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Original research paper

FEEDING OF COMMON CARP ON FLOATING FEEDS FOR ENRICHMENT OF FISH FLESH WITH ESSENTIAL FATTY ACIDS

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ABSTRACT: Based on the formulations of the ingredient mixtures and setting up the details of the extrusion technology 4 floating complementary carp feeds were developed suitable for inclusion of high oil levels by vacuum coating technology. The experimental feeds were manufactured in pilot scale and the efficiency of oil supplementation after application of vacuum coating was checked by GC determination of feed fatty acid composition. The amounts of fortifying long chain polyunsaturated fatty acid (LcPUFA) containing oils – *Mortierella alpine* oil, containing 40% arachidonic acid in triglyceride form (ARA); eicosapentaenoic (EPA) and docosahexaenoic (DHA) ethyl esters – were as follows: Feed CF2: 6.0% Linseed oil (LSO); CF3: 3.5% LSO + 1.5% Arachidonic acid (ARA) + 1% DHA; CF4: 6.0% Fish oil (FO); CF5: 4.5% FO + 1.5% ARA. The control sinking feed (CF1) was supplemented with 6.0% Soybean oil (SBO). The fatty acid composition determined by capillary gas chromatography well reflected the fortification. The experimental feeds were tested in a feeding experiment with common carp under intensive RAS rearing conditions. The experimental feeds and the control sinking one were fed to fish in parallel groups at constant water temperature (average: 23.9±0.3 ºC), with an average oxygen saturation of 90±2 %. Fish were grown steadily, the best growth rate (SGR) and feed conversion rate (FCR) both were found in the FO fed group (1.17±0.00 %/day and 2.02±0.12 g/g). In the feeds highest levels for 18:3n3, 20:4n6 and 22:6n3 were 27.6%, 9.2%, and 11.2%, respectively. Whereas for the above mentioned fatty acids the highest levels of 7.3%, 2.3%, and 2.3%, respectively, were found in the carp fillets, the estimated EPA+DHA contents of the fillet were 5 to 12 times higher in the fillets of fish fed on fortified feeds than in those of fish fed on control feed CF1. Fortified floating feeds can be applied as complementary feed along with sufficient levels of natural food in fish ponds in suitable feeding regimes. A suitable feeding regime was suggested to apply the complementary feeds.

Key words: *extruded fish feed, fatty acid enrichment, fish flesh*

INTRODUCTION

Hungary and Serbia are similar countries in respect of fish production and fish production capacity, in fish consumption and in cardiovascular disease (CVD) mortality rates. The area for pond culture in Hungary is around 25 000 ha and roughly 70% of the produced fish is common carp (Pintér, 2009). In Serbia, the total area covered by warm water fish farms is about 12 000 ha and approximately 97 percent of the fish farms are located in the Northern part of Serbia, in Vojvodina. Common carp is the predominant species here, too (FAO, 2011). Compounded feeds are used now mainly in carp nursery ponds or in some carp cage culture units.

In the carp rearing ponds, fish are fed mainly on cereal grains like wheat, corn and barley, depending on price and local availability. However, an increased usage of pelleted feeds in carp culture is a necessity to increase the production and fish quality. This is especially important when the human nutritional value of the carp flesh is intended to be increased.

The per capita fish consumption was 5.2 kg in 2006 in Serbia (FAO, 2011) and approx 6.0 kg in live weight in Hungary (4.16 kg fish meat/per capita in 2008 in Hungary – Pintér,2009), being among the lowest in Europe. The CVD mortalities are over 50% in both countries (Knezevic & Grozdanov, 2009; Bényi, 2008).

It is well established that consumption of fish rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) favourrably affects CVD mortality (e.g. Chris-Etherton et al., 2002; Simopoulos, 2008). At present the main health concern is connected to increase the consumption of omega–3 and omega–6 long chain polyunsaturated fatty acids (LcPUFA) like arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The Global Omega–3 Summits (http://www.omega3summit.org/) dealt extensively with these issues (Omega–3 Summit, 2011, 2012, 2013). The experts also agreed that "Tissue concentrations of LC–Omega–3 (relative to LC–Omega–6) are the key variable for health not dietary intakes. Biomarkers need to be standardised and used as public health targets." An Omega–3 Index of 8–11, meaning that Omega–3 in HUFA over 50% would protect 98% of population of the industrial countries (Omega–3 Summit, 2011). The Omega–3 index is the sum of 2 prominent long-chain n–3 fatty acids [i.e. eicosapentaenoic acid (EPA) and docosahexaenoic acids (DHA)] in erythrocyte membranes and is expressed as a percentage of total erythrocyte fatty acids (FAs) as suggested by Harris and von Schacky (2004). The Global Omega–3 Summit in 2013 debated on the "Role of Omega–3 and Omega–6 in Health", on the "Importance of Ratio and Bioavailability", and on "Consumer Awareness of Omega-3s and its Benefits" (Omega–3 Summit, 2012).

Necessity of increasing the environmental sustainability of both feed and fish production in respect of lowering release of waste nutrient into the environment is also a serious concern (Naylor et al., 2000; Roth et al., 2000; Bartley et al., 2007; Ellingsen et al., 2009).

Ensuring the maintaining sustainability of polyunsaturated fatty supply is attempted by applying gene manipulation plants, for instance genes for enhanced production stearidonic acid (18:4n–3) and EPA and DHA were introduced into various oilseed production plants (Nichols et al., 2010). Food grade arachidonic acid produced in *Mortierella alpine* is approved for trading in European Union (Nr. 2008/968/EC Commission Decision).

In the present study we planned to apply an extrusion technology for carp feed production from selected ingredients and with precise setting of the extrusion parameters for conditioning and extrusion. Efficiency was planned to be tested by feeding the new, floating type feed for carp supplemented with omega–3 and omega–6 fatty acids (FAs) with proved health benefits (Del Prado et al., 2001; Chris-Etherton et al., 2002; Harris and von Schacky 2004; Harris, 2008; Harris et al., 2009;). The health benefits of the improved quality of the produced carp meat in respect of cardiovascular diseases was planned to be studied in a mammalian animal model (rat). As common carp is widely consumed in both Hungary and Serbia, it is expected that our results – when applied in the practice of carp culture – can contribute to lowering the risk of cardiovascular deceases in the region.

MATERIALS AND METHODS

Proximate analysis methods for determination of the gross nutrient contents of feed ingredients, feeds and fillets

Gross nutrient content data of feed ingredients, feeds and fillets were determined by standard methods with modifications in some cases.

Table 1.
Proximate composition (g/kg) and metabolisable energy content of experimental feeds

Feeds	CF1 Sinking control (SBO)	CF 2 Linseed oil (LSO)	CF 3 LSO+ ARA+DHA	CF 4 Fish oil (FO)	CF 5 FO+ARA
Dry matter	931	941	936	939	936
Crude protein	310	327	322	322	323
Crude fat	80	78	64	75	67
Crude fiber	28	19	19	24	21
Crude ash	91	65	68	70	66
N-free extract	422	452	463	447	459
Starch	278	217	245	218	272
NDF	104	105	99	93	118
ADF	28	21	21	24	32
ADL	7	8	11	12	9
ME (MJ/kg)	12.68	13.52	13.05	13.21	13.22

Shortly, dry matter content was determined by oven drying at 105 ºC for 4 hours. The oven dried samples were combusted on ceramic hot plate, than the crucibles were placed into muffle furnace for crude ash determination at 550 ºC for 4 hours.

Crude protein was determined by the Kjeldahl–method using digestion block (SPEED DIGESTOR K–436, Buchi, Switzerland) and distillation method (DISTILLATION UNIT K–350, Buchi, Switzerland). Ammonia was titrated through the titration of the residual sulfuric acid (0.05 M) by NaOH (0.1 M). Crude fat was determined by Soxhlet method using semi–automatic system (SOXTEC HT 1043, Tecator, Denmark) and petroleum ether of 40–60 ºC boiling range. Crude fiber content was determined directly from low fat samples. If the presumable fat content was higher than 10%, the determination was done after the extraction of fats with chloroform. The samples were hot digested consecutively for 30 min with sulfuric acid (0.26 mole/liter) and 30 min with potassium hydroxide (0.23 mole/liter), then filtered onto filter crucibles, dried and weighed.

The proximate composition of the experimental feeds presented in Table 1 were determined by AOAC (2005) methods and metabolizable energy contents according to Csengeri and Majoros (2004).

Experimental fish

The experimental fish were collected in December of 2012 then quarantined and upon arrival to the RAS system special antibacterial and antiparasitic treatments were applied with formalin and salt solutions. The carefully selected and acclimatized fish were then weighted and experimental groups distributed into the compartments rearing tanks (12 individuals/compartments). The feeding experiment was performed in 2.0 m^3 plastic square–shaped tanks divided into two compartments (about 1.0 m^3 each) by a screen wall equipped along the one diagonal of each tank. The tanks were covered by wide–meshed net to avoid jumping out of the fish from the rearing containers. Water inflow into the tanks was regulated to ensure oxygen concentration of the outflow water at least 80% of saturation. This required – depending from water temperature, fish biomass and daily feeding rate – a turnover rate about 2 times per hour. During the experiment water temperature and oxygen saturation were measured twice per day (at about 8 and 19 hour), water chemistry analysis were performed twice per day.

Initially all diets was applied at a daily amount of 90% of the previously determined maximal consumption. This value was converted to % of metabolic fish weight (kg $^{0.8}$) and further this feeding rate was be used for the calculation of the daily ration according to the new weight attained at the end of each week. Fish was weighed at weekly intervals in anesthetised condition. Feeding was performed by

automatic belt feeders during 10 hours per day. Each day at morning uneaten feed residues and faeces was siphonned from each tank and tank bottoms and walls was cleaned from bio-films. Fish behaviour and health was inspected continuously and in needed cases adequate medical treatments was applied either locally on the body surface and/or in a form of bathing in the solution of the requested substances.

In the carp feeding experiments five soybean meal based (36%) diets were tested. The diets were formulated as listed above to have approximately 30-32% final protein and 8-8.5% final oil contents. Each diet was supplied to duplicate groups.

The growth and feed conversion efficiency for the weekly periods, as well as for the whole duration was characterised by daily growth rate (SGR) and feed conversion rate (FCR) as follow:

$$SGR = 100 \times (\ln w_t - \ln w_0) \times t^{-1} \quad (\%.day^{-1})$$

$$FCR = F \times (W_t - W_0)^{-1} \quad (g.g^{-1})$$

where w_0; w_t – initial and final average weight; W_0; W_t – initial and final biomass; t – duration of the investigated period in days.

The effect of different feed types on fish condition was evaluated by the condition factor (CF) as follows:

$$CF = 100 \times w_t \times L_t^{-3} \quad (\%)$$

where l_t – total length of fish (cm)

The effect of different feed types on size distribution was characterised with the proportions (H) of the coefficients of variation (CV) of the initial and final average body weights:

$$H = 100 \times CV_t \times CV_0^{-1} \quad (\%)$$

Additionally the effect of different feeds on slaughtering proportion (SP) was calculated as proportion of obtained fillet weight (*Fil*) to life fish weight as follows:

$$SP = 100 \times Fil \times w_t^{-1} \quad (\%)$$

Treatment effects was examined for statistically significant differences in dw/dt; SGR, FCR, CF and H by one–way analysis of variance (one–way ANOVA).

During each week of the experimental period all groups received the same daily feeding rates, which were in the range of 2.5–3.0% of the metabolic weight (kg $^{0.8}$). These values were determined considering 1) the previously obtained production performance data; 2) overall health condition and behaviour of the fish; – and mainly - 3) to attain about 1 kg average body weight in each group at the end of the 8 weeks lasted treatments.

The study was conducted in accordance with the European Community guidelines on the care and use of experimental animals and according to the respective Hungarian rules (243/1998. (XII. 31.) Korm. rend. and 66/2009. (IV. 2.) Korm. rend.) and to the local institutional regulation (Regulation on Animal Experiments: Ig/258-3/2002). The experimental protocol was submitted to the Institute Committee of Animal Experiments at HAKI (Munkahelyi Állatkísérleti Bizottság – MÁB, Szarvas, Hungary).

Determination of fatty acid composition

Before lipid extraction all feed samples were grinded on laboratory mill with cooling (FOSS Knifetec™ 1095, Tecator technology, Denmark). Lipids were extracted from the samples of 5 grams by using cold extraction process, which involves mixing/homogenizing with chloroform: methanol mixture (2:1) and the extracts were purified according to the method by Folch et al. (Folch et al., 1957). Aliquots of 20 mg of total lipid samples were trans-esterified by method that uses 14% (w/w) boron trifluoride/methanol solution (Sigma Aldrich, MO, USA).

The fatty acid composition of the carp feed total lipids were determined in Folch's extracts (Folch et al., 1957) after esterification in BF_3-Methanol (Metcalfe and Schmitz, 1961). Obtained fatty acid methyl esters (FAME) samples were analyzed by gas chromatography in an Agilent 7890A system (Agilent Technologies, Santa Clara, CA, USA) equipped with flame ionization detector (FID) and auto-injection module for liquid samples. FAME samples

were injected in n–heptane solution with a split ratio of 30:1 onto a fused silica capillary column (SP-2560, 100 m x 0.25 mm I.D., 0.20 µm; ,SUPELCO, Bellefonte, NY, USA). Carrier gas was high purity Helium at a constant flow rate of 1.5 ml/min. Column temperature was programmed: 140 °C for 5 min – 3 °C/min up to 240 °C – hold for 10 min. Data acqui-sition and integration was done by using the software of Agilent 7890A system. The fatty acid methyl ester peaks were identified by comparison of retention times with retention times of peaks from standard fatty acid methyl ester mix (FAME Mix, Cat. no. 4–7885; SUPELCO, Bellefonte, NY) and with data from internal data library, based on previous experiments and fatty acid methyl ester determination on GC–MS.

For quantification of the fatty acid (FA) composition, the FID detector signals were integrated and from the peak areas of FAME, the fatty acid composition was calculated and expressed as g FA per 100 g of FA according to method described by Karlović and Andrić (1996).

The fatty acid composition of the total lipids of the carp fillet samples were determined after esterification in t–butyl–methyl–ether with trimethyl–sulfonium hydroxide /TMSH/. Trimethyl–sulfonium hydroxide reproducibly converts fatty acids of phospholipids and acyl–glycerols into fatty acid methyl esters (FAME) and the transesterification can be performed at room temperature (Müller et al., 1993). The obtained fatty acid methyl esters samples were analyzed by gas chromategraphy in a Thermo FOCUS GC system (Thermo Scientific, Part of Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with flame ionization detector (FID) and with AI 3000 autoinjector. FAME samples of 1 µl were injected with a split ratio of 100:1 onto a fused silica capillary column (TRACE TR-FAME GC Column – 60 m x 0.25 mm x 0.25 µm; Thermo Scientific). Carrier gas was high purity Helium at a constant flow of 20 cm/sec.

Column temperature was programmed from 140 °C (hold for 5 min) – 4 °C/min up to 240 °C. Data acquisition and integration was done by using the software of Thermo FOCUS system.

SFA, MUFA, etc. fatty acid groups includes all of the identified FAs belonging to their groups.

Statistics

One–way analysis of variance (ANOVA Tukey–tests) was applied to explore significant differences between feed performance and fillet fatty acid composition data.

RESULTS AND DISCUSSION

Formulation and manufacturing of experimental feeds

Based on the literature data proving crucial role of long chain polyunsaturated fatty acids (LcPUFAs) in human metabolism and their relation to the prevention of cardiovascular diseases, the experimental feeds were planned to provide enriched levels of LcPUFAs in the carp flesh for human nutrition as well as for the planned animal model experiments. One sinking pellet and 4 extruded–expanded pellets supplemented with different oils were designed for using as complementary feeds in pond culture of common carp (*Cyprinus carpio*). Basic feed formulations were obtained by the linear programming with updated composition data using the SOLVER program of the EXCEL's Tools (Microsoft Office Excel 2003 SP3) adapted to feed formulation (Csengeri, 1999). After evaluation of the manufacturing possibilities and comerciallization aspects, a basic ingredient mixture was formulated which contained 36% of defatted soybean meal as major component (Table 2).

The calculated composition of basic formula was as follows: 34.25% crude protein, 2.79 % crude fat, and 3.86% crude fiber with lysine 1.92%, methionine 0.68%, meth+cys 1.2%.

Fish feed was produced on twin-screw extruder (Mu Yang MY 90, China) with a screw diameter of 85 mm, length–to–diameter ratio of 20:1, and maximum temperature of 135 °C. Extruder was equipped with differential diameter conditioner (DDC).

Table 2.
Basic soybean meal based formula

Ingredient	Percentage
Soybean meal	36
Corn	30
Wheat	10
Corn gluten	10
Fish meal (70% of protein	10
Yeast	3
Premix	1

The used linseed oil and fish oil were obtained from local Serbian market. The fortifying oils – *Mortierella alpine* oil, containing 40% arachidonic acid (ARA); eicosapentaenoic (EPA) and docosahexaenoic (DHA) ethyl esters – were supplied by PRAEVIDEO Kft. (Budapest, Hungary).

The amounts of the added oils were as follows: CF2: 6.0% Linseed oil (LSO); CF3: 3.5% LSO + 1.5% Arachidonic acid (ARA) + 1% DHA; CF4: 6.0% Fish oil (FO); CF5: 4.5% FO + 1.5% ARA. The control sinking feed (CF1) was supplemented with 6.0% Soybean oil (SBO).

Addition of oil was done on laboratory vacuum coater (model F–6–RVC, Forberg International AS, Norway) with capacity of 6 L per batch.

The floating feeds were developed for inclusion of oils with high levels of long-chain polyunsaturated fatty acids. The applied extrusion technology involved a process of cooking steamed ingredients under pressure at elevated temperature.

The planned amounts of the added oils (CF2: 6.0% Linseed oil /LSO/; CF3: 3.5% LSO + 1.5% Arachidonic acid /ARA/ + 1% DHA; CF4: 6.0% Fish oil /FO/; CF5: 4.5% FO + 1.5% ARA.; control sinking feed, CF1: 6.0% Soybean oil /SBO/) were reflected in the fatty acid compositions of the developed floating feeds (Table 3).

Raw material preparation plays an important role in the final production of extruded aquaculture feeds therefore preliminary measurement were made to determine the composition of the ingredients which were then finely grinded.

Advantages of the extrusion cooking process for aquaculture feed production include the control of pellet density, increased feed conversion rate, greater feed stability in water, and better production efficiency and versatility. Extrusion is gradually replacing the pelleting systems, where only heavy density sinking aquaculture feeds can be processed. The pellet-mill provides a very low cook on the formula such as 20–25% starch gelatinisation (gelatinisation starts at around 55–70 °C (Coultate, 1989)), while the extruder has yielded cook value of more than 90% starch gelatinization (Kiang, 1999).

Preconditioning at a moisture content of 25% for 150 seconds will be resulted in a degree of cook of 33%. Increasing the moisture level to 30% will be resulted in a degree of cook of 47%. These are typical moisture and associated degrees of cook levels achieved in the DDC cylinder (Kearns, 1989). Floating feeds typically are in the density range of 320 to 400 grams per litre (Kearns, 1993). Floating feeds result in better feed conversion due to the fact that the feed consumption can be monitored and adjusted to decrease feed wastages. Sinking aquatic feeds are typically in the density range of 400 to 600 grams per litre. Sinking aquatic feeds are designned to feed slow eating bottom feeding species. Slow sinking aquatic feeds are typically in the density range of 390 to 410 grams per litre (Kearns, 1993). These types of feeds are being used in the salmon production, especially in the floating cages in the ocean. The slow sinking feeds then have a better chance to be consumed prior to their exiting the net bottom.

The 4 applied extruded feeds and the control sinking one (pellets size: 9 mm diameter) were fed to common carp specimens in parallel groups at constant water

temperature with an average value of 23.9±0.3ºC, with an average oxygen saturation of 90±2%. The measured water chemistry parameter (pH; KOI; NH_4–N; NO_2–N; NO_3–N) remained in the optimal range for carp rearing.

The growth pattern of the fish was rather regular; continuous, non–interrupted weight increases were observed in all groups. During 7 weeks of the experiment weights were increased satisfactorily; by about 60–70% per treatment relatively to the initial weight.

The production performance data are summarized in Table 4. By SPSS one–way analysis of variance (ANOVA test) significant differences were found between the values of total length (L_t), slaughterring proportion (SP) and daily growth rate (SGR), but not significant differences were revealed among the other parameters.

The highest daily growth rate (SGR) was measured in group fed with fish oil based feed.

Table 3.
Percentage of main fatty acids in experimental carp feeds

Feeds	CF1 Sinking control (SBO)	CF 2 Linseed oil (LSO)	CF 3 LSO+ ARA+DHA	CF 4 Fish oil (FO)	CF 5 FO+ ARA
Fatty acid	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD
14:0	0.43±0.02	0.40±0.03	0.47±0.03	2.96±0.13	2.70±0.12
16:0	11.46±0.14	8.06±0.11	7.91±0.16	12.72±0.25	12.32±0.20
16:1	0.59±0.08	0.62±0.07	0.56±0.09	3.32±0.09	2.97±0.13
18:0	4.70±0.11	3.43±0.10	4.18±0.04	3.08±0.09	4.29±0.22
18:1n9c	23.52±0.23	22.85±0.13	17.86±0.20	24.06±0.27	21.20±0.20
18:2n6c	47.42±0.20	32.83±0.32	25.78±0.23	19.85±0.24	19.11±0.19
18:3n3	7.08±0.07	27.57±0.24	17.17±0.23	4.52±0.14	2.62±0.10
20:4n6	0.11±0.04	0.16±0.09	7.56±0.23	0.47±0.06	9.22±0.19
20:5n3	0.36±0.04	0.43±0.09	1.09±0.10	4.14±0.19	3.66±0.07
22:6n3	0.80±0.07	0.97±0.06	11.21±0.23	7.32±0.20	6.23±0.09
SFA	17.43±0.47	12.46±0.36	13.76±0.39	19.37±0.55	20.70±0.67
MUFA	26.14±0.58	25.02±0.47	20.71±0.81	34.96±1.03	30.39±0.86
PUFA	56.43±0.47	62.52±0.91	65.53±1.14	45.67±0.98	48.91±0.79
PUFA/SFA	3.24	5.02	4.76	2.36	2.36
∑ n-6	48.19±0.29	32.99±0.52	34.10±0.58	26.86±0.45	33.88±0.53
∑ n-3	8.24±0.18	28.97±0.39	30.11±0.56	17.85±0.53	14.16±0.26
n-6/n-3	5.85	1.14	1.13	1.51	2.39

Table 4.
Production performance of common carp fed with different feeds [1]

Feeds	CF1 Sinking control (SBO)	CF 2 Linseed oil (LSO)	CF 3 LSO+ ARA+DHA	CF 4 Fish oil (FO)	CF 5 FO+ ARA
w_t (g)	959±36 [a]	984±55 [a]	933±21 [a]	1028±10 [a]	974±31 [a]
L_t (cm)	35.4±0.3 [ab]	36.0±0.1 [ac]	35.3±0.1 [b]	36.2±0.3 [c]	35.7±0.2 [abc]
CF_t (%)	2.15±0.04 [a]	2.15±0.12 [a]	2.14±0.05 [a]	2.18±0.02 [a]	2.17±0.08 [a]
H (%)	197±32 [a]	216±60 [a]	200±63 [a]	206±23 [a]	204±14 [a]
SP (%)	39.5±1.1 [ac]	41.1±0.1 [ab]	41.9±0.5 [b]	38.9±0.3 [c]	42.2±0.6 [b]
SGR (%.day^{-1})	1.05±0.04 [ab]	1.06±0.09 [ab]	0.97±0.05 [a]	1.17±0.00 [b]	1.05±0.06 [ab]
FCR (g.g $^{-1}$)	2.22±0.10 [a]	2.20±0.25 [a]	2.36±0.08 [a]	2.02±0.12 [a]	2.32±0.22 [a]

[1] The initial weight, length and condition factor of the fish were 578±5 g; 32.4±0.3 cm, and 1.79±0.04 %, respectively.

[2.] Values are means ± SD of two replicates. Values by row with different letters are significantly different ($P < 0.05$).

Table 5.
Proximate composition of pooled fillets samples obtained from experimental fish

Feeds fed to fish	CF1 Sinking control (SBO)	CF 2 Linseed oil (LSO)	CF 3 LSO+ ARA+DHA	CF 4 Fish oil (FO)	CF 5 FO+ARA
Contents in the fillets (g/100 g)					
Dry matter	29.98	30.80	30.71	29.65	30.93
Crude protein	17.66	18.31	17.23	18.24	17.33
Crude fat	8.10	11.15	10.84	6.40	7.12
Crude ash	1.16	1.09	1.10	0.97	1.10

Table 6.
Percentage of main fatty acids in carp fillets [1]

Feed groups	CF1 Sinking control	CF 2 Linseed oil (LSO)	CF 3 LSO+ARA+DHA	CF 4 Fish oil (FO)	CF 5 FO+ARA
Fatty acid	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD
14:0	0.88±0.02 [a]	0.91±0.01 [a]	0.90±0.01 [a]	1.73±0.03 [b]	1.50±0.08 [c]
16:0	16.73±0.10 [a]	15.80±0.10 [a]	16.33±0.46 [a]	18.34±0.25 [b]	17.62±0.28 [b,a]
16:1	4.94±0.05 [a]	5.56±0.06 [a]	5.81±0.30 [a]	6.95±0.26 [b]	6.20±0.59 [a]
18:0	5.25±0.18	4.59±0.18	4.74±0.28	4.49±0.13	4.66±0.27
18:1n9c	41.04±0.34	41.79±0.12	41.89±1.36	41.69±0.28	42.60±0.80
18:2n6c	21.17±0.16 [a]	15.17±0.08 [b]	13.96±0.16 [c]	11.88±0.04 [d]	11.49±0.04 [d]
18:3n3	1.76±0.04 [a]	7.28±0.38 [b]	4.17±0.10 [c]	1.22±0.02 [a,d]	1.04±0.04 [d]
20:4n6	0.75±0.07 [a]	0.44±0.00 [b]	1.85±0.01 [c]	0.46±0.03 [b]	2.26±0.09 [d]
20:5n3 (EPA)	0.06±0.06 [a,c]	0.19±0.05 [a,b]	0.15±0.00 [a,c]	0.31±0.00 [b,c]	0.25±0.03 [c]
22:6n3 (DHA)	0.21±0.04 [a]	0.75±0.07 [b]	2.34±0.04 [c]	2.17±0.21 [c]	1.88±0.19 [c]
SFA	23.93±0.04 [a]	22.24±0.08 [b]	22.87±0.21 [a,b]	25.53±0.20 [c]	24.80±0.57 [c]
MUFA	50.04±0.35 [a]	51.61±0.01 [a,b]	52.24±0.95 [b]	55.06±0.25 [c]	54.99±0.34 [c]
PUFA	25.22±0.27 [a]	25.07±0.09 [a]	23.77±0.28 [b]	17.46±0.30 [c]	18.43±0.47 [d]
PUFA/SFA	1.05±0.01 [a]	1.13±0.00 [a,b]	1.04±0.02 [a,c]	0.68±0.01 [d]	0.74±0.04 [d]
∑ n-6	23.06±0.25 [a]	16.36±0.16 [b]	16.63±0.14 [b]	13.09±0.11 [c]	14.61±0.07 [d]
∑ n-3	2.17±0.02 [a]	8.71±0.25 [b]	7.14±0.14 [c]	4.38±0.19 [d]	3.82±0.40 [d]
n-6/n-3	10.65±0.01 [a]	1.88±0.07 [b]	2.33±0.03 [b]	2.99±0.11 [c]	3.84±0.38 [d]
EPA+DHA fortification (%)	100	490	1280	750	720

[1] Values are means±SD of two replicates. Values in rows with same letters are not significantly different (P<0.05).

However, – rather surprisingly – the slaughterrring proportion (SP) was the lowest in this group. It is supposed that this phenomenon could be explained mainly with the fat deposition either in the body cavity, or in other internal organs (i.e. liver, kidney etc) – but the measurement of these parameters were not performed at the fish processing time.

There are few literature data regarding the protein content in common carp feeds for rearing them exclusively on dry diets. However, all these data suggest that feeds for intensive rearing of this species should have protein higher than 35%. Therefore, it seems that our feeds with around 32% of crude protein can be applied as complementary feed in pond culture conditions in presence of natural food.

During the present experiment the fish were fed at fixed ration level at constant temperature of around 24 ºC. In the rearing season in fish ponds, in Hungarian and Serbian climate conditions, the water

temperature is generally lower than this in April, May and late August and September, October.

In fish pond culture of carp usually *ad libitum* feeding is applied fitted to the varying water temperature. Furthermore, the natural food availability is uneven during the season (see for example Ruttkay, 2000), therefore, the beneficial effect of the floating feeds can be expected.

The measured crude fat contents of the carp fillets were in the range of 6.4 to 11.1% (Table 5) as expected from earlier observations (Csengeri et al., 2009; 2011). The fatty acid composition of fillets (Table 6) reflected that of the experimental feeds (*cf*. Table 3). The fatty acid composition, however, differs from the planned/expected values. The differences are probably due to the late start of carp feeding experiment: fish fed on traditional cereal feeding prepared themselves for overwintering with oleic acid deposition and the oleic acid diluted the deposited LcPUFA amounts. The protein to energy ratio of carp feeds lower then the optimal for intensive feeding, was planned for oil enrichment of carp fillets under practical pond conditions.

Although, under the presented intensive rearing conditions, the expected high LcPUFA fortification levels were not well manifested, calculating the EPA+DHA content of the fillet from the data for crude fat (Table 5) and fatty acid composition (Table 6), 5 to 13 times enrichment can be seen in the fillets of fish fed on fortified feeds as compared to control feed fed group (Table 6).

When the fortified floating feeds will be applied along with sufficient levels of natural food in the fish ponds in a suitable feeding regime, the required enrichment of carp flesh will be achieved. For a suitable feeding regime we suggest to apply the complementary feeds to such rearing conditions, in which the natural food supply is high for the whole spring and summer, and the application of the long chain polyunsaturated fatty acid fortified feeds would start in mid of August and it would last until the water is “warm” enough for the fish to take food and till harvesting. The higher food conversion ratios (FCR) observed in this intensive rearing experiment (Table 4) will then be well compensated. Another improvement of the FCR would come from the control of dosage through the observable food consumption of the fish. Further experiments under pond fish conditions would confirm these expectations.

CONCLUSIONS

The formulations of the suitable ingredient mixture and details of the extrusion technology were designed to develop 4 floating carp feeds suitable for inclusion of high oil levels by vacuum infusion. The experimental feeds were manufactured and the oil supplementation was done and feed fatty acid composition well reflected the fortification. The experimental feeds were tested under intensive rearing conditions. Although the expected high LcPUFA fortification levels were not well manifested, however, the estimated EPA+DHA contents of the fillet were 5 to 12 times higher in the fillets of fish fed on fortified feeds than in those of fish fed on control feed.

Fortified floating feeds will be applied as complementary feed along with sufficient levels of natural food in the fish ponds in suitable feeding regimes. For a suitable feeding regime we suggest to apply the complementary feeds to such rearing conditions in which the natural food supply is high for the whole spring and summer, and the application of the long chain polyunsaturated fatty acid fortified feeds will start in mid of August and it would last until the autumn harvesting.

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REFERENCES

1. AOAC. Official Methods of Analysis of AOAC International, 18th ed. Association of Official Analytical Chemists, Washington DC, USA (2005).

2. Bartley, D.M., Brugère, C., Soto, D., Gerber, P., Harvey, B. (eds). (2007). Comparative assessment of the environmental costs of aquaculture and other food production sectors: methods for meaningful comparisons. FAO/WFT Expert Workshop. 24–28 April 2006, Vancouver, Canada. *FAO Fisheries Proceedings. No. 10. Rome, FAO.* 2007. 241p. – ftp://ftp.fao.org/docrep/fao/010/a1445e/a1445e.pdf;
3. Bényi, Mária (Ed.) (2008). NÉPEGÉSZSÉGÜGYI JELENTÉS – 2008. *ANTSZ-OSZMK nem–fertőző betegségek epidemiológiája osztálya,* Budapest
4. Chris–Etherton, P.M., Harris, W.S. & Appel, L.J. (2002). Fish consumption, fish oil, omega–3 fatty acids, and cardiovascular disease. *Circulation*,*106*, 2747-2757.
5. Coultate, T.P. (1989). FOOD – The Chemistry of Its Components. 2nd Ed. Royal Society of Chemistry, London, pp. 28–35.
6. Csengeri I., Gál D., Kosáros T., Pekár F., Bakos J., Potra F., Kovács Gy., Feledi T., Fazekas J., Biró J., J. Sándor Zs., Gy. Papp Zs., Jeney Zs., Rónyai A. (2011). A haltakarmányozás halliszt és halolaj nélkül? (Fish feeding without fishmeal and fish oil?). *Állattenyésztés és Takarmányozás*, *60 (3),* 281–294.
7. Csengeri, I. (1999). Feed formulation. In. Csengeri, I., Gy. Papp, Zs., Verreth, J., van Weerd, J.H., Pócsi, L., Majoros, F., Lanari, D.. (Eds.): Fish Nutrition. Lecture notes; *EC INCOFIT project: incoFIT Tempus S Jet No 11266–96*, Debrecen–Szarvas–Wageningen –Udine; 1999. pp. 25 + program attachments.
8. Csengeri, I., Majoros F. (2004). 6. Halak táplálóanyag–szükséglete. (Nutrient requirements of fishes – In Hungarian) In: Magyar Takarmánykódex Bizottság: MAGYAR TAKARMÁNYKÓDEX Vol. II. Gazdasági állatok táplálóanyag–szükséglete, takarmányok kémiai összetétele és mikotoxin határértékek a takarmánykeverékekben. *OMMI*, Budapest, 2004. pp. 88–93. (ISBN 963 86097 5 3)
9. Csengeri, I., Potra, F., Fazekas, J., Rónyai, A. (2009). Halolaj és növényi olajok hatása a ponty növekedésére és a filé esszenciális zsírsav tartalmára. (In Hungarian) (Effect of fish and vegetable oils on the growth and fillet fatty acid contents). 33rd. National Conference on Fisheries Science, May 27-28, 2009, *HAKI*, Szarvas, Hungary book of abstracts, p. 30–31. – lecture abstract)
10. Del Prado M, Villalpando, S., Elizondo, A., Rodríguez, M., Demmelmair, H., Koletzko, B. (2001). Contribution of dietary and newly formed arachidonic acid to human milk lipids in women eating a low–fat diet. *Am J Clin Nutr*., *74(2)*, 242–7.
11. Ellingsen, H., Olaussen, J.O., Utne, I.B. (2009). Environmental analysis of the Norwegian fishery and aquaculture industry – A preliminary study focusing on farmed salmon. *Marine Policy, 33,* 479–488.
12. European Union 2008. Commission Decision Nr. 2008/968/EC authorising the placing on the market of arachidonic acid–rich oil from *Mortierella alpina* as a novel food ingredient. *European Union Commission,* Brussels.
13. FAO (2011). National Aquaculture Sector Overview Serbia. *FAO*, Rome. http://www.fao.org/fishery/countrysector/naso_serbia/en
14. Folch, J., Lee, M., Sloane Stanley, G.H. (1957). A simple method for isolation and purification of total lipids from animal tissue. J*. Biol. Chem*., 226**,** 497–509.
15. Harris, W.S. (2008). The omega–3 index as a risk factor for coronary heart disease. *Am J Clin Nutr.*; *87(suppl),* 1997S–2002S.
16. Harris, W.S., Mozaffarian, D., Rimm, E., Kris-Etherton, P., Rudel, L.L., Appel, L.J., Engler, M.M.. Engler, M.B,. and Sacks, F. (2009). Omega–6 Fatty Acids and Risk for Cardiovascular Disease: A Science Advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation, 119,* 902-907. http://circ.ahajournals.org/cgi/content/full/119/6/902
17. Harris, W.S., von Schacky, C. (2004). The Omega–3 Index: a new risk factor for death from coronary heart disease? *Preventive Med., 39,* 212–220.
18. Karlović, Đ., Andrić, N. (1996). Kontrola kvaliteta semena uljarica. *Tehnološki fakultet*, Novi Sad, 1996
19. Kearns, J. (1989). Preparation of fish and shrimp feeds. In: T.H. Applewhite (ed.) Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs. *The American Oil Chemists Society*, 1989, pp. 152–161.
20. Kearns, J. (1993). Extrusion of aquatic feeds. *Extrusion Communique*, *6(4),* 7–8.
21. Kiang, M-J. (1999). The principles of extruding fish feeds. *Feed Tech*., 3(6), 48–50.
22. Knezevic, Tanja, Grozdanov, Jasmina (Eds.) (2009). Health of Population of Serbia – Analytical Study 1997–2007. *Institute of Public Health of Serbia*, Belgrade, ISBN 978–86–7358-048–7, pp. 176.
23. Metcalfe, L.D., Schmitz, A.A. (1961). The Rapid Preparation of Fatty Acid Esters for Gas Chromatographic Analysis. *Anal. Chem*., *33***,** 363–364.
24. Müller, K. –D., Nalik, H. P., Schmid, E.N., Husmann, H. and Schomburg, G. (1993). Fast identification of mycobacterium species by GC analysis with trimethylsulfonium hydroxide (TMSH) for transesterification. *J. High Resol. Chromatogr. 16,* 161–165.
25. Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M. (2000): Effect of aquaculture on world fish supplies. *Nature*, *405,* 1017-1024.

http://www.eve.ucdavis.edu/catoft/eve101/Protected/PDF/lit/Naylor_etal_2000.pdf),

26. Nichols, P.D., Petrie, J., and Singh S. (2010). Review on the Long–Chain Omega–3 Oils–An Update on Sustainable Sources. *Nutrients*, *2,* 572–585; doi:10.3390/nu2060572
27. Omega–3 Summit, (2011). The Global Omega–3 Summit on Nutrition, Health and Human behaviour – Sustainable Lc-Omega–3 for a Better World – Consensus statetment http://home.scarlet.be/~tpm12374/omega3summit/pdf/ConsensusStatements.pdf)
28. Omega–3 Summit, (2012). GLOBAL OMEGA–3 SUMMIT 2012 - Optimal Omega-3 Intake from Sustainable Sources (http://www.omega3summit.org/pdf/omega3summit2012.pdf)
29. Omega–3 Summit, (2013). OMEGA–3 SUMMIT 2013 – Product Availability and Purity – How to Influence Policy and Grow Consumer Awareness (http://www.omega3summit.org/pdf/omega3summit2013.pdf)
30. Pintér, K. 2009. Magyarország halászata. *Halászat (Budapest)*, *102*, 49–54.
31. Roth, E., Rosenthal, H., Burbridge, P. (2000). A discussion of the use of the sustainability index: 'ecological footprint' for aquaculture production. *Aquat. Living Resour*. *13,* 461–469.
32. Ruttkay A. (2000). Fish feeding research in Hungary – 1895-1995. In: I. Csengeri, A. Szító, Zs.Gy. Papp, and A.G.J. Tacon (eds): Fish and Crustacean Nutrition Methodology and Research for Semi–intensive Pond-based farming Systems. HALÁSZATFEJLESZTÉS 23 – *Fisheries Development, 23*, *HAKI,* Szarvas, Hungary, pp. 21–41.
33. Simopoulos, A.P. (2008). The importance of the omega–6/omega–3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp. Biol. Med., 233,* 674–688.

ИСХРАНА ШАРАНА ПЛУТАЈУЋОМ ХРАНОМ У ЦИЉУ ОБОГАЋЕЊА РИБЉЕГ МЕСА ЕСЕНЦИЈАЛНИМ МАСНИМ КИСЕЛИНАМА

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Сажетак: На бази формулисања смесе сировина и параметара екструдирања, развијене су 4 плутајуће хране за шарана погодне за додавање високих нивоа уља коришћењем технологије вакуум замашћивања. Експериментална храна је произведена у пилот погону, а ефикасност додавања уља применом вакуум замашћивања је проверена одређивањем масних киселина хране методом гасне хроматографије. Количине додатих уља која садрже полинезасићене масне киселине дугачког ланца (LcPUFA) – *Mortierella alpine* уља са 40% арахидонске киселине (ARA) у форми триглицерида и етил естара еикосапентаноичне (EPA) и докосахексаноичне (DHA) киселине, су биле следеће. Храна CF2: 6.0% ланеног уља (LSO); CF3: 3.5% LSO + 1.5% арахидонске киселине (ARA) + 1% DHA; CF4: 6.0% рибљег уља (FO); CF5: 4.5% FO + 1.5% ARA. У контролну тонућу храну (CF1) је додато 6.0% сојиног уља (SBO). Произведена храна је тестирана у огледу на шарану гајеном у интензивним условима. Шаран је храњен експерименталном плутајућом и контролном тонућом храном у паралелним групама при константној температури воде (просечно 23.9±0.3 ºC) и са просечном засићеношћу кисеоником од 90±2%. Најбољи прираст (SGR) и конверзија хране (FCR) су били у групи која је добијала храну са рибљим уљем (1.17±0.00%/дан and 2.02±0.12 g/g). У храни највиши нивои за 18:3n3, 20:4n6 и 22:6n3 масне киселина су били 27.6%, 9.2% и 11.2%, респективно. Док су за горепоменуте масне киселине највиши утврђени нивои у месу шарана били 7.3%, 2.3% и 2.3%, респективно, садржај EPA+DHA је био 5 до 12 пута већи у месу рибе храњене експерименталном храном него у месу рибе која је добијала контролну храну CF1. Плутајућа храна обогаћена есенцијалним масним киселинама може бити примењена као додатна храна у рибњацима са довољно природне хране и при одговарајућем режиму исхране.

Кључне речи: *екструдирана храна за рибе, обогаћење масним киселинама, рибље месо*

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