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# Effects of dietary vegetable oil supplementation on fillet quality traits, chemical and fatty acid composition of African catfish (*Clarias gariepinus*)

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## Abstract

The effects of dietary fish oil (FO), soybean oil (SO) and linseed oil (LO) (12% crude fat content each) in African catfish (*Clarias gariepinus*) diets were tested on the fillet flesh quality, chemical and fatty acid (FA) composition, after 3 and 6 weeks of feeding. The bodyweight gain of fish and the fillet dry matter, crude protein and crude fat content was not different among the divergent treatments. High (>20%) total n3 FA supplementation significantly increased the moisture loss of fillet (FO, LO). Applying the simple FA dilution model (JOBLING 2004a, 2004b), the incorporation dynamics of the most largely dosed FAs were accurately predictable after 3 weeks ($R^2$ between observed and estimated data for total n3 FAs: FO 0.95, LO 0.73 and for α-linolenic acid, LO 0.97). In the fillet FA composition the metabolism of n3 acids was more pronounced. The large provision of α-linolenic acid (LO) had a pronounced effect on the longchain, polyunsaturated n3 FA proportions (eicosapentaenoic and docosapentaenoic acids), while no effect was experienced on docosahexaenoic acid. This study suggests that daily bodyweight gain is not, while fillet flesh quality and FA composition is slightly compromised when fish oil is substituted for vegetable oils.

**Keywords**: African catfish, fatty acid incorporation, fillet, flesh quality, vegetable oils

## Zusammenfassung

### Der Einfluss von Pflanzenfetten auf die Fleischqualität, chemische Zusammensetzung und das Fettsäuremuster beim Afrikanischen Wels *(Clarias gariepinus)*

Untersucht wurde der Einfluss einer Zugabe von Fischöl (FO), Sojaöl (SO) und Leinöl (LO) mit einem Rohfettgehalt von jeweils 12% auf die Fleischqualität, chemische Zusammensetzung und das Fettsäuremuster beim Afrikanischen Wels über eine Zeitdauer von 3 bis 6 Wochen. Verglichen mit der Kontrollvariante ergaben sich bei den drei Versuchsvarianten keine Einflüsse auf den Körperzuwachs, den Rohfett-, Eiweiß- sowie Trockensubstanzanteil. Das höhere n3 Fettsäureangebot (>20%) gegenüber der Kontrolle verminderte bei den FO und LO Varianten in hohem Maße das Wasserbindungsvermögen des Fleisches. Durch die Anwendung des Modells für Fettsäureverdünnung nach JOBLING (2004a, 2004b) war die Inkorporationsdynamik der wichtigsten Fettsäuren drei Wochen nach Zugabe der

unterschiedlichen Futterfette mit hoher Genauigkeit schätzbar. Die $R^2$ Werte zwischen gemessenen und geschätzten Werten betrugen bei FO 0,95; LO 0,73 und für die α-Linolensäure 0,97. Der n3 Fettsäuremetabolismus war im Fischfleich stärker ausgeprägt. Die hohe Versorgung mit α-Linolensäure übte einen großen Einfluss auf die n3 Fettsäuren besonders auf die Eicosapentaensäure sowie den Docosapentaensäuregehalt aus, während der Docosahexaensäureanteil nicht verändert wurde. Die Ergebnisse zeigen, dass die Zugabe von Pflanzenöl den Körperzuwachs nicht beeinflusste jedoch einen negativen Effekt auf die Fleischqualität und die Fettsäurezusammensetzung des Fischfleisches bewirkte.

**Schlüsselwörter**: Afrikanischer Wels, Fettsäureinkorporation, Filet, Fleischqualität, Pflanzenfett

## Introduction

Highly unsaturated, essential n3 fatty acids have beneficial health effects and are more and more frequently ingested via fish or fish products (BURR 1981). Just like other vertebrate species, fish also need essential fatty acids (FAs), albeit their qualitative and quantitative requirements strongly differ from those of humans (GREENE and SELIVONCHICK 1987). Freshwater fish, including also carnivorous species, as compared to seawater fish, are superior in converting both n3 and n6 precursor FAs to highly unsaturated, longchain fatty acids, due to their origin in environs basically lacking these FAs (AGABA *et al.* 2005). Accordingly, studies on the dietary fatty acid incorporation and their consequent metabolism in both marine and freshwater fish are intensely performed. In marine fish, fatty acid incorporation experiments are highly successful for the accurate prediction of the fillet fatty acid composition (JOBLING 2004a, 2004b), as well in the pre-defined modification of the fillet FA profile, e.g. for the production of cardioprotective human diets (TORSTENSEN *et al.* 2004).

Besides a very robust knowledge on the fatty acid metabolism of sea- and freshwater species (GREENE and SELIVONCHICK 1987, HENDERSON and TOCHER 1987), the contribution of dietary fatty acids to the fillet quality attributes is less known. A basic mode of action of dietary highly unsaturated FAs may be connected to the fact that they are effectively incorporated into the cellular membranes (HENDERSON and TOCHER 1987). Thus, an altered cellular lipid profile may ultimately lead to severe modifications of the fillet quality attributes, as it has been demonstrated for the thawing loss process of catfish fillets (BAKER 1997). The link between dietary fatty acids and fillet water-holding capacity may be the cellular membrane rigidity, as influenced by the diet (DOBRETSOV *et al.* 1977).

The experimental results of WING-KEONG *et al.* (2003) supported evidence that vegetable oil, e.g. palm oil may be a successful alternative for the frequently used fish oil in African catfish feeds. As far as the authors are aware, the effects of relatively easily accessible vegetable oils on the conventional fillet flesh quality indices have been less investigated. Therefore, the aim of the present approach was to test the performance of two vegetable oils in shaping the fillet fatty acid profile and quality, as compared to the classical fish oil.

## Material and methods

*Experimental fish, feeding and culture facilities*

African catfish stock was obtained from the Tuka fish farm (Szarvas Fish Ltd., Szarvas, Hungary), where rearing was carried out in an intensive system using mixed geothermic water supply. At the average size of 1 kg, juveniles were transported to the Fish Laboratory of the Kaposvár University. At the beginning of the experiment, the initial body weight was 1 026±121 g ($n$=374). Stocking density (60-65 kg 1 000 $L^{-1}$) was similar to normal farming conditions. The experimental unit had a total useful volume of 10 000 L attached to a simple bio-filter unit and a 1 600 L settling tank from where the water was pumped back to the fish keeping units. The daily water replacement rate was about 10% of the useful volume. The water flow rate was adjusted to 2.5 L/min and the temperature was 28±0.5 °C. Water temperature was measured daily over the 42-day rearing interval with laboratory thermometer (±0.1 °C). Experimental fish were weighed at the initial point and after 3 and 6 weeks in the study. Each time 5-5 fish from each dietary treatment (one fish from one tank) were killed after anaesthesia (Norcaicum, para-amino-benzoic-acid-ethylester, Egis, Budapest, Hungary), according to MATUK (1987). After dissection, fillet samples were analyzed for chemical composition according to standard AOAC (1990) methods. The crude fat and crude protein (total N × 6.25) contents were interpreted on a dry matter basis. The analysis of the diets was identical with that of fillet samples.

The diet before and in the study was characterized by the chemical and fatty acid composition given in Table 1. The experimental feeds contained, besides the basic 6% crude fat content 6% added oil from different sources: fish oil, and two vegetable oils, soybean and linseed oil.

The feeding lasted for 42 days, which was preceded by a 14-day conditioning period. During conditioning, fish were kept in the same system as during the trial. In this period fish were fed a commercial catfish diet (basal diet) containing 60 g $kg^{-1}$ of crude fat. Diets were fed six times daily, from 8 to 18 h, according to appetite. Each diet was fed in 5 aerated tanks working as a part of a recirculation system. The bodyweight gain of fish showed no significant differences, while the feed conversion ratio of fish fed on FO (1.625) was significantly higher ($P$<0.05) than that of the SO (1.235) and LO (1.330) groups.

Experimental tanks were covered with black plastic, which was removed only for the short periods of feeding and cleaning. Dark environment and frequent feeding helped to avoid stress and aggression among fish.

*Fillet flesh quality investigation*

Fillet pH was measured at 45 min and 24 h *post mortem*, by a Testo 205 precision pH meter (Testo AG, Lenzkirch, Germany). The colour (CIE Lab, $L^*$ – lightness, $a^*$ – redness, $b^*$ – yellowness) of the fresh fillet was determined by a Minolta ChromaMeter 300 apparatus (Minolta, Osaka, Japan). Dripping loss was determined by the method of HONIKEL (1998). To determine the so-called cooking loss, fillet samples (100 g) were closed into sealed bags and were cooked at 75 °C for 20 min. The exudate weight, as expressed in the percentage of the initial sample weight was referred to as cooking loss. The thawing loss was determined by the same manner, i.e. samples (25 g) were frozen (–20 °C) and

thawed to room temperature after 2 days. Moreover, fillet dry matter content was determined by drying to constant weight at 103 °C, and the Warner-Bratzler shear force of 15 x 15 mm fillet quadratic cuts was determined from the cooked samples, on a ZwickRoell Z105 precision equipment (Zwick GmbH & Co. KG, Ulm, Germany). The peak shearing force value was recorded from 3 cuts and the mean values were given.

Table 1
Chemical and fatty acid composition of the diets in the study
*Chemische und Fettsäurezusammensetzung der Fütterungsvarianten*

| | Basal diet | Soybean oil | Linseed oil | Fish oil |
|---|---|---|---|---|
| *Chemical composition* | | | | |
| Dry matter (DM), % | 86.5 | 86.1 | 87.2 | 87.8 |
| Crude ash, % DM | 7.7 | 7.77 | 7.55 | 7.66 |
| Crude protein, % DM | 53.4 | 47.9 | 48.3 | 47.4 |
| Crude fat, % DM | 6.0 | 12.1 | 12.1 | 12.0 |
| Crude fiber, % DM | 2.30 | 2.10 | 2.22 | 2.27 |
| *Fatty acid composition* | | | | |
| C12:0 | 0.11 | 0.05 | 0.05 | 0.07 |
| C14:0 | 0.86 | 1.71 | 1.62 | 4.35 |
| C14:1 n5 | 0.13 | 0.06 | 0.05 | 0.12 |
| C15:0 | 0.18 | 0.21 | 0.20 | 0.49 |
| C16:0 | 21.19 | 14.63 | 12.92 | 15.63 |
| C16:1 n7 | 4.79 | 2.06 | 2.00 | 3.97 |
| C17:0 | 0.37 | 0.27 | 0.25 | 0.53 |
| C17:1 n7 | 0.19 | 0.35 | 0.27 | 0.67 |
| C18:0 | 5.75 | 4.11 | 3.54 | 2.72 |
| C18:1 n9 | 27.34 | 20.63 | 18.26 | 15.78 |
| C18:1 n11 | | 1.98 | 1.82 | 2.26 |
| C18:2 n6 t | | 0.12 | 0.05 | 0.41 |
| C18:2 n6 c | 33.16 | 34.45 | 25.42 | 15.94 |
| C18:3 n6 | 0.06 | 0.04 | 0.04 | 0.08 |
| C18:3 n3 | 1.73 | 4.46 | 19.14 | 2.66 |
| C20:0 | 0.12 | 0.31 | 0.21 | 0.23 |
| C20:1 n9 | 0.63 | 2.12 | 1.97 | 6.94 |
| C20:2 n6 | 0.2 | 0.27 | 0.27 | 0.34 |
| C20:3 n3 | 0.07 | 0.05 | 0.05 | 0.07 |
| C20:3 n6 | 0.01 | 0.10 | 0.12 | 0.15 |
| C20:4 n6 | 0.43 | 0.29 | 0.28 | 0.42 |
| C20:5 n3 | 0.7 | 2.53 | 2.50 | 5.52 |
| C22:1 n9 | 0.02 | 2.36 | 2.14 | 8.19 |
| C22:5 n3 | 0.16 | 0.63 | 0.66 | 1.01 |
| C22:6 n3 | 1.66 | 6.02 | 5.95 | 11.06 |
| C24:0 | | 0.04 | 0.03 | 0.05 |
| C24:1 n9 | 0.1 | 0.16 | 0.17 | 0.31 |
| Σ n3 | 4.32 | 13.69 | 28.30 | 20.33 |
| Σ n6 | 33.86 | 35.28 | 26.18 | 17.36 |
| Σ n6 / Σ n3 | 7.84 | 2.58 | 0.93 | 0.85 |
| Σ monoenoic | 33.20 | 29.72 | 26.69 | 38.24 |
| Σ PUFA | 38.18 | 48.96 | 54.48 | 37.69 |
| Unsaturation index[1] | 121.5 | 166.4 | 188.8 | 181.3 |
| Average fatty acyl chain length[2] | 17.54 | 18.07 | 18.09 | 18.50 |

[1]1(Σ monoenoic FA)+2(Σ dienoic FA)+3(Σ trienoic FA), [2]Σ (% each FA × number of carbons in the chain)/(% total FAs)

*Fatty acid composition*

The fatty acid composition was determined from the complex fat content of 5 g samples. After the extraction (FOLCH *et al.* 1957), fatty acids were converted to methyl esters by means of $BF_3$ and methanol. Fatty acid methyl esters were analysed on an Agilent Technologies (Santa Clara, CA, USA) 6890 N type capillary gas-liquid chromatograph system, with a SP-23804 capillary column (30 m × 0.25 mm inside diameter, 0.20 µm film, Supelco, Bellefonte, PA, USA) and a flame ionization detector. Characteristic operating conditions were: injector temperature: 270 °C, detector temperature: 300 °C, helium flow: 28 cm $s^{-1}$. The oven temperature was programmed from 80 to 205 °C and increased by 2.5 °C $min^{-1}$, 5 min at 205 °C, increased from 205 to 250 °C at 10 °C $min^{-1}$, and 5 min at 250 °C. Individual fatty acids were identified based on their retention times, as assessed from a standard fatty acid mixture (Mixture Me 105, Larodan Fine Chemicals, Malmö, Sweden). The so-called unsaturation index (UI) was defined as the number of double bonds in 100 fatty acyl chains.

*Statistical analysis*

Fatty acid and fillet quality (flesh quality and chemical composition) data between groups were compared by multivariate analysis of variance, with the Tukey post hoc test. Bodyweight was handled as covariant, while treatment duration and fat source were set as fixed factors in the model.

The FA incorporation was tested on the basis of the so-called dilution hypothesis (JOBLING 2004a, 2004b). The basis of the hypothesis is that the increasing addition of a fatty acid into the diet induces a change in the tissue fatty acid proportion that can be predicted as follows:

$$P_T = P_R + \frac{P_I - P_R}{\frac{Q_T}{Q_I}} \quad (1)$$

where $P_T$ is the percentage of the given fatty acid in the fish tissue at the time point $T$, $P_I$ is the initial fatty acid percentage in the tissue, while $P_R$ is the percentage of the fatty acid in the tissues of the reference group, i.e. in a group with a rather long fatty acid supplementation period. $Q_I$ and $Q_T$ represent the information on the fish growth, i.e. this measure is applied to express the body or fillet weight gain. Based on the hypothetic assumption that fillet fat content shows only minor changes within the rearing, the fillet weight was the trait used to characterize the growth in our study. The model includes the assumption that fish grow continuously throughout their lifespan (ASHTON *et al.* 2005). The so-called reference groups were those receiving 6 weeks of experimental oil supplementation, and the estimation was performed for those receiving the oil supplementations for 3 weeks.

In all instances SPSS 10 for Windows (1999) was used for the statistical analysis.

## Results

*Effects of different dietary fatty acid supplementation on the fillet flesh quality and bodyweight gain*

Statistically proven effect of the dietary fat source was experienced only on two fillet flesh quality traits (pH45 and $b^*$), while the duration of dietary fatty acid supplementation had marked effect on most of the traits. The effects and the between-sampling differences are indicated in Table 2. Clearly defined effect of the treatment duration was found on pH45, pH24, $a^*$, dripping loss and thawing loss. As seen in Table 2, bodyweight had a significant influence on the fillet pH24 value, dry matter content and shear-resistance. The values of average daily gain (g $day^{-1}$) were as follows: 12.7 (FO), 12.4 (LO) and 13.3 (SO), without statistically significant differences.

*Influence of different fatty acid supplementation on the fillet chemical composition*

Interestingly, neither fillet dry matter content, nor the crude fat and the crude protein content showed statistically significant alterations during the entire experiment.

*Effects of different fatty acid supplementation on the fillet fatty acid profile*

As expected, both the dietary fat source and the duration of the feeding had marked effects on fillet fatty acids. Detailed fillet FA compositional data are summarized in Table 3, handling the three dietary treatments (FO, LO, SO) in a separated manner.

The proportion of total n3 fatty acids increased in the FO and SO groups, while in the LO group no statistically proven increment was shown. The proportion of α-linolenic acid (C18:3 n3, ALA) increased in all groups, though the SO group reached lower proportions, as compared to the FO, in spite of the ca. two-fold higher ALA supplementation of the latter diet. The fillet proportion of eicosapentaenoic acid (C20:5 n3, EPA) increased according to the dietary supplementation. Interestingly, while the SO and LO diets contained similar EPA amounts, the final fillet proportion of EPA was slightly higher in the LO samples. The docosapentaenoic (C22:5 n3, DPA) and docosahexaenoic acid (C22:6 n3, DHA) proportions in the fillet increased according to the graded dietary uptake of these acids.

The total n6 fatty acids showed either no change (SO, LO), or decreased, in parallel with the decreased dietary uptake (FO). The proportion of linoleic acid (C18:2 n6, LA) decreased only in the fillet samples of the FO group, according to the drastically decreased LA proportion of the diet. In contrast, arachidonic acid (C20:4 n6, AA) did not show marked proportional reduction in either of the treatments, albeit the SO and LO diets contained lower amounts of this acid, as compared to the basal diet.

The markedly altered dietary n3 and n6 doses were effective in the reduction of the n6/n3 fatty acid ratio in the FO and LO groups. The unsaturation index (UI) increased in the former two groups, while the average fatty acyl chain length increased in all three groups to the end of the 6th week.

Table 2
Chemical composition and flesh quality traits of African catfish in the three different treatments ($n$=5 in each case)
*Chemische Zusammensetzung und Fleischqualität vom Afrikanischer Wels in der Untersuchung*

| | Linseed oil (LO) supplementation | | | Soybean oil (SO) supplementation | | | Fish oil (FO) supplementation | | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TD, week | 0 | 3 | 6 | 0 | 3 | 6 | 0 | 3 | 6 | | | | |
| | mean±SD | mean±SD | mean±SD | mean±SD | mean±SD | mean±SD | mean±SD | mean±SD | mean±SD | FS | D | FS×D | BW |
| BW, g | 1185.0±160.0$^{a}$ | 1310.6±347.0$^{b}$ | 1580.0±169.2$^{c}$ | 1185.0±160.0$^{a}$ | 1030.0±42.6$^{a}$ | 1606.0±472.3$^{b}$ | 1185.0±160.0$^{a}$ | 1138.2±304.9$^{a}$ | 1809.0±396.9$^{b}$ | ns | ns | ns | ns |
| AFM, g | 25.0±11.0$^{a}$ | 42.7±25.6$^{b}$ | 47.1±27.5$^{b}$ | 25.0±11.0$^{a}$ | 32.1±2.74$^{b}$ | 52.1±36.8$^{c}$ | 25.0±11.0$^{a}$ | 22.6±1.99$^{a}$ | 96.0±12.7$^{b}$ | ns | ns | ns | ns |
| pH45 | 6.93±0.04$^{b}$ | 6.45±0.25$^{a}$ | 7.04±0.11$^{b\boldsymbol{B}}$ | 6.93±0.04 | 6.73±0.08 | 6.87±0.18$^{\boldsymbol{A}}$ | 6.93±0.04$^{b}$ | 6.56±0.06$^{a}$ | 6.73±0.12$^{a\boldsymbol{AB}}$ | 0.109 | 0.030 | 0.140 | ns |
| pH24 | 6.01±0.01$^{a}$ | 5.88±0.08$^{b}$ | 5.92±0.06$^{ab}$ | 6.01±0.01 | 5.90±0.10 | 5.89±0.05 | 6.01±0.01$^{b}$ | 5.88±0.04$^{a}$ | 5.95±0.09$^{ab}$ | ns | 0.092 | ns | 0.098 |
| L$^{*}$ | 48.1±1.28 | 50.8±4.49 | 49.1±3.44 | 48.1±1.28 | 50.6±0.66 | 50.2±2.86 | 48.1±1.28$^{a}$ | 53.4±1.18$^{b}$ | 50.22±1.18$^{a}$ | ns | ns | ns | ns |
| a$^{*}$ | −1.65±1.88 | −2.11±1.23 | −3.19±0.44 | −1.65±1.88 | −2.06±1.18 | −1.34±1.90 | −1.65±1.88 | −1.90±0.91 | −2.11±0.73 | ns | ns | ns | ns |
| b$^{*}$ | 4.41±1.75 | 5.23±0.67$^{A}$ | 4.46±1.34 | 4.41±1.75 | 5.42±1.13$^{B}$ | 5.15±0.10 | 4.41±1.75 | 7.21±0.37$^{A}$ | 4.55±0.86 | 0.096 | 0.061 | ns | ns |
| DM, % | 22.8±1.11 | 22.2±0.54 | 23.1±0.35 | 22.8±1.11 | 22.1±0.53 | 22.9±1.14 | 22.8±1.11 | 21.8±1.04 | 22.6±0.93 | ns | ns | ns | 0.023 |
| CP, % DM | 73.6±5.80 | 77.3±3.12 | 77.9±2.41 | 73.6±5.80 | 79.1±1.50 | 73.0±2.18 | 73.6±5.80 | 80.4±2.67 | 73.6±4.59 | ns | 0.005 | 0.038 | ns |
| EE, % DM | 23.6±5.85 | 20.3±3.45 | 19.7±2.54 | 23.6±5.85 | 18.2±1.90 | 24.2±2.95 | 23.6±5.85 | 16.9±2.16 | 24.0±4.67 | ns | 0.010 | 0.051 | ns |
| DL, % | 1.79±0.18$^{a}$ | 2.81±0.59$^{b}$ | 1.79±0.34$^{a}$ | 1.79±0.18 | 2.17±0.45 | 1.84±0.16 | 1.79±0.18$^{a}$ | 2.36±0.44$^{b}$ | 1.81±0.17$^{a}$ | ns | 0.001 | ns | ns |
| CL, % | 7.78±1.51 | 11.1±3.36 | 11.8±3.73 | 7.78±1.51$^{a}$ | 11.7±1.89$^{b}$ | 8.59±0.80$^{a}$ | 7.78±1.51$^{a}$ | 11.61±1.57$^{b}$ | 11.71±0.95$^{b}$ | 0.153 | ns | ns | ns |
| TL, % | 2.98±1.31$^{a}$ | 2.64±0.98$^{a}$ | 3.81±0.16$^{b}$ | 2.98±1.31 | 3.01±0.75 | 3.68±0.18$^{a}$ | 2.98±1.31$^{a}$ | 2.18±0.49 | 3.93±1.07$^{b}$ | ns | 0.087 | ns | ns |
| WB SF, N/mm$^{2}$ | 0.04±0.00$^{b}$ | 0.03±0.01$^{a}$ | 0.03±0.01$^{ab}$ | 0.04±0.00$^{b}$ | 0.02±0.01$^{a}$ | 0.03±0.00$^{ab}$ | 0.04±0.00$^{b}$ | 0.02±0.01$^{a}$ | 0.03±0.01$^{ab}$ | ns | ns | ns | 0.031 |

TD treatment duration, SD standard deviation, FS fat source, D duration, BW body weight, AFM abdominal fat mass, DM dry matter, CP crude protein, EE ether extract, DL dripping loss, CL cooking loss, TL thawing loss, WB SF Warner Bratzler shear force, UI unsaturation index, A FA CH L Average FA chain length

$^{a,b,c}$different small superscripts represent significat differences within treatment, $^{A,B,C}$different capital superscripts refer to significant differences between the 3rd week data of the single treatments, $^{\boldsymbol{A,B,C}}$different boldface, capital, italic superscripts refer to significant differences between the 6th week data of the single treatments, ns not significant

Table 3
The alteration of fillet fatty acid composition along the three divergent supplementation treatments ($n$=5 in each case) (legend: see Table 2)
*Veränderungen der Fettsäurezusammensetzung nach der Fütterung von verschiedenen Futtersorten (Legende: siehe Tabelle 2)*

| | Linseed oil (LO) supplementation | | | Soybean oil (SO) supplementation | | | Fish oil (FO) supplementation | | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TD, week | 0 | 3 | 6 | 0 | 3 | 6 | 0 | 3 | 6 | | | | |
| | mean± SD | mean± SD | mean± SD | mean± SD | mean± SD | mean± SD | mean± SD | mean± SD | mean± SD | FS | D | FS×D | BW |
| C14:0 | 1.56± 0.12 | 1.63± 0.16 | 1.52± 0.24$^{A}$ | 1.56± 0.12 | 1.62± 0.01 | 1.56± 0.16$^{B}$ | 1.56± 0.12$^{a}$ | 1.86± 0.40$^{ab}$ | 2.34± 0.02$^{bA}$ | 0.000 | ns | 0.040 | 0.097 |
| C14:1 n5 | 0.04± 0.01$^{b}$ | 0.04± 0.00$^{b}$ | 0.03± 0.01$^{a}$ | 0.04± 0.01 | 0.04± 0.01 | 0.03± 0.00 | 0.04± 0.01$^{b}$ | 0.04± 0.00$^{b}$ | 0.03± 0.00$^{a}$ | 0.110 | 0.000 | 0.021 | ns |
| C15:0 | 0.21± 0.02 | 0.18± 0.02 | 0.20± 0.02$^{A}$ | 0.21± 0.02 | 0.20± 0.00 | 0.18± 0.01$^{B}$ | 0.21± 0.02 | 0.23± 0.04 | 0.24± 0.01$^{A}$ | 0.030 | ns | ns | ns |
| C16:0 | 22.58± 0.23$^{b}$ | 21.9± 0.91$^{b}$ | 20.4± 0.36$^{aA}$ | 22.6± 0.23$^{b}$ | 21.7± 0.71$^{ab}$ | 21.5± 0.70$^{aAB}$ | 22.6± 0.23$^{b}$ | 22.7± 1.24$^{b}$ | 21.0± 0.20$^{aB}$ | ns | 0.018 | ns | ns |
| C16:1 n7 | 3.96± 0.20$^{b}$ | 3.63± 0.32$^{b}$ | 3.06± 0.19$^{a}$ | 3.96± 0.20$^{b}$ | 3.53± 0.11$^{a}$ | 3.23± 0.18$^{a}$ | 3.96± 0.20$^{b}$ | 3.75± 0.10$^{b}$ | 3.39± 0.13$^{a}$ | 0.042 | 0.005 | ns | 0.023 |
| C17:0 | 0.25± 0.01 | 0.23± 0.02 | 0.24± 0.01 | 0.25± 0.01 | 0.24± 0.01 | 0.24± 0.02 | 0.25± 0.01 | 0.26± 0.02 | 0.24± 0.00 | ns | ns | ns | ns |
| C17:1 n7 | 0.23± 0.01 | 0.20± 0.01 | 0.19± 0.05 | 0.23± 0.01$^{b}$ | 0.19± 0.01$^{a}$ | 0.19± 0.01$^{a}$ | 0.23± 0.01$^{b}$ | 0.21± 0.01$^{a}$ | 0.20± 0.01$^{a}$ | ns | ns | ns | ns |
| C18:0 | 7.17± 0.23 | 7.63± 0.06$^{B}$ | 7.47± 0.62 | 7.17± 0.23$^{a}$ | 7.39± 0.16$^{abA}$ | 7.90± 0.35$^{b}$ | 7.17± 0.23$^{a}$ | 7.00± 0.27$^{aAB}$ | 7.87± 0.26$^{b}$ | ns | ns | ns | ns |
| C18:1 n9 | 29.26± 0.71 | 28.8± 0.95 | 27.8± 1.65 | 29.3± 0.71 | 28.8± 1.00 | 28.7± 0.74 | 29.3± 0.71 | 27.7± 1.90 | 27.4± 1.06 | ns | ns | ns | 0.104 |
| C18:1 n11 | 1.71± 0.12 | 1.64± 0.09 | 1.55± 0.08 | 1.71± 0.12 | 1.72± 0.01 | 1.64± 0.12 | 1.71± 0.12 | 1.77± 0.03 | 1.66± 0.05 | 0.006 | ns | ns | 0.022 |
| C18:2 n6 c | 16.76± 0.97 | 16.5± 0.55$^{A}$ | 16.8± 1.77$^{B}$ | 16.8± 0.97 | 17.5± 0.56$^{A}$ | 18.8± 1.92$^{A}$ | 16.8± 0.97$^{b}$ | 16.3± 0.53$^{bB}$ | 13.3± 0.64$^{aB}$ | 0.000 | ns | ns | ns |
| C18:2 n6 t | 0.04± 0.00 | 0.04± 0.00 | 0.04± 0.00 | 0.04± 0.00 | 0.04± 0.00 | 0.05± 0.01$^{B}$ | 0.04± 0.00 | 0.04± 0.01 | 0.05± 0.01 | ns | ns | ns | ns |
| C18:2 (9c, 11t) | 0.03± 0.01 | 0.02± 0.00 | 0.03± 0.01 | 0.03± 0.01$^{b}$ | 0.02± 0.00$^{a}$ | 0.02± 0.00$^{a}$ | 0.03± 0.01 | 0.02± 0.00 | 0.02± 0.00 | ns | ns | ns | ns |
| C18:2 (9t, 11c) | 0.25± 0.02 | 0.34± 0.14$^{B}$ | 0.43± 0.01$^{B}$ | 0.25± 0.02 | 0.32± 0.10$^{A}$ | 0.30± 0.09$^{C}$ | 0.25± 0.02$^{a}$ | 0.41± 0.18$^{a}$ | 0.64± 0.02$^{bA}$ | 0.003 | ns | ns | ns |
| C18:3 n3 | 3.09± 0.35$^{a}$ | 5.45± 1.45$^{b}$ | 6.05± 0.24$^{bC}$ | 3.09± 0.35$^{a}$ | 3.38± 0.13$^{ab}$ | 3.71± 0.18$^{bB}$ | 3.09± 0.35$^{a}$ | 3.59± 0.77$^{aA}$ | 4.91± 0.22$^{bA}$ | 0.000 | ns | ns | 0.000 |
| C20:2 n6 | 0.53± 0.03 | 0.51± 0.04 | 0.50± 0.05 | 0.53± 0.03 | 0.50± 0.03 | 0.56± 0.09$^{B}$ | 0.53± 0.03$^{b}$ | 0.51± 0.04$^{ab}$ | 0.46± 0.04$^{a}$ | ns | ns | 0.053 | 0.026 |
| C22:0 | 0.07± 0.01 | 0.07± 0.00$^{A}$ | 0.08± 0.01$^{A}$ | 0.07± 0.01$^{a}$ | 0.08± 0.00$^{aB}$ | 0.09± 0.01$^{b}$ | 0.07± 0.01 | 0.08± 0.01$^{B}$ | 0.08± 0.01$^{B}$ | 0.005 | 0.007 | ns | ns |
| C20:3 n3 | 0.53± 0.04 | 0.57± 0.04 | 0.57± 0.07$^{B}$ | 0.53± 0.04$^{a}$ | 0.55± 0.05$^{a}$ | 0.67± 0.07$^{bA}$ | 0.53± 0.04$^{b}$ | 0.58± 0.03$^{b}$ | 0.41± 0.01$^{aB}$ | 0.004 | ns | 0.001 | ns |
| C22.1 n9 | 0.00± 0.00$^{a}$ | 1.13± 0.31$^{b}$ | 1.12± 0.23$^{bB}$ | 0.00± 0.00$^{a}$ | 1.07± 0.14$^{b}$ | 1.15± 0.12$^{b}$ | 0.00± 0.00$^{a}$ | 1.58± 0.60$^{b}$ | 2.59± 0.33$^{cB}$ | 0.000 | ns | 0.009 | 0.005 |
| C20:4 n6 | 0.58± 0.09 | 0.55± 0.07 | 0.48± 0.05 | 0.58± 0.09 | 0.55± 0.07 | 0.53± 0.13 | 0.58± 0.09 | 0.56± 0.03 | 0.48± 0.04 | ns | ns | 0.036 | 0.000 |
| C22:2 n6 | 0.03± 0.00 | 0.03± 0.00 | 0.04± 0.01 | 0.03± 0.00 | 0.03± 0.01 | 0.03± 0.00 | 0.03± 0.00 | 0.03± 0.00 | 0.03± 0.00 | 0.057 | ns | ns | ns |
| C20:5 n3 | 1.23± 0.17 | 1.21± 0.24 | 1.40± 0.02$^{B}$ | 1.23± 0.17 | 1.36± 0.03 | 1.15± 0.06$^{A}$ | 1.23± 0.17$^{a}$ | 1.50± 0.40$^{a}$ | 2.19± 0.19$^{bB}$ | 0.000 | ns | 0.004 | 0.035 |
| C22:4 n6 | 0.11± 0.02 | 0.11± 0.02 | 0.09± 0.01 | 0.11± 0.02 | 0.10± 0.00 | 0.09± 0.01 | 0.11± 0.02$^{b}$ | 0.11± 0.01$^{b}$ | 0.08± 0.01$^{a}$ | ns | 0.103 | ns | 0.074 |
| C22:5 n3 | 0.79± 0.04 | 0.79± 0.06 | 0.85± 0.08$^{B}$ | 0.79± 0.04 | 0.80± 0.07 | 0.76± 0.03$^{A}$ | 0.79± 0.04$^{a}$ | 0.81± 0.12$^{a}$ | 0.99± 0.02$^{bB}$ | 0.011 | ns | 0.035 | 0.007 |
| C22:6 n3 | 4.42± 0.05 | 4.63± 0.36 | 4.98± 0.55$^{B}$ | 4.42± 0.05$^{a}$ | 4.62± 0.31$^{a}$ | 4.50± 0.21$^{aA}$ | 4.42± 0.05$^{a}$ | 4.94± 0.90$^{a}$ | 6.20± 0.14$^{bB}$ | 0.006 | ns | 0.058 | 0.021 |
| Σ saturated | 32.09± 0.24$^{b}$ | 31.9± 0.58$^{b}$ | 30.1± 0.84$^{aA}$ | 32.1± 0.24$^{b}$ | 31.5± 0.24$^{a}$ | 32.0± 0.27$^{abB}$ | 32.1± 0.24 | 31.8± 0.62 | 32.0± 0.02$^{B}$ | 0.010 | ns | 0.002 | ns |
| Σ monenoic | 35.21± 0.54 | 35.5± 1.03 | 35.2± 0.65 | 35.2± 0.54 | 35.4± 1.02 | 34.6± 0.24 | 35.2± 0.54 | 35.2± 1.62 | 34.9± 0.40 | ns | ns | ns | 0.029 |
| Σ PUFA | 28.44± 0.67$^{a}$ | 29.4± 2.55$^{ab}$ | 31.9± 0.98$^{bB}$ | 28.4± 0.67$^{a}$ | 30.0± 1.34$^{ab}$ | 30.6± 0.30$^{bA}$ | 28.4± 0.67 | 29.4± 2.01 | 29.6± 0.88$^{AB}$ | 0.097 | ns | 0.003 | 0.004 |
| Σ n3 | 9.34± 0.36 | 11.0± 3.31 | 13.2± 0.11$^{B}$ | 9.34± 0.36$^{a}$ | 10.1± 0.44$^{b}$ | 10.1± 0.37$^{abC}$ | 9.34± 0.36$^{a}$ | 10.8± 2.14$^{a}$ | 14.5± 0.13$^{bB}$ | 0.031 | ns | 0.024 | 0.001 |
| Σ n6 | 18.06± 1.05 | 17.5± 0.98 | 18.0± 2.02$^{B}$ | 18.1± 1.05 | 18.7± 0.54 | 19.4± 0.67$^{A}$ | 18.1± 1.05$^{b}$ | 17.6± 0.62$^{b}$ | 14.4± 0.64$^{a}$ | 0.000 | ns | 0.000 | 0.014 |
| Σ n6 / Σ n3 | 1.89± 0.22$^{b}$ | 1.44± 0.34$^{a}$ | 1.33± 0.07$^{aB}$ | 1.89± 0.22 | 1.83± 0.01 | 1.86± 0.01$^{A}$ | 1.89± 0.22$^{b}$ | 1.68± 0.36$^{b}$ | 1.05± 0.08$^{aC}$ | 0.001 | ns | 0.006 | 0.003 |
| Σ n9 | 29.52± 0.72$^{a}$ | 30.3± 0.52$^{ab}$ | 31.5± 1.62$^{b}$ | 29.5± 0.72 | 30.6± 0.60 | 30.5± 0.68 | 29.5± 0.72 | 29.7± 1.43 | 30.7± 0.84 | ns | 0.077 | ns | ns |
| UI | 119.31± 0.93$^{a}$ | 124.0± 8.70$^{ab}$ | 131.6± 3.86$^{bB}$ | 119.3± 0.93 | 125.8± 4.91 | 124.3± 0.39$^{B}$ | 119.3± 0.93$^{a}$ | 125.1± 8.83$^{ab}$ | 134.8± 0.76$^{bA}$ | ns | ns | 0.035 | 0.002 |
| A FA CH L | 16.93± 0.05$^{a}$ | 17.15± 0.26$^{ab}$ | 17.40± 0.02$^{b}$ | 16.93± 0.05$^{a}$ | 17.25± 0.03$^{b}$ | 17.28± 0.01$^{b}$ | 16.93± 0.05$^{a}$ | 17.22± 0.05$^{b}$ | 17.30± 0.06$^{b}$ | ns | ns | 0.062 | 0.052 |

*Testing of the fatty acid dilution model*

The dietary fatty acid incorporation process was tested with the application of the dilution model of JOBLING (2004a), worked out for carnivorous fish. The estimation was performed for the dataset obtained after 3 weeks onset of the fatty acid supplementation. Table 4 contains the determination coefficients obtained from the correlation analysis between the observed and estimated fatty acid results, for the 3 types of dietary oil supplementation. Only the major or mostly interesting fatty acids were evaluated with the application of the model, only for the cases where the dietary fatty acid component dose increased, as compared to the basal diet.

As it is visible in Table 4, the estimation was the most robust in the cases where the dietary supplementation meant an expressed elevation of the given fatty acid, i.e. ALA in the LO group, EPA in the FO group, and total n3 fatty acids in the FO and LO groups.

Table 4
Determination coefficients ($R^2$) between observed and estimated (by means of the dilution model) fatty acid data at the 3rd week of trial ($n$=5 in each case)
*Zusammenhang zwischen gemessenen und geschätzten Werten ($R^2$) nach dem Modell JOBLING (2004a, 2004b) nach der dritten Versuchswoche*

| Fatty acid | Fish oil | Soybean oil | Linseed oil |
|---|---|---|---|
| C18:3 n3 | ns | 0.42 | 0.97 |
| C20:5 n3 | 0.83 | ns | 0.85 |
| C22:5 n3 | ns | ns | ns |
| C22:6 n3 | ns | ns | ns |
| Total n3 | 0.95 | 0.56 | 0.73 |

## Discussion

*Effects of different fatty acid supplementation on the body weight gain and fillet flesh quality*

The results of the present approach aiming the partial substitution of fish oil for vegetable oils demonstrate that the weight gain of fish was not influenced negatively by the vegetable oils. In the study of WING-KEONG *et al.* (2003) on African catfish, palm oil and sunflower oil resulted in higher growth performances, as compared to high n3 PUFA diets, containing cod liver oil. A number of studies reported similar results, when relatively saturated fatty acid compositional diets led to better growth characteristics of African catfish (HOFFMAN *et al.* 1995, LEGENDRE *et al.* 1995). In contrast, Channel catfish (*Ictalurus punctuatus*) was reported to utilize fish oil better for growth, as compared to vegetable oils (MANNING *et al.* 2006).

The compositional alteration of the fillet, as induced by the divergent dietary fat types was statistically proven by the pH and in the yellow color component ($b^*$), although the between-sampling differences within the divergent treatments (i.e. SO, LO and FO) were negligible. Moreover, FLIS *et al.* (2007) also reported the alteration of the muscle $b^*$ component as a result of dietary manipulation. The fact that the effect of treatment

duration, as compared to that of fat source, was more expressed on the fillet quality traits suggests that 3 weeks on a finishing diet may not always lead to the demanded quality alterations of the fillet. This assumption is supported by the recommendations of TORSTENSEN *et al.* (2004), applying a 25-week period for the fillet fatty acid profile modification of Atlantic salmon, and also by that of BAKER (1997), reporting an 56-day treatment period for African catfish.

Possibly the mainly interesting finding on the fillet quality is the marked influence of fat source and treatment duration on the water holding capacity of fillet, as expressed by different exudative moisture losses. Though significance was not proven for the effects of fat source (only for cooking loss, $P$=0.153), the treatment duration affected the dripping loss and also the thawing loss. The tissue moisture retention ability of African catfish has been shown to be influenced by the oxidation level and PUFA proportion of the dietary fat (BAKER 1997), and by rainbow trout as well by age (WERNER *et al.* 2008). Interestingly, the dripping loss was higher in LO and FO groups at the 3rd week, as compared to the data obtained at the end of the trial (6th week). In the authors opinion, this may be a result of a longer-term adaptation, namely the one-step change to the LO and FO diets may have led to a progressive in vivo lipid peroxidation, which was later compensated by an adaptation of the antioxidant enzymes. A highly similar phenomenon was experienced by SZABÓ *et al.* (2004) in rabbit skeletal muscles, and Nile Tilapia (2007, unpublished observation), when a diet with saturated fatty acids was changed to a highly unsaturated one. Unfortunately, in the present study *in vivo* lipid peroxidation was not determined, albeit in rabbits it has been characterized by the malondialdehyde concentration of the muscle.

Concerning the shearing force of the fillet, the present results are supported by those of REGOST *et al.* (2003), reporting that total substitution of fish oil either for linseed oil or soybean oil does not measurably influence the shearing resistance of fillet, in turbot (*Psetta maxima*).

### *Effects of different fatty acid supplementation on the fillet fatty acid profile and metabolism*

Analyzing the dietary fatty acid incorporation into fillet complex lipids, there is no defined mathematical model besides the simple dilution models of ROBIN *et al.* (2003) and JOBLING (2004a, 2004b) for the interpretation of the process. Analyzing the precursor-product fatty acid relationships needs to fulfil the condition that the final fillet fatty acid profile is, at least partly, shaped by the endogenous fatty acid synthetic processes, i.e. elongation and desaturation (RUYTER *et al.* 2003). This is, however, rather true in freshwater fish, especially under intensive rearing conditions and growth (JOBLING 2004b).

The results found for ALA are interesting considering the fact that a ca. two-fold higher ALA supplementation (SO), as compared to the FO diet, induced lower tissue ALA proportions. Naturally, the ca. one magnitude higher ALA supplementation led to markedly higher tissue proportions in the LO treatment. The background of the fate of ALA may be the well-known phenomenon that ALA desaturation and elongation of fish is augmented when instead of fish oil vegetable oils are fed, as reported by BELL *et al.* (2001) in Atlantic salmon.

The effect of the high dietary provision of the precursor fatty acid (ALA) was also reflected in the tissue proportion of EPA. In the formation of the tissue EPA levels a very expressed dietary effect and the role of endogenous synthesis was supposed. The former assumption is based on the finding that double amounts of dietary EPA (FO vs. SO) led to a nearly double tissue proportion of this fatty acid in the FO fillets. In contrast, identical dietary EPA supplementation in the LO and SO groups led ultimately to higher tissue EPA levels in the LO group, suggesting the role of endogenous fatty acid transformation, based on ALA as precursor.

In the tissue proportion of DPA some slight effects of the dietary precursor (i.e. ALA) load might be supposed, since by totally identical dietary DPA, and as well EPA levels (SO and LO), the fillet in the LO treatment tended to contain higher DPA proportions, however without statistical significance. A similar tendency was supposed for DHA, where all the dietary fatty acids antecedent in the n3 biosynthetic pathway were dosed in identical proportions in the SO and LO diets, except ALA, while a significantly higher DHA level was found in the fillet fatty acid profile of the LO fish.

The present results concerning the final products of the n3 FA biosynthesis suggest that the substitution of fish oil with vegetable oils does not lead to identically beneficial fatty acid profile of the catfish fillet. In our study, however, the dietary ensured proportions of EPA, DPA and DHA were higher in the FO diet, resulting ultimately in slightly higher proportions of these acids, most probably resulting from direct incorporation.

It was hypothesized that that the relative dietary overload of a precursor does not unconditionally lead to an increased tissue proportion of the product fatty acid. Similar findings were reported by BELL *et al.* (2006), in Atlantic cod fillet, where the feeding of stearidonic acid (C18:4 n3), another n3 precursor, led to reduced levels EPA and DHA, as compared to FO. From our study it seems that high dietary n3 supplementation, as compared to the natural diets, primarily activated the steps of the n3 biosynthesis pathway, to the detriment of the n6 FAs. This latter finding was proven by the practically minimal change of the n6 FAs in the tissue, and was supported by RUYTER *et al.* (2000), reporting that the rate of conversion between LA and AA is thus influenced by the dietary balance of n3 fatty acids. Since both biosynthetic processes (i.e. n3 and n6 desaturation) are mediated by Δ6 desaturase, the large dietary ALA amount may out compete LA for the enzyme (MILLER *et al.* 2007). Moreover, according to STUBHAUG *et al.* (2005), the affinity of elongating and desaturating enzymes is higher towards the n3 fatty acid family, as compared to the n6 or n9 ones.

*Dilution model of fatty acid incorporation*

According to JOBLING (2004a, 2004b), the supplementation of finishing diets with the desired fatty acids in increased quantities may lead to precisely predictable fillet fatty acid profiles. By testing this hypothesis, we only used a relatively short feeding period, but intensive keeping conditions. In our experience, only the incorporation of the fatty acids in drastically increased dietary amounts occurs highly efficiently, as proven by the results of Table 4. The rather high accuracy of the predictions supports the hypothesis that the alteration in the fillet fatty acid profile, as induced by the dietary treatment is a

dilution (JOBLING 2004a, ROBIN *et al.* 2003). However, the endogenous fatty acid synthesis has also to be considered as a determination factor of the overall fillet fatty acid profile. Since the fat content of fillet contains rather high proportions of storage triglycerides, and fish are growing, the endogenous modification of dietary fatty acids is of secondary importance. In contrast, dietary fatty acids are more directly incorporated, with only minor elongation or desaturation (JOBLING 2004a).

*Conclusions*

The different vegetable oil supplementation, as compared to the fish oil, did not negatively affect the daily bodyweight gain, while high dietary n3 fatty acid levels were shown to augment fillet moisture loss. Analyzing the n3 fatty acid synthetic pathway it was found that an uncommonly high dietary n3 precursor level may not unconditionally implicate high n3 longchain PUFA levels in fillet of African catfish. Feeding vegetable oils, the correlative changes of fillet FA profile were well predictable for the major dietary FAs by a simple dilution model.

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