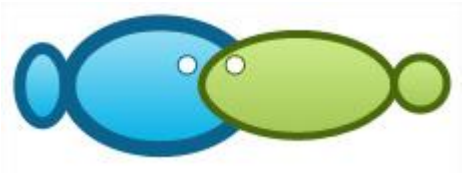

Comparison of carp filet quality produced in semi-intensive pond system using different type of feeds

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Abstract. The aim of the study was to compare the long-term effects of formulated diet containing only plant ingredients with commercial and traditional cereal based diets in pond culture of common carp (*Cyprinus carpio* L.). The main objective of the present experiment was to investigate the impact of dietary linseed oil to the composition of the fish flesh, especially to the level of long chain polyunsaturated fatty acids (Lc-PUFA) in carp filet. The effect of nutritional history to some filet storage parameters and composition data were also evaluated. The results have shown significant differences in the total fat and Lc-PUFA content of the filets between the treatments. The high source of linolenic acid (α-LNA) in the linseed oil supplemented diet did not raised the eicosapentaenoic (EPA) and docosahexaenoic (DHA) level in the flesh compared to the cereal-based diet during whole life feeding. However, the fish fed with composed diet containing plant ingredients is more suitable for human consumption in context of fat content, PUFA and storage compared to the carp produced traditionally.
Key Words: linseed oil, linolenic acid, bio-conversion, storage, meat quality.

Introduction. Nowadays, in the pursuit of conscious nutrition, there is an increasing demand for good quality food ingredients, including high-quality fish meat. In addition to commercially available frozen or canned fish food with marine origin, more and more people are looking for local and fresh fish to make our delicious, traditional fish dishes. In Hungary, the most important aquaculture fish species is the common carp (*Cyprinus carpio*), representing approximately 80% to the total annual fish production of the country (Kiss 2019). C. *carpio* is produced in extensive or semi intensive pond rearing system in polyculture, and in the latter case supplementary cereal feeding is applied in addition to the natural available food supply (Woynarovich et al 2010). The quality of the fish produced in the pond culture highly depends on the natural biomass production of the ponds, which is affected naturally by water temperature and availability of nutrients (Tkaczewska et al 2014). Therefore, due to this reason the nutritional value of the *C. carpio* is variable.

Little attention has been paid to the standardization of pond fish farming concerning the quality of fish flesh, while the composition of meat may be influenced by feeding and can improve the main characteristics that are important for human nutrition. According to our previous studies (Lengyel et al 2001) the fat content of *C. carpio* cultured traditionally is very high (9-15% of wet weight basis) and the proportion of long chain polyunsaturated fatty acids (Lc-PUFA) such as eicosapentaenoic (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA) essential for human nutrition is relatively low (1-2%). Similar wide interval for fat content in muscle has been reported by Trenovszki et al (2011) from five Hungarian fish farms and by Klobukowski et al (2018) in five different farms in Poland. Replacing cereals with composed feeds in most of the cases has a positive impact on muscle composition and decrease its fat content (Markovic et al 2016). Data on different cultured fish species claim that the fatty acid composition of the edible parts can be influenced by the diet (Steffens & Wirth 2007).

The main objective of the present experiment was to investigate the impact of dietary linseed oil to the composition of the fish flesh, especially to the level of Lc-PUFA in *C. carpio* filet at the end of 3-years feeding period. Previous experiments demonstrated that several freshwater fish are able for bioconversion of linoleic acid (LIN) and α-linolenic acid (α-LNA) to higher homologues from dietary sources (Ljubojevic et al 2015; Župan et al 2016). Since vegetable oils are rich in these fatty acids feeding continuously would moderate the Lc-PUFA deficiency of the fish. Efficacy of biosynthesis of EPA, DHA and ARA in pond reared conditions from the precursors as α-LNA and LIN were assessed on order to demonstrate the hypothesis of enrichment in essential fatty acids (EFA) of the filets. The flesh quality tests have been extended to a number of shelf-life studies, such as the determination of degree of rancidity and the food safety aspect of heavy metals.

Material and Method. The ARRAINA project supported by EU FP7 programme was dedicated to study the long-term effect of plant based supplementary feed in *C. carpio* monoculture during the whole life cycle. For this purpose, two semi-intensive supplementary feeds were formulated and used during three rearing seasons in ponds with intensive natural food production. As control traditional cereal based diets were used.

Experimental diets. Two semi-intensive supplementary feeds for pond feeding of *C. carpio* were formulated and used during three rearing seasons with intensive natural food production. As control traditional cereal based diets were used (treatment CT). In the formulations, standard ingredients available and commonly used for fish feeds in Hungary were utilized. One group of the feeds was without fish meal (FM) and fish oil (FO) composed from local plant sources (PM) and linseed oil (VO) (treatment P), the second feed contained moderate levels of FM (14 to 16%) and FO (1.65 to 2.20%) (treatment F). The pairs of the plant-based and marine-based feeds were formulated to be isoproteinous (30%), isolipidic (7.4%) similar crude fiber (2.8-3.0%), isoenergetic (18-18.2 MJ kg^{-1}), similar n-3/n-6 ratio, but differing in LNA/LIN acid ratio (Table 1).

Experimental animals and conditions. The trial on *C. carpio* monoculture started in 2013, when the fries were stocked into six (each about 1,700 m^2) earthen ponds, previously treated with cow manure (3 t ha^{-1}) with stocking density 20,000 fish ha^{-1}. After overwintering in the second year the fingerlings were restocked into the same ponds as previous year with stocking density of around 5,000 fish ha^{-1}. In the third year the fish outsourcing was 1,000 fish ha^{-1}, respectively. During the growing season the water temperature in ponds fluctuated between 16 and 30°C. The daily amount of feeds varied between 1.5 and 3.2% of metabolic body weight (MBW%: $kg^{0.8}$). All animal experiments described comply with the guidelines of the European Union Council (2010/63/EU) and have been approved by the Ethical Committee of HAKI (1/2002), which was established according to Hungarian State law (10/1999. (I. 27.)) and operated according to different Hungarian State laws concerning animal experiments, transportation of animal, welfare etc. (40/2013. (II.14)).

Sample collection. At the end of 3rd year (after 862 rearing days and 519 feeding days) growth performance of fish and dressing yield of edible part was evaluated. Fish filet samples were collected for proximate and fatty acid composition measurements. Storage quality of fish filets during 5 days of storage at 4°C was evaluated considering the antioxidant vitamin level and peroxidation status of flesh. Additionally, heavy metal content (Cr, Ni, Pb, Cd, As) was checked from the edible part of *C. carpio* as a safety issue in the application of originally different sources of feeds in pond nutrition.

Table 1

Formulation and composition of the feeds

Treatments	*CT*	*F*	*P*
Ingredients		w %	
Fish meal (60%)	-	14.0	0.0
Winter wheat meal	-	20.5	16.5
Maize	-	6.5	27.5
Full-fat soya	-	27.5	9.5
Soybean meal (46%)	-	6.5	29.5
Blood meal	-	5.0	8.0
Yeast, feed grade	-	5.0	5.0
Vitamin -mineral mix	-	2.0	2.0
Fish oil	-	2.0	0
Linseed oil	-	0	2.0
Proximate composition		g 100 g^{-1} (w.w.)	
Dry matter	96.36	91.86	92.50
Crude protein	10.05	30.18	29.57
Crude fat	1.20	7.38	7.43
Crude ash	8.24	5.96	4.21
Crude fibre	3.24	2.87	3.36
Fatty acid composition		w % FA (w.w.)	
16:0	15.29	14.59	11.29
18:2ω6	55.66	31.29	40.16
18:3ω3	4.44	6.04	18.75
20:4ω6	0.16	0.53	0.07
20:5ω3	0.32	2.45	0.07
22:6ω3	0.18	5.95	0.19
Total SFA	17.79	21.63	16.45
Total MUFA	18.56	28.30	23.80
Total n-6	56.00	32.69	40.27
Total n-3	4.94	15.36	19.01
n-3/n-6	0.09	0.47	0.47
Total PUFA	60.94	48.06	59.29
Trace metal composition		mg kg^{-1} (d.w.)	
Ca	968	11400	4340
Cr	<2.5	<2.5	<2.5
Cu	<2.5	18.1	15.3
Fe	280	406	364
Mg	1080	2560	2110
Mn	11.5	36.3	38.4
Ni	<2.5	<2.5	2.95
P	2200	10230	6380
Pb	<2.5	<2.5	<2.5
Zn	16.5	74.2	55.8

SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, n-6 - omega-6 fatty acids, n-3 - omega-3 fatty acids, CT - control, F - fish-based diet, P - plant-based diet, w.w. - wet weight, d.w. – dry weight.

Analytical methods. The chemical compositions of fish and feed were analyzed by standard methods of the AOAC (1998) (Table 1). The experimental diet's total carbohydrate (TC) and gross energy (GE) values were calculated as TC = 100 – (crude protein + crude fat + crude fibre + ash), with GE = values of carbohydrates, proteins and lipids of 17.2, 23.6 and 39.5 KJ g^{-1}, respectively (Halver & Hardy 2002). The fatty acid compositions of fish and feed samples were analyzed by capillary gas chromatography (AGILENT 6890N) according to the method by Folch et al (1957). The vitamin E

measurements were done through adaptation of methods of Ochoa et al (1992). For the trace metal determination ICP-OES technique was applied. The peroxidation status of the samples was determined by measuring the malondialdehyde formation after photochemical reaction with tio-barbutiric acid (TBA) based on the method developed by Vyncke (1975) and modified by Juncher et al (2001).

Statistical methods. To compare and evaluate the results, we used SPSS 22.0 for Windows. All data were tested with one-way analysis of variance (ANOVA) with Tukey's Post Hoc test. The statistical IDs marked with different letters translate into a deviation on a significance level of $p<0.05$.

Results

Biometric and slaughtering indices. There were no significant differences between the body weight and body length of the fish at end of the trial, but the smallest weighted group was the CT group. We observed differences in some indicators of the dressing yield (Table 2), mainly for head index and hepatosomatic index, when the highest values were observed for the CT group and lowest for F group. There were no differences in fileting yield. Surprisingly, ovary was not present in P group's female individuals, absolutely. However, male specimens had a significant amount of milt.

Table 2

Biometric indices and composition of the filet (wet weight)

Treatments	*CT*	*F*	*P*
Body weight (g)	1873±228	2102±238	2139±240
Total length (mm)	488±23	495±17	480±32
Head index (%)	20.4±0.8^{a}	17.9±0.9^{b}	19.7±1.1^{a}
Filleting yield (%)	38.8±3.1	40.6±2.4	41.2±3.4
Hepatosomatic index (%)	2.9±0.7^{a}	2.1±0.3^{b}	2.6±0.5^{ab}
Gonadosomatic (%) - female	2.8±2.6	7.5±3.0	0
Proximate composition and mineral content of the filet			
Crude fat (%)	11.3±1.4^{a}	7.1±0.2^{b}	6.6±0.1^{c}
Crude protein (%)	15.04±0.03^{a}	16.34±0.52^{b}	16.67±0.09^{b}
Crude ash (%)	0.99±0.02^{a}	1.05±0.02^{b}	1.04±0.03^{b}
Ca (mg kg^{-1})	601.7	644.2	571.2
Mg (mg kg^{-1})	500.8	544.7	524.0
P (mg kg^{-1})	1728.3	1896.7	1833.3
K (mg kg^{-1})	2893.3	2950.0	2996.7
Fe (mg kg^{-1})	10.8	12.2	10.4
Cu (mg kg^{-1})	0.672	0.745	0.659
Zn (mg kg^{-1})	7.5	13.0	7.8
As (µg kg^{-1})	50.79	107.63	30.95
Ni (µg kg^{-1})	85.62	56.71	65.46
Pb (µg kg^{-1})	<LD*	<LD	<LD
Cr (µg kg^{-1})	<LD	<LD	<LD
Cd (µg kg^{-1})	<LD	<LD	<LD

Mean ± standard deviation. The biometric data is the average of 9 fish from each treatment, the proximate and mineral composition data are pooled sample from 3 fish individuals per ponds. Values followed by different letters in the same line are significantly different ($P<0.05$). LD: limit of detection 2.50 mg kg^{-1} (ppm) * outlier values from pond 51 were detected: 2.61 mg kg^{-1} and 6.98 mg kg^{-1} MRL (w.w.) EC (1881/2006) Pb: 300 µg kg^{-1}, Cd: 50 µg kg^{-1}.
CT- control, F- fish based diet, P- plant based diet.

Assessment of flesh quality and safety. The results have shown significant differences in the total crude fat content of the filets between the treatments. At the market size fish significantly higher fat deposition was found in CT group (11% w.w.) compared to F and P (7.1 and 6.6%) groups (Table 2). In our study significant

differences in protein and ash was observed between CT and composed diet groups, as well. Saturated (Total SFA) and monoenoic (Total MUFA) fatty acids were present in significantly higher ratio in the CT group compared to other groups, as well as the total lipid content (Table 3).

Table 3

Fatty acid content of filet after harvest in 2015

Treatments	*CT*	*F*	*P*
Fatty acid		mg g^{-1} FA (w.w.)	
16:0	18.03±2.29[a]	13.63±0.41[b]	11.64±1.03[c]
18:0	6.48±0.64[a]	3.88±0.33[b]	3.39±0.36[b]
18:1ω9	46.72±4.94[a]	26.84±1.26[b]	23.40±2.78[b]
18:2ω6	7.42±1.02[a]	11.69±0.27[b]	12.69±1.15[b]
18:3ω3	0.87±0.11[a]	1.77±0.13[b]	3.65±0.38[c]
18:3ω6	0.13±0.03[a]	0.13±0.02[a]	0.17±0.01[b]
20:2ω6	0.28±0.03[a]	0.31±0.02[a]	0.32±0.03[b]
20:3ω6	0.32±0.05[a]	0.26±0.01[b]	0.37±0.02[a]
20:3ω3	0.04±0.02[a]	0.08±0.04[a]	0.14±0.01[b]
20:4ω6	0.77±0.12[a]	0.61±0.03[b]	0.69±0.04[a]
20:5ω3	0.45±0.12[a]	0.97±0.05[b]	0.55±0.10[a]
22:5ω3	0.21±0.08[a]	0.37±0.02 [b]	0.24±0.03[a]
22:6ω3	0.76±0.21[a]	2.57±0.09[b]	0.95±0.05[a]
Total lipid mg g^{-1}	100.85±9.66[a]	77.71±2.17[b]	71.14±5.52[b]
Total SFA	25.78±2.86[a]	18.97±0.66[b]	15.98±1.41[c]
Total MUFA	59.09±5.91[a]	35.41±1.31[b]	30.49±3.38[c]
Total n-6	9.19±1.29[a]	13.19±0.28[b]	14.04±1.25[b]
Total n-3	2.42±0.51[a]	5.94±0.28[b]	5.69±0.58[b]
Total PUFA	11.61±1.49[a]	19.13±0.46[b]	20.09±1.82[b]
EPA + DHA	1.21±0.32[a]	3.54±0.10[b]	1.50±0.15[a]
ARA	0.77±0.12[a]	0.61±0.03[b]	0.69±0.04[a]

Mean ± standard deviation. Values are means of three pooled samples; values within the same line with different letters are significantly different ($P<0.05$). CT - control, F fish-based diet, P - plant-based diet, w.w. - wet weight.

The total PUFA content, total n-3 and total n-6 level was significantly higher in F and P group, but not differing between each other. EPA and DHA was naturally highest in the F group (due to the marine source) but at the same time there was no significant difference between the content of these fatty acids in CT and P group. Fatty acid composition of the red and white muscles was compared in 2-years old individuals, data are presented in Table 4. In most of the FA significant differences were recorded between CT and F group. The P group were differing from others in the case of n-3 FA homologues (18:3n-3, 20:3n-3; 20:5n-3; 22:6n-3). Sum of EPA and DHA amount determined in 2-years old and 3-years old fish muscle is presented on Figure 1. The measured value in the market size fish flesh for F group reached 3.54 mg g^{-1}, while in P and CT groups only 1.50 mg g^{-1} and 1.21 mg g^{-1} were determined (Table 3). It was found increasing level of oleic acid in F and P group after three-year feeding period (F: 29.55%; P: 28.17% in 2014 harvest and F: 35.37%; P: 33.69% in 2015 September).

Table 4

Fatty acid composition (w%) of muscle (2-years old fish) (w.w.)

Specification	*Red muscle*			*White muscle*		
	CT	*F*	*P*	*CT*	*F*	*P*
	w%					
14:0	0.72±0.07^{a}	1.11±0.07^{b}	0.60±0.11^{a}	0.73±0.02^{a}	1.14±0.26^{b}	0.64±0.08^{a}
16:0	16.76±0.57^{ab}	17.07±0.46^{a}	15.75±1.01^{b}	16.59±0.06^{ab}	17.27±0.16^{a}	15.77±0.49^{b}
18:0	6.81±0.21^{a}	4.76±0.26^{b}	4.65±0.25^{b}	7.26±0.24^{a}	4.79±0.37^{b}	4.72±0.01^{b}
18:1ω9	46.45±2.57^{a}	29.24±0.93^{b}	26.68±0.86^{b}	47.15±2.00^{a}	27.85±2.88^{b}	26.15±3.75^{b}
18:2ω6	6.95±0.76^{a}	17.50±1.13^{b}	19.85±1.89^{b}	6.81±0.86^{a}	16.46±3.43^{b}	19.17±3.30^{b}
18:3ω3	0.95±0.27^{a}	2.48±0.20^{b}	7.05±0.55^{c}	0.89±0.05^{a}	2.36±0.43^{b}	7.04±0.9^{c}
20:2ω6	0.29±0.04^{a}	0.52±0.06^{b}	0.62±0.08^{b}	0.27±0.04^{a}	0.47±0.01^{b}	0.51±0.05^{b}
20:3ω6	0.42±0.07^{a}	0.46±0.03^{a}	0.96±0.11^{b}	0.34±0.02^{a}	0.41±0.06^{a}	0.77±0.12^{b}
20:3ω3	0.07±0.00^{a}	0.19±0.01^{b}	0.36±0.03^{c}	0.06±0.00^{a}	0.16±0.01^{b}	0.31±0.02^{c}
20:3ω9	0.81±0.08^{a}	0.05±0.04^{b}	0.13±0.06^{b}	0.66±0.04^{a}	0.04±0.03^{b}	0.11±0.02^{c}
20:4ω6	1.14±0.19^{a}	1.19±0.14^{a}	2.07±0.17^{b}	0.74±0.05^{a}	1.05±0.16^{b}	1.49±0.09^{c}
20:5ω3	0.52±0.06^{a}	2.07±0.27^{b}	1.43±0.49^{c}	0.35±0.02^{a}	1.83±0.06^{b}	1.05±0.30^{c}
22:5ω3	0.26±0.02 ^{a}	0.78±0.08^{b}	0.70±0.10^{b}	0.18±0.01^{a}	0.66±0.02^{b}	0.51±0.02^{c}
22:6ω3	1.21±0.40^{a}	6.37±0.82^{b}	3.38±0.53^{c}	0.82±0.08^{a}	6.06±1.23^{b}	2.68±0.08^{c}
Total SFA	25.76±2.25^{a}	24.68±1.70^{a}	23.80±1.04^{a}	27.07±2.52^{a}	28.88±6.81^{a}	28.42±7.52^{a}
Total MUFA	59.18±2.41^{a}	40.56±1.14^{b}	36.38±1.87^{b}	59.75±1.70^{a}	38.49±3.93^{b}	34.93±3.87^{b}
Total n-6	9.42±1.12^{a}	20.27±1.31^{b}	24.34±1.90^{b}	8.68±0.96^{a}	18.97±3.18^{b}	22.68±3.29^{b}
Total n-3	3.13±0.24^{a}	12.24±1.24^{b}	*13.35±0.72*^{b}	2.40±0.17^{a}	11.47±0.81^{b}	*11.92±0.62*^{b}
Total PUFA	12.55±1.11^{a}	32.51±2.54^{b}	*37.69±1.72*^{c}	11.08±0.79^{a}	30.43±2.37^{b}	*34.60±3.92*^{b}
EPA+DHA	1.73±0.42^{a}	8.44±1.07^{b}	4.81±0.71^{c}	1.17±0.10^{a}	7.90±1.16^{b}	3.72±0.21^{c}
ARA	1.14±0.19^{a}	1.19±0.14^{a}	2.07±0.17^{b}	0.74±0.05^{a}	1.06±0.16^{b}	1.49±0.09^{c}

Mean ± standard deviation. Values are means of four replicates of individual samples; values within the same line with different letters are significantly different (P<0.05).
CT - control, F - fish-based diet, P - plant-based diet, w.w. - wet weight .

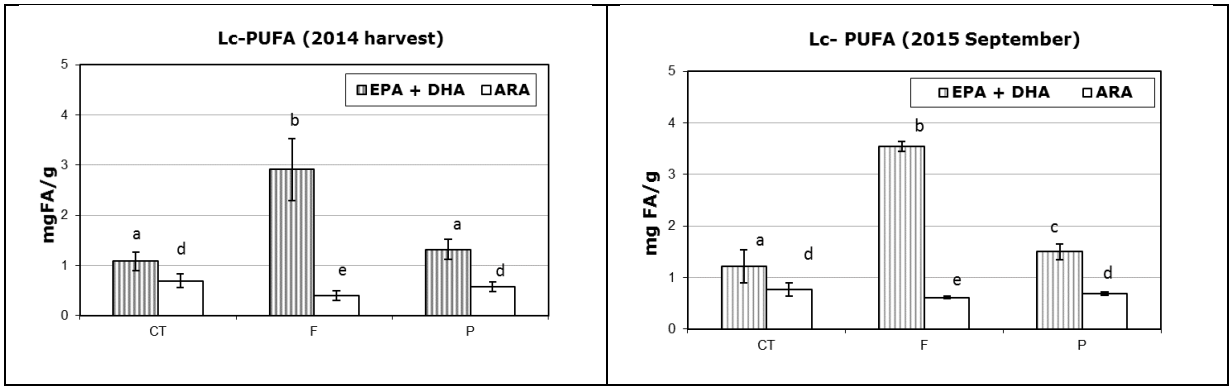

Figure 1. Lc-PUFA level (w.w.) in the edible part (filet) of *Cyprinus carpio* after 2- and 3 years of feeding (Mean ± standard deviation. Values are means of three replicates of individual samples; values within the same fatty acids with different letters are significantly different (P<0.05). Values between fatty acids are not considered. CT- control, F- fish based diet, P- plant based diet).

The content of trace metals in the filet is presented in Table 2. Significantly, higher zinc was detected in F group (13 mg kg^{-1}), compared with groups with plant origin (P: 7.8 mg kg^{-1} and CT: 7.5 mg kg^{-1}), furthermore the phosphorus was differed in CT and F treatments. The heavy metal levels were below the food safety maximum residue levels (EC regulations 1881/2006) in the most of the analyzed elements (Cd, Ar, Cr, Pb, Ni) except in pond nr. 51, were relatively high level of Pb detected (outlier values were: 2.61 mg kg^{-1} and 6.98 mg kg^{-1}).

Dietary effect on the storage of the meat. Level of malondialdehyde, as a peroxidation product in the muscle of fish, during 5 days of storage is presented in Figure 2. Significant differences were observed between the treatments during the storage period; meantime the lowest values in the CT group were detected. In the last day of sampling the curve with MDA concentration turn to saturation in both three treatments. Antioxidant vitamin E level in the muscle of carp has been determined at the beginning of the storage period, as well. Concentration of vitamin E varied between 11 and 22 mg kg^{-1}, the highest level was found in the F group (Figure 3). Significant differences were found between the treatments due to the presumable dietary differences.

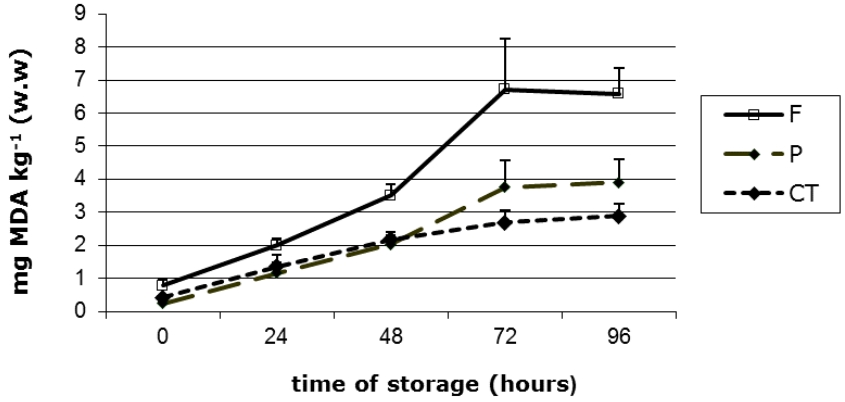

Figure 2. Peroxidation status of *Cyprinus carpio* flesh during storage at 4°C (Data point shows the mean of three replicates for sampling period. Bars represent the standard deviation. CT - control, F - fish-based diet, P - plant-based diet).

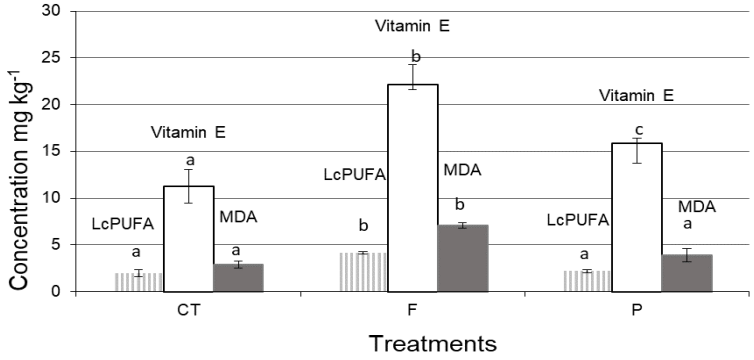

Figure 3. Level of antioxidant vitamin E, Lc-PUFA, and malondialdehyde (w.w.) in the filet of *Cyprinus carpio* from different treatments determined after 72 hours of storage (Same columns indicated with different letters are significantly different (P<0.05); CT- control, F- fish based diet, P- plant based diet).

Discussion. Growth of the fish at harvest after the whole production season was not significantly different within treatments, even though the average weight of the P group fish was the highest. Flesh composition of the market size carp (average body weight 2,000 g) has been studied for nutritional value for human consumption. Assessment of the filet fat content has shown remarkable lower level in our studies in groups fed with composed feeds (F: 7.1% and P: 6.6%) compared to *C. carpio* produced in intensive feeding tank systems (11-15%) (Csengeri et al 2011) or in intensive pond rearing systems (11-12%) (Csengeri et al 1999) using similar nutritive content feeds. This could be attributed to low stocking densities applied in the semi-intensive pond technology in our case. Similarly, low level (3.0%) was obtained in pond culture using extruded feed by Trbovic et al (2013) for feeding the market size *C. carpio*, but 11.6% fat was detected when corn was applied. It was demonstrated that the intramuscular fat deposition in carp have been caused by high level of dietary carbohydrate (Keshavanath et al 2002). Hereafter different studies reflected the fact that decreasing the protein (increasing the carbohydrate ratio) content in composed diet leads to higher fat in muscle tissue (Ljubojevic et al 2015). Considering this observation feeding with complete diet using plant meal and plant oil is more preferable compared to cereals in contest of filet fat composition.

The amount of PUFA was significantly lower in CT group meanwhile level of them was not differing in F and P groups. Similar trend was demonstrated by Csengeri et al (2011) using different lupine and linseed level in the feed. In this case PUFA content of the muscle, which are important for human nutrition, was differing significantly (50% higher) from the control fish fed with wheat. In our study sum of EPA+DHA was relatively low in the filet of market size carp fed without any marine sources (Figure 1), but are still higher (P: 1.50±0.15 mg g^{-1} and CT: 1.21±0.32 mg g^{-1}) compared with data obtained by Tribovic et al (2013), where only 0.476 mg g^{-1} and 0.184 mg g^{-1} EPA+DHA was determined when maize and plant based extruded feed was administered in pond rearing system.

Previous studies showed that *C. carpio* is able to convert linolenic acid to a higher homologue (Olsen 2011), but in this survey a moderate conversion rate was observed even though high level of dietary LNA was available throughout feeding period. The white and red muscle tissue has been known to have different fat content and fatty acid profile (Mraz & Pickova 2009). Our data presents similar trend with significant differences in term of EPA+DHA level (Table 4). We compared the filet quality of market size fish (3-years old) with the filet of 2-years old fish in harvest season and we observed fattening of fish at the end of production cycle. Zajic et al (2013) reported that after harvest, in the starvation period changes are observed in the fat content and in the fatty acid profile of the filet. As a consequence of the decreasing atmospheric temperature, synthesizing oleic acid for energy storage and body maintenance is fasted (Csengeri 1996). Thus, consideration of their external factors on the quality of *C. carpio* flesh should be taken into account. Surprisingly the younger aged fish contained almost similar EPA+DHA

amount (2014: 1.31 mg g^{-1}) than the market size fish (2015: 1.50 mg g^{-1}) (Figure 1) and these amounts are one third of the recommended daily amount from EPA+DHA for humans (500 mg day^{-1}) (EFSA 2012). These findings may suggest that consumption of younger *C. carpio* individuals are as good as the table size fish filets in respect of EFA.

The trace metals in the market size fish filet are in strong correlation with dietary level. Trenovszki (2013) reported zinc level of 5-10 mg kg^{-1}, copper 0.2-0.6 mg kg^{-1} in carps from different farms, which are close to our findings. In the case of iron, levels in our fish samples were much higher compared to samples from another region presented in the above-mentioned publication (1-5 mg kg^{-1}). It is well known, that soil composition in the Körös-river basin contain elevated level of iron, which could be found in vegetation or in the aquatic living beings (Sandor et al 2001). The Ca/P ratio of the filet was almost 1:3 in each of the cases (CT: 2.87; F: 2.94; P: 3.21), which demonstrate that the fish is a highly valuable food for human nutrition. The data on heavy metals obtained in our study are comparable with previous results from Hungarian sampling sites in ponds, where values of 329±62 μg kg^{-1} Ni, 3.1±0.2 μg kg^{-1} Pb, 317±77 μg kg^{-1} Cr and 2.0±0.5 μg kg^{-1} Cd (w.w) were detected (Oncsik et al 2009). Origin of Pb contamination found in one of the ponds is unknown.

Relationships between the meat composition and rancidity processes were assessed which could be attributed to the dietary effects and to the feeding history of the animals. The changes in fish lipids directly or indirectly are responsible for rancidity of the filet. Lipid oxidation in fish concerns fatty individuals. Lipid peroxidation characteristics, such as amount of the tio-barbutiric acid reactive substance are produced of consecutive reaction phase of peroxidation. Trenovszki et al (2011) investigated whether the amount of malondialdehyde (MDA) indicating the intensity of lipid peroxidation processes varies, for instance how is affected the duration period during storage. They concluded that higher quantities of grain during the feeding period (corn, wheat and sunflower) contributed to higher, less valuable saturated fatty acids (SFA) in the muscle of fish. However, in the aspect of storage, the level of PUFA should be lower. In the present study levels of MDA were situated between 0.2 and 0.7 mg kg^{-1} on the first day of storage (time zero), the highest values found in the F group. According to Trenovszki et al (2011) findings peroxidation status varies in such intervals (0.3-1.5 mg kg^{-1}) in the surveyed *C. carpio* muscles right after slaughtering and mainly differing on the feeding conditions of the fish. In this study the level of MDA increased quickly during the storage when at the 3rd day the growing tendency has slowed down and remained constant. At the 72nd hour of storage significant differences were observed between the treatments (Figure 2), the lowest values were measured in the control group. This observation is in connection with earlier findings of our research group, where total volatile nitrogen was measured and correlated with organoleptic studies (Lengyel et al 2000). Pacheco-Aguilar et al (2000) studied the post mortem changes in Monterey sardine muscle stored at 0°C for 15 days. They found level of MDA between 5 and 38 mg kg^{-1} with the highest level at 11th days of storage. They concluded that quality of fish is optimal up to 5 days of storage at zero degrade based on the evaluation of different post-mortem parameters.

Sources of feed ingredients were different in the F and P groups, however, the dietary level in the vitamin premix was similar in both composed feeds. Vitamin E measurement of the feeds was not performed in the present study. It is well documented that increased dietary PUFA increases the requirement of vitamin E in fish, but there are still questions on the mechanism of α-tocopherol (α-TOAc) retention (Hamre 2011). Schwarz et al (1988) demonstrated that increased PUFA at a constant level of dietary lipid increases the vitamin E requirement in *C. carpio*. Unfortunately, tissue vitamin E levels were not given in their article, and it is not known whether the dietary PUFA had any effect on the retention of vitamin E. Atlantic salmon fed different levels of α-TOAc adjust their body levels to the dietary concentrations after approximately 3 months (Hamre & Lie 1995, 1997). There is a linear relationship between dietary and whole-body α-TOH, when dietary concentrations range between 0 and 300 mg kg^{-1}.

Relationship between the antioxidant vitamin status of the filet, grade of rancidity and content of essential fatty acid content of the filet was evaluated (Figure 3). It was assumed that vitamin E content of fish muscle will improve flesh quality and shelf life

through protection against lipid oxidation. We have found positive correlation between the rancidity and PUFA content of the filet, but it seems that vitamin E level of the filet has no influence on the MDA level of the filet. Based on the results reviewed by Hamre (2011), unclear correlations should be found, because induction of lipid oxidation in the filets is dependent on many factors, e.g. fatty acid composition of the filet lipid, vitamin E levels, storage conditions and time.

Conclusions. Our results show that linolenic acid, which is present in large volumes in linseed oil, can be transformed moderately to a higher homologue - even from juvenile stage. During all life cycle feeding some fatty acid synthesis was detected in P group, but the biosynthesis capacity was not as high as was expected based on previous studies. Moreover, the hypothesis of dynamic bioconversion trough all life cycle feeding with precursors of Lc-PUFA was not detected and percent of essential fatty acids in the filet remain unchanged. In this context in order to produce high quality *C. carpio* filet preferable for human nutrition is necessary to apply such feeding regime where in the last growing period fish meal and fish oil containing feed is used. Using plant meal and plant oil in feeds for growing of *C. carpio* is appreciable compared to cereal based diet, better growth parameters and better nutrient composition of the filet can be attained.

Peroxidation status of fish flesh was significantly higher when marine origin diet was applied even at the beginning or during of storage. It seems the vitamin E level in feeds has not influenced the MDA formation in *C. carpio* filet. Similarly, the trace metals level was only different in such elements that are different in plants and aquatic organism. Levels of toxic metals were almost below the food safety maximum residue levels in all of the analyzed samples. In conclusion, the linseed oil dietary group gained better production parameters during the 3 years trial, but more important information for the consumers is that the quality of the fish is more suitable for human consumption in context of fat content, EFA composition and storage compared to the other *C. carpio* flesh.

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