and linolenic (18:3 n-3; 53%) acids, respectively. SLO diet was prepared with equal proportion of cod liver, safflower and linseed oils. Two diets were similar in terms of n-3/n-6 FA ratios (0.8 and 1.1 for SO and SLO diets, respectively) but somewhat different in terms of 18:3 n-3 contents (0.7 and 18.1% total FA, respectively). The ingredient composition of the diets was formulated to meet the protein and lipid requirements of Eurasian perch (Kestemont et al., 2001; Xu et al., 2002). According to a previous experiment demonstrating the beneficial effect of astaxanthin supplementation in perch (Blanchard et al., submitted for publication),

Table 2
Fatty acid composition (% of total fatty acids)* of the experimental diets containing different LnA/LA ratio and fed to juvenile Eurasian perch

	Diets			
	CO	SO	LO	SLO
14:00	4.43±0.09[a]	3.03±0.03[a,b]	3.02±0.00[a,b]	1.57±0.01[b]
16:00	13.50±0.09[a]	11.71±0.12[b]	11.36±0.25[b]	9.35±0.08[c]
18:00	2.48±0.03[a]	2.43 ±0.02[a]	2.66±0.05[b]	2.66±0.04[b]
Σ SFA	20.41±0.22[a]	17.17±0.11[b]	17.03±0.30[b]	13.58±0.11[c]
16:1n-7	6.63±0.10[a]	4.39±0.05[b]	4.38±0.17[b]	2.22±0.03[c]
18:1n-9	13.65±0.09[a]	13.09±0.21[b]	14.89±0.04[c]	14.49±0.07[c]
18:1n-7	3.35±0.07[a]	2.62±0.06[b]	2.71±0.16[b]	1.63±0.03[c]
20:1n-9	8.81±0.10[a]	5.88±0.10[b]	6.05±0.22[b]	3.15±0.03[c]
22:1n-9	7.46±0.07[a]	4.88±0.09[b]	5.12±0.35[b]	2.55±0.03[c]
Σ MUFA	39.90±0.08[a]	30.85±0.30[b]	33.15±0.93[b]	24.04±0.13[c]
Σ n-4 PUFA	0.93±0.03[a]	0.63±0.00[b]	0.56±0.01[c]	0.28±0.01[d]
18:2n-6	2.06±0.01[a]	25.18±0.01[b]	6.08±0.06[c]	28.10±0.00[d]
20:4n-6	0.72±0.01[a]	0.56±0.00[b]	0.54±0.00[b]	0.41±0.00[c]
Σ n-6 PUFA	2.79±0.03[a]	25.73±0.02[b]	6.62±0.06[c]	28.51±0.00[d]
18:3n-3	0.89±0.01[a]	0.67±0.01[a]	18.43±0.14[b]	18.06±0.14[b]
18:4n-3	2.03±0.02[a]	1.34±0.02[b]	1.37±0.02[b]	0.70±0.00[c]
20:4n-3	0.91±0.01[a]	0.59±0.01[b]	0.60±0.01[b]	0.32±0.00[c]
20:5n-3 (EPA)	9.35±0.04[a]	6.79±0.01[b]	6.79±0.07[b]	4.19±0.03[c]
22:5n-3	1.58±0.01[a]	1.06±0.03[b]	1.06±0.02[b]	0.59±0.00[c]
22:6n-3 (DHA)	13.91±0.10[a]	10.50±0.07[b]	10.67±0.15[b]	7.49±0.08[c]
Σ n-3 PUFA	28.66±0.20[a]	20.94±0.00[b]	38.92±0.37[c]	31.35±0.27[d]
LnA/LA ratio	0.43±0.00[a]	0.03±0.00[b]	3.03±0.01[c]	0.64±0.01[d]
n-3/n-6 ratio	10.29±0.04[a]	0.81±0.00[b]	5.88±0.00[c]	1.10±0.01[d]
DHA/EPA ratio	1.49±0.00[a]	1.55±0.01[b]	1.57±0.01[b]	1.79±0.00[c]

* Results are expressed as mean±SD of three replicate analyses of different samples of each diet. Different letters indicate significant differences between diet for each fatty acid, $P<0.05$.
CO: Cod liver oil; SO: Safflower oil and CO (1/2); LO: Linseed oil and CO (1/2); SLO: CO and SO and LO (1/1/1). SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.