ORIGINAL ARTICLE

Culture of common carp (*Cyprinus carpio*) with defined flesh quality for prevention of cardiovascular diseases using finishing feeding strategy

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Abstract

OBJECTIVES: Fish is the major source of n-3 polyunsaturated fatty acids (n-3 PUFA) which are well known to have positive effects in prevention of cardiovascular diseases. This study investigated the possibility to produce common carp with defined flesh quality using finishing feeding strategy and predict changes of fillet FA by a dilution model.

METHODS: During the 110-day experiment, fish were fed diets with two different vegetable oils (rapeseed/linseed blend, VO; olive oil, OO) only, or with a subsequent fish oil (FO) finishing treatment for 30 or 60 days. Fillet FA composition was measured and data were compared to the ones predicted by the dilution model.

RESULTS: The FO finishing treatment resulted in the higher percentage of SFA (from 19.1% to 23.6%; $p<0.001$), MUFA (from 46.8% to 51.9%; $p<0.001$), n-3 PUFA (from 3.6% to 7.4%; $p<0.001$) and lower n-6 PUFA (from 30.5% to 16.9%; $p<0.001$) and n-6/n-3 ratio (from 8.7 to 2.3; $p<0.001$) in groups previously fed the VO diet and in lower MUFA percentage (from 67% to 63%; $p<0.001$) and n-6/n-3 ratio (from 8.2 to 2.8; $p<0.001$) and higher n-3 PUFA percentage (from 1.5% to 4.5%; $p<0.001$) in group previously fed the OO diet. The dilution model gave a good prediction for fillet FA changes (slope of the regression line 0.97–1.00; R^2 value of 0.992–0.996).

CONCLUSION: The finishing feeding strategy is suggested for production of common carp with a required flesh FA composition for purposes of special nutritional needs, especially for primary and secondary prevention of cardiovascular disease.

Abbreviations:

DHA	- docosahexaenoic acid (22:6n-3)
EPA	- eicosapentaenoic acid (20:5n-3)
FA	- fatty acids
FAME	- fatty acid methyl esters
FO	- fish oil
HUFA	- highly unsaturated fatty acids (20 ≥ carbons, 3 ≥ double bonds)
MUFA	- monounsaturated fatty acids
OO	- olive oil
PUFA	- polyunsaturated fatty acids
SFA	- saturated fatty acids
VO	- vegetable oil blend (rapeseed/linseed blend)

INTRODUCTION

The n-3 highly unsaturated fatty acids (n-3 HUFA; 20 ≥ carbons, 3 ≥ double bonds), especially eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are beneficial for human health (Mozaffarian & Rimm 2006). These fatty acids (FA) play an important role in biological functions, including brain development, inflammatory response, homeostasis and prevention of cardiovascular disease (Calder 2006; Calder & Yaqoob 2010). Fish is the major dietary source of n-3 HUFA and worldwide promoted as healthy and beneficial for human health, especially in prevention of cardiovascular diseases. Several specific dietary recommendations have been developed related to fish consumption and intake of n-3 FA by nutrition and health authorities. For the general population, two servings of fish per week or 250 mg of EPA and DHA per day are recommended (EFSA 2009). Patients with documented cardiac heart disease and hypertriglyceridemia are advised to have daily intake of EPA+DHA even higher up to 1 and 2–4 g, respectively (Kris-Etherton 2002). However, it is difficult for the public to meet the nutritional recommendations for the FA by a diet since the FA composition of fish flesh is highly variable and influenced by many factors, mainly by feeding (Mráz & Pickova 2011). Therefore it would be valuable if fish producers could produce fish with high and defined content of n-3 HUFA.

Feed sources rich in n-3 HUFA, such as fish oil, are becoming scarce, while algae and various microorganisms that supply n-3 HUFA are expensive and not yet available on a commercial scale. Therefore it would be of great economic and sustainability value if a feeding strategy could be devised where these feedstuffs are not used for the entire feeding period but only for the final part, thus saving resources. In line with this there is a need to be able to predict changes of fish FA composition during the course of feeding.

Such a finishing feeding strategy has been suggested and developed for carnivorous fish species, including medium fatty fish species such turbot (*Psetta maxima*) (Robin *et al.* 2003), fatty fish such as Atlantic salmon (*Salmo salar*) (Jobling 2003 and 2004b) and lean fish species such as Atlantic cod (*Gadus morhua*) (Jobling *et al.* 2008) and Murray cod (*Maccullochella peelii peelii*) (Turchini *et al.* 2006). The results so far have been promising and a finishing feeding strategy for commercial applications has been proposed. The overall conclusions from all these different studies are in agreement with a general dilution model suggested by Robin *et al.* (2003).

Common carp (*Cyprinus carpio*) is one of the most cultured fish species in the world (FAO 2008). Thus, from a worldwide nutrition perspective, a method to increase the amount of n-3 HUFA in carp fillet is valuable. Optimization of the FA composition of carp has been examined in previous studies (Domaizon *et al.* 2000; Chen *et al.* 2011; Mráz *et al.* 2012; Steffens 1997; Steffens & Wirth 2007). Mráz & Pickova (2011) concluded that adjusting the lipid composition in the feed is the most effective tool to achieve the desired n-3 HUFA content. However, previous studies have not investigated the response of muscle FA composition to dietary changes and the duration of finishing feeding period needed to achieve desired changes in n-3 HUFA content. Therefore the aim of this study was to examine the applicability of the finishing feeding strategy in common carp production with defined and tailored flesh quality for specific needs in human nutrition.

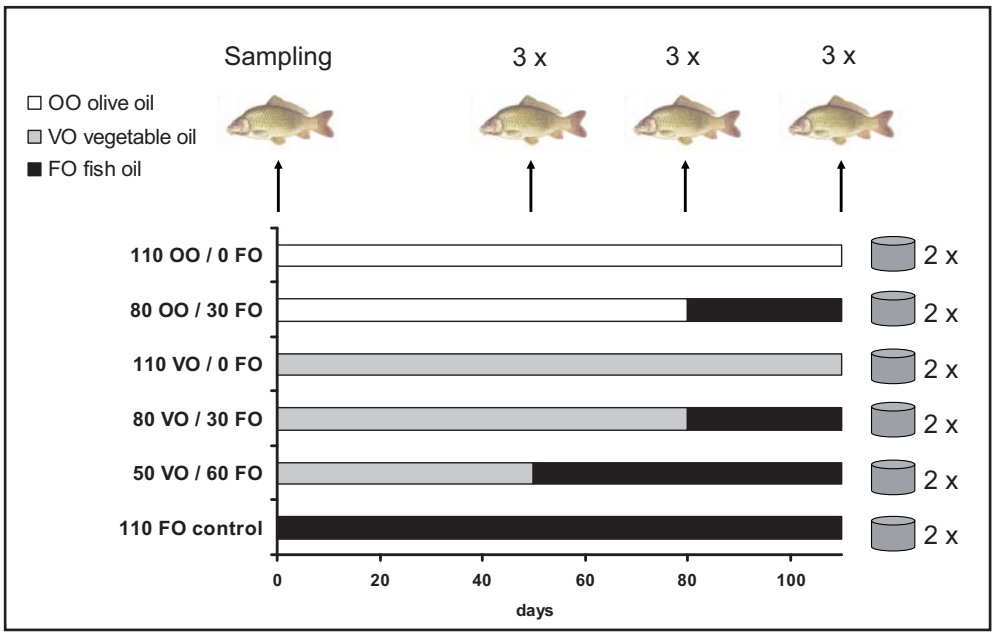

Fig. 1. Experimental design of the 110-day feeding trial. Fish (10 per tank, duplicate tanks per treatment) were fed one of three diets containing vegetable oil (VO), olive oil (OO) or fish oil (FO) alone for 110 days, or with a 30 or 60 day finishing period with FO. The different diets were: 110 OO/0 FO; 80 OO/30 FO; 80 OO/30 FO; 110 VO/0 FO; 110 VO/0 FO; 80 VO/30 FO; 50 VO/60 FO and 110 FO control. Fish were sampled at 0, 50, 80 and 110 days.

MATERIALS AND METHODS

Diets

The experimental diets used were based mainly on vegetable components (Table 1). No fish meal was used, in order to avoid high background levels of n-3 HUFA in the diet. The diets differed only in lipid source. The basal diet contained either a blend of vegetable oils (VO; rapeseed/linseed 3:2) or olive oil (OO) and the finishing feeding diet contained fish oil (FO). All diets were manufactured by extrusion. The proximate and FA composition of the experimental diets are listed in Table 1.

Tab. 1. Formulation (g 100g^{-1}), proximate composition (% on as is basis) and FA composition (% of identified FAs) of the experimental diets.

	VO Vegetable oil	OO Olive oil	FO Fish oil
Soybean meal	30	30	30
Wheat	18	18	18
Soycomil[a]	15.1	15.1	15.1
Maize	12	12	12
Fish oil	0	0	9
Linseed oil	3.6	0	0
Rapeseed oil	5.4	0	0
Olive oil	0	9	0
Corn gluten[b]	6	6	6
Wheat germ	5	5	5
Yeast vitex[c]	3	3	3
Aminovitan KPd[d]	0.6	0.6	0.6
Salt	0.5	0.5	0.5
Limestone	0.4	0.4	0.4
DL-methionine[d]	0.4	0.4	0.4
Dry matter	94.8	95.3	96.9
Protein	34.1	34.4	34.6
Fat	8.5	8.3	8.8
Fiber	3.5	3.1	3.7
Carbohydrates	44.1	44.8	44.2
Ash	4.6	4.7	5.6
SFA[e]	11.8	15.5	27.9
MUFA[f]	31.0	61.1	34.6
PUFA[g]	57.2	23.3	37.5
18:2n-6	53.0	21.7	16.2
20:2n-6	0	0	0.3
20:4n-6	0	0	0.3
18:3n-3	4.2	1.7	2.7
18:4n-3	0	0	2.2
20:5n-3	0	0	6.3
22:5n-3	0	0	0.6
22:6n-3	0	0	8.8
n-6/n-3	12.7	13.1	0.8

[a] ADM (Archer Daniels Midland Company), Olomouc, Czech Republic; [b] Bodit Tachov, s.r.o., Stribro, Czech Republic; [c] Biocel, a.s., Paskov, Czech Republic; [d] Zavod Biochemickych Sluzeb, s.r.o., Slusovice, Czech Republic; [e] SFA: saturated fatty acids; [f] MUFA: monounsaturated fatty acids; [g] PUFA: polyunsaturated fatty acids

Fish and experimental design

Two-year-old common carp (*Cyprinus carpio*) of the mirror scaly type with an average weight of 780g were used for the experiment. The fish were transferred from an earthen pond to the experimental facility at the Research Institute of Fish Culture and Hydrobiology in Vodnany, Czech Republic. Six fish were sampled to determine the initial lipid content and composition (data not shown). The fish were placed in tanks (1 m^3) and divided into 12 groups of 10 fish each. The tanks were supplied with oxygenated water from a recirculating system after mechanical and biological filtration (flow 0.1 l s^{-1}; dissolved oxygen 7–10 mg l^{-1}; temperature 20°C). The water level in the tanks was set to 0.4 m (tank volume 400 l). The fish were subjected to a light:dark (12h:12h) regime. During the 110-day experiment, the fish groups were fed only VO (110 VO/0 FO), only OO (110 OO/0 FO) or only FO (110 FO control), or VO or OO with a subsequent 30 or 60-day FO finishing treatment (80 OO/30 FO, 80 VO/30 FO, 50 VO/60 FO), with duplicate groups for each treatment. The experimental design is shown in Figure 1. The diets were supplied by automated continual feeders for 9 hours at a feeding ratio of 1.5% of current biomass. The fish stock biomass was determined every second week. Three fish were randomly sampled from each tank after 50, 80 and 110 days for lipid analysis. Samples of the fillets were immediately frozen in liquid nitrogen and stored at –80 °C until further analysis.

Lipid analysis

Lipid analyses were performed as described in detail by (Mraz & Pickova 2009). In brief, lipids from the fillet and feed samples were extracted with hexane and isopropanol according to Hara & Radin (1978). The FA were methylated (Appelqvist 1968) and the fatty acid methyl esters (FAME) were analyzed with a gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with flame ionization detector and split injector and fitted with a 50 m long × 0.22 mm i.d. × 0.25 µm film thickness BPX 70 fused-silica capillary column (SGE, Austin, TX, USA) according to (Fredriksson Eriksson & Pickova 2007). The FA were identified by comparison with a standard FA mixture (GLC standard 461, Nu-Chek Prep, Elysian, MN, USA) and specific retention times. Peak area integration was performed using Star chromatography workstation software version 5.5 (Varian AB, Stockholm, Sweden). The FA were quantified using internal standard methyl 15-methylheptadecanoate (Larodan Fine Chemicals AB, Malmö, Sweden).

Dilution model

The data obtained from lipid analyses of fillet tissues from groups 80 OO/30 FO, 80 VO/30 FO and 50 VO/60 FO were compared against predicted data calculated according the dilution model designed by Robin *et al.* (2003) and shown in Equation 1, as verified by Jobling (2004a).

$P_T = P_R + [(P_0 - P_R) / (Q_T/Q_0)]$ (Equation 1), where

P_T = Predicted percentage of a fatty acid at time T

P_R = Percentage of a fatty acid measured at time T in the fillet of control fish continuously fed the reference/finishing diet

P_0 = Percentage of a fatty acid in the fillet of tested fish at the beginning of finishing feeding period

Q_T = Quantity of total fatty acids in the tested fish at time T

Q_0 = Quantity of total fatty acids in the tested fish at the beginning of the finishing feeding period.

The predicted percentage of specific FA, e.g. EPA at a specific time point (P_T) (in this case the end point after 110 days), was calculated by taking the percentage of the specific FA measured at time T (110 days) in fish continuously fed the finishing diet (P_R = value for 110 FO control at 110 days) and the corresponding percentage in the other experimental groups (80 OO/30 FO, 80 VO/30 FO or 50 VO/60 FO) at time point P_0 (50 or 80 days), directly before the finishing feeding period. Q_0 was taken as the average total FA content (lipid content × body mass) of the experimental fish before the finishing feeding period and Q_T as the final total FA content of fish from the corresponding group at the end of the experimental period. All values represent the mean of six replicates (three fish per duplicate tank).

Statistical analysis

Where applicable (n>2), all data are presented as mean values ± standard deviation (SD) and differences were regarded as significant at $p<0.05$. The SAS General Linear Model (GLM), Tukey´s test (SAS Institute Inc., Cary, NC, USA, version 9.2) was used to compare fillet FA composition among the dietary treatments.

RESULTS

Survival, growth and feed conversion data for the different groups are presented in Table 2. The fillet lipid content was not affected by any dietary treatment over the course of the feeding trial ($p>0.05$). The final fillet lipid content varied between 9–10% at the end of the trial.

Replacing OO or VO with FO as the lipid source in the diet of common carp resulted in fillets with clearly different FA profiles (Figure 2). In the groups previously fed the VO diet the percentage of SFA (from 19.1% to 23.6%; $p<0.001$), MUFA (from 46.8% to 51.9%; $p<0.001$), n-3 PUFA (from 3.6% to 7.4%; $p<0.001$), EPA (from 0.36% to 1.53%; $p<0.001$) and DHA (from 0.71% to 3.16%;$p<0.001$) were positively correlated to the length of the FO finishing feeding period while the percentage of n-6 PUFA (from 30.5% to 16.9%; $p<0.001$) and the n-6/n-3 ratio (from 8.7 to 2.3; $p<0.001$) were negatively correlated. In the group previously fed the OO diet, the finishing treatment resulted in a lower percentage of MUFA (from 67% to 63%; $p<0.001$), a lower n-6/n-3 ratio (from 8.2 to 2.8; $p<0.001$) and a higher percentage of n-3 PUFA (from 1.5% to 4.5%; $p<0.001$), EPA (from 0.24% to 0.84%; $p<0.001$) and DHA (from 0.49% to 1.54%; $p<0.001$). The percentages of EPA and DHA both increased linearly with cumulative FO consumption (R^2 value >0.99) (Figure 3).

Although the fillet FA composition (Figure 2) changed significantly in response to the dietary FA composition (Table 1), the FA composition in the fillet did not reach that in the feed. The most obvious differences were seen in percentage of MUFA and PUFA (Figure 2 and Table 1). The percentage of MUFA in the VO and FO diet was 31% and 35%, respectively. The percentage of MUFA was considerably higher in the fillet, varying between 47% and 54% ($p<0.001$). The PUFA content in the VO and FO diet was 57% and 38%, respectively, but the corresponding values in the fillet were significantly lower and varied from 20% to 34% ($p<0.001$).

At the end of the experiment, the observed FA composition in the fillet samples from fish receiving the finishing feed (80 OO/30 FO, 80 VO/30 FO and 50 VO/60 FO) was compared against the values predicted using the dilution model designed by Robin *et al.* (2003) (Figure 4). The data showed that the dilution model gave a good prediction for the 10 most important FA or FA groups in the fillet of common carp, with a slope of the regression line close to 1 (0.97, 0.99 and 1.00, respectively) and with an R^2 value of 0.996, 0.993 and 0.992, respectively. Similar regression statistics were obtained for all FA identified (data not shown).

DISCUSSION

This study investigated possibility to produce common carp with defined flesh quality (high content of n-3 PUFA, EPA, DHA) for prevention of cardiovascular

Tab. 2. Fish performance (data presented are mean of duplicate values).

	Survival (%)	Final body weight (g)	Feed conversion (kg feed kg yield^{-1})
110 VO/0 FO	100	1610	1.76
80 VO/30 FO	95	1466	1.96
50 VO/60 FO	100	1407	1.73
110 OO/0 FO	100	1648	1.57
80 OO/30 FO	100	1491	1.74
110 FO control	95	1624	1.69

Abbreviations: VO, vegetable oil mixture; FO, fish oil; OO, olive oil

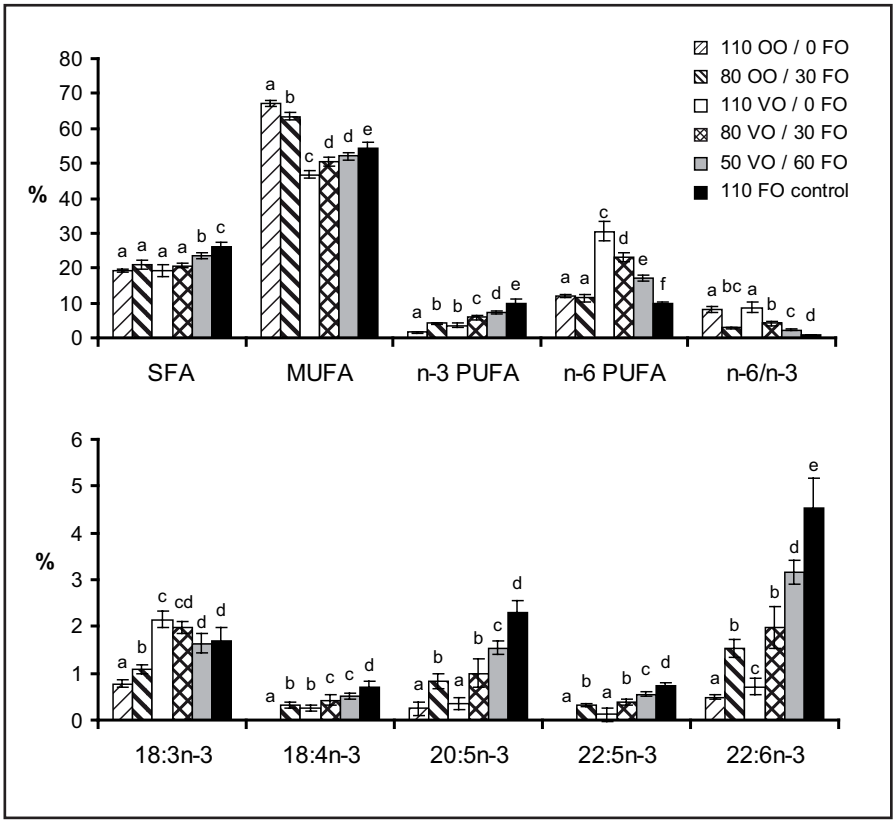

Fig. 2. Fatty acid (FA) composition (% of identified) in the fillet of the experimental fish at the end of the experiment (n=6; mean ± SD). Different letters indicate significant difference among the treatments. Abbreviations: SFA = saturated FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA.

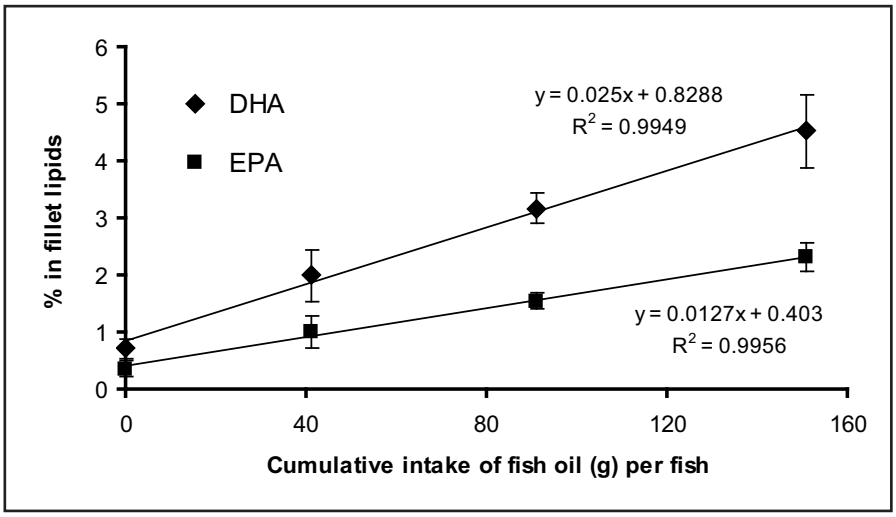

Fig. 3. Percentage of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (% of total identified FA) in fish fillet lipids in relation to the cumulative intake of fish oil (g) per fish (n=6; mean ± SD).

diseases using finishing feeding strategy and predict changes of fillet FA by a dilution model.

In the present study, the fillet FA composition highly reflected the FA composition of the diet and was significantly correlated to the length of the feeding period. This agrees with previous findings that it is possible to boost the content of beneficial EPA and DHA in fish fillet by n-3 HUFA supplementation prior to harvest (Bell *et al.* 2004; Torstensen *et al.* 2005, re Atlantic salmon; Benedito-Palos *et al.* 2009, re gilthead sea bream; Steffens 1997; Steffens & Wirth 2007, re common carp; Turchini *et al.* 2006, re murray cod).

However, when the dietary composition was used as the reference value for prediction of the FA composition in the groups continuously fed the same diet (110 FO control, 110 VO/0 FO and 110 OO/0 FO; Figure 5) the observed percentage of MUFA were significantly higher than the predicted values ($p<0.001$) while percentage of PUFA were significantly lower ($p<0.001$). This could indicate that the carp synthesized a significant amount of MUFA de novo from excess energy, or that MUFA are the preferred FA group for storage in common carp. This is supported by the fact that regardless of the low amount of MUFA in the natural and supplemented diet

of carp, MUFA is the major FA group stored in carp fillet and is probably produced from energy obtained from cereals (Buchtová *et al.* 2010, Mráz *et al.* 2012).

Using the dilution model proposed by Robin *et al.* (2003) showed to give an excellent prediction of the FA composition in fillet of carp of marketable size. This confirms previous findings for carnivorous fatty fish species such as Atlantic salmon (Jobling 2003), where the lipids are predominantly represented by storage fat (triacylglycerols). The dilution model is clear in its straightforwardness and can therefore be applicable for fish farmers, enabling production of high quality fish as well as minimizing the use of expensive feed. A small disadvantage is that the model does not account for the FA composition of the feed used, but for the FA composition in the fillet of fish continuously fed the finishing diet, which is hence needed as a reference value. However if the reference value has been established for a species and diet once it might be used continually.

The European Food Safety Authority recommends a daily intake of 250 mg EPA+DHA per person (EFSA 2009) and two servings of oily fish per week. A 200 g serving of carp from the 110 FO control and 110 VO/0 FO group contained 1190 mg and 180 mg EPA+DHA, respectively. According to the predictions by the dilution model and experimental values obtained here, we concluded that the finishing feeding treatment needs to be applied for 70 days to achieve the recommended daily value of 250 mg EPA+DHA in two 200 g servings a week (250 mg × 7 days = 1750 mg/2 servings = 875 mg/serving). Reducing FO feeding to this shorter period would significantly reduce fish production costs and lead to more sustainable use of limited FO resources.

Currently common carp is mostly produced in ponds on the basis of natural feed (plankton and benthos) with cereal supplementation. Since there are huge differences in natural productivity among ponds there is also a huge variability of FA composition in carp flesh (Mráz & Pickova 2012). As a consequence there is no standard of quality which makes it difficult to advertise carp as a healthy product. The finishing feeding strategy could therefore be used in production of carp with defined flesh quality to fulfill dietary needs for humans, especially in connection to cardiovascular recovery. Fish farmers could easier control the final carp flesh quality and produce fish with standardized and tailored quality. In line with this they could declare the content of n-3 PUFA and EPA+DHA on the product label which could increase the market value of carp and support consumption of this locally produced fish. From the consumers point of view this would be desirable as they thereby could easier meet the nutritional recommendations. It would also be easier to set up dietary interventions using carp in prevention and treatment of cardiovascular diseases.

In conclusion, the finishing feeding strategy is suggested for the production of common carp with tailored flesh FA composition, for contributing to healthy fat profile of e.g. EPA and DHA content, for cardiovascular disease prevention of the Central Europe population.

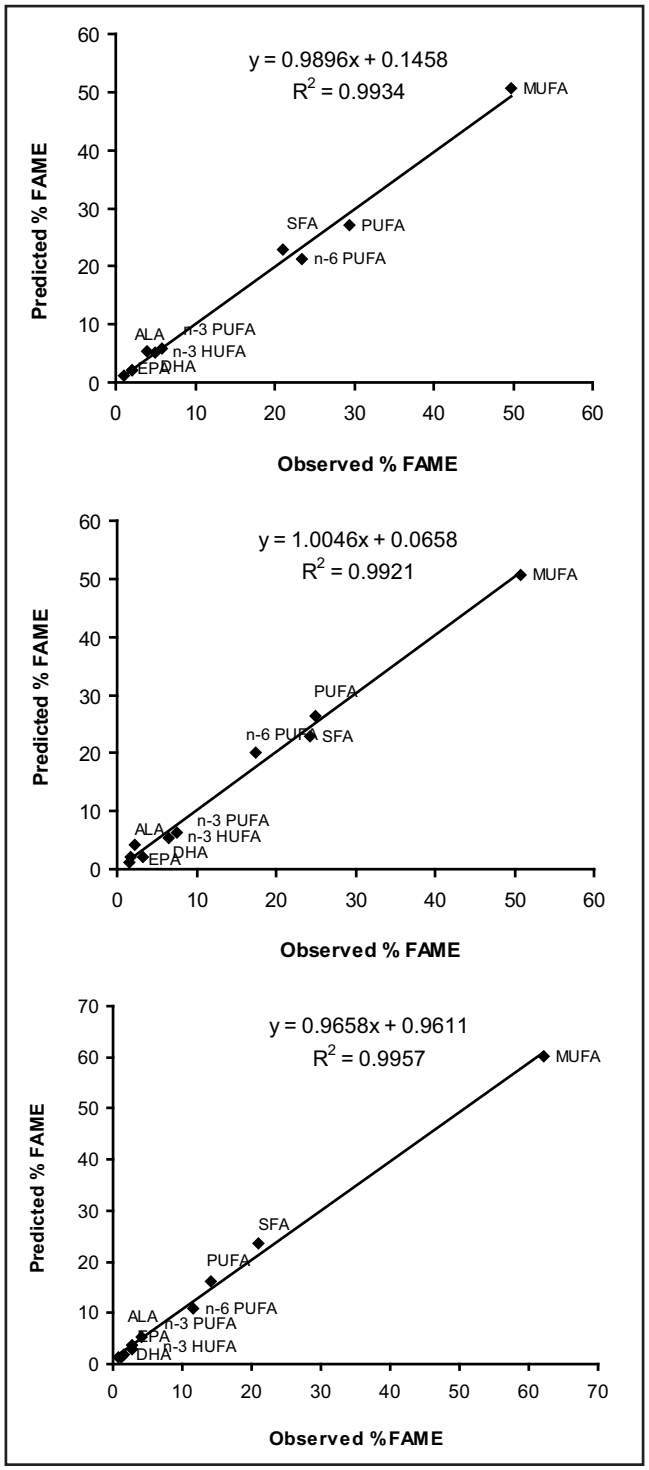

Fig. 4. Prediction plot of fillet fatty acid composition (%) for carp groups A) 80 VO/30 FO; B) 50 VO/60 FO; and C) 80 OO/30 FO. The measured values represent the mean for 6 fish. The thick line shows the regression line. FAME = fatty acid methyl esters, for other abbreviations see Figure 2.

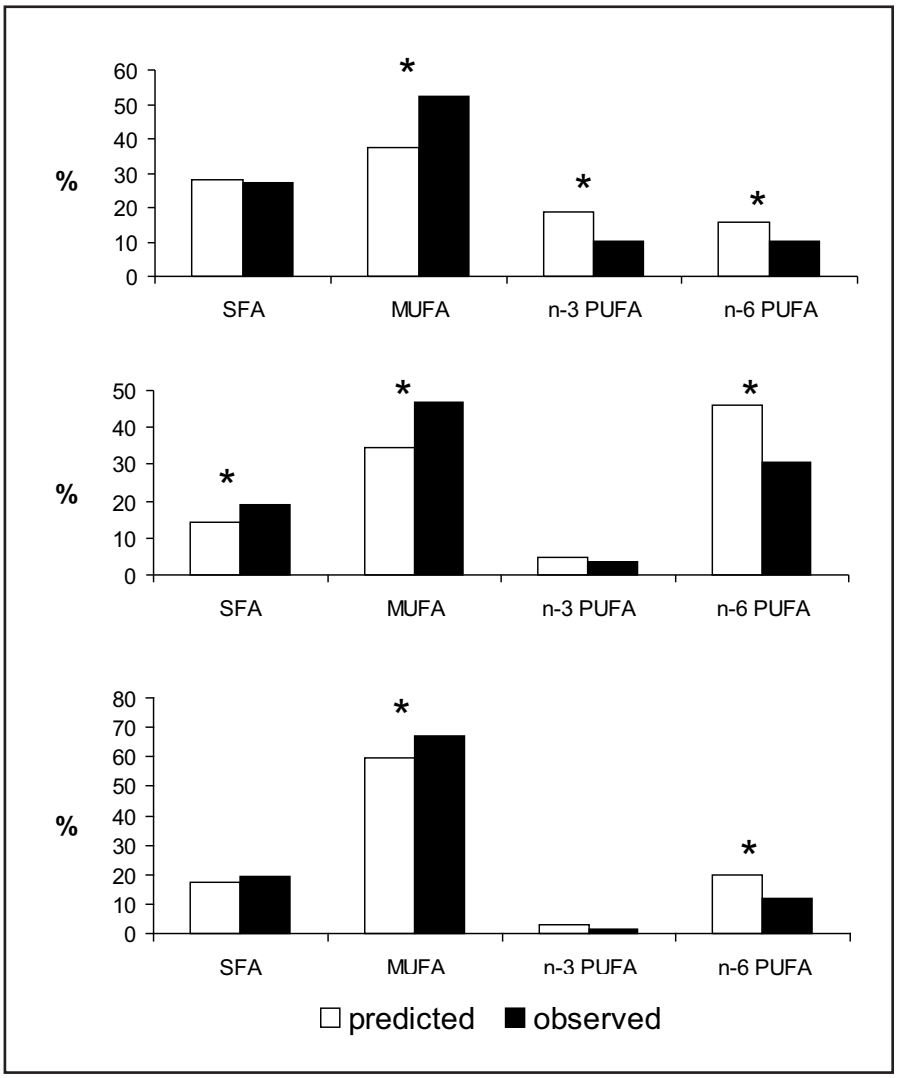

Fig. 5. Observed fatty acid composition (%) in fillet of fish from the carp groups A) 110 FO control; B) 110 VO/0 FO; and C 110 OO/0 FO, compared with the composition predicted by the dilution model when the fatty acid composition of the FO, VO and OO diet, respectively, was used as the reference value. * indicates significant difference ($p<0.05$) between predicted and observed data. For abbreviations see Figure 2.

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