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The Breed Effect on Productivity and Meat Nutrient **Compsition of Fish**

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Summary

Productivity and meat quality of carp (Cyprinus carpio L.), silver carp (Hypophthalmichthys molitrix), grass carp (Ctenopharyngodon idella), catfish (Silurus glanis), zander (Stizostedion lucioperca) and tench (Tinca tinca), which were grown in policulture on natural food in pond fertilised with livestock manure, were compared. The amount of protein ranged from 15.74% (tench) to 19.26% (zander). Lipid contens (%) in the fillets of zander, tench, catfish, carp, silver carp and grass carp was 0.38; 0.79; 2.19; 2.42; 3.82 and 5.24, respectively. The total cholesterol content was the highest in the silver carp fillets (62.32 mg/100 g) and the lowest in catfish (34.34 mg/100 g). The total amount of saturated fatty acids was the highest in silver carp (33.05%) and the lowest in catfish (24.97%). The total polyunsaturated fatty acids was however the highest in tench (44.50%) and the lowest percentage of PUFA was in silver carp (29.95%) and the lowest ratio n-3/n-6 was in common carp (0.92). The chemical composition and quantity of n-3 fatty acids varied largely by the fish species. In summary, rearing fish in polyculture on natural food with the use of agricultural limestone and livestock manure is feasible and productivity and nutrient content varies by the fish species.

Keywords: Productivity, Nutrient composition, Fatty acid profile, Fish species, Livestock manure, Natural food, Pondpolyculture

Yetiştiriciliğin Balık Eti Besin Kompozisyonu ve Balıkların Verimliliği Üzerine Etkisi

Özet

Bu çalışmada, çiftlik hayvanlarının dışkısı ile gübrelenmiş polikültür havuzlarında doğal gıdalarla beslenen sazangiller grubu balıkların [sazan (Cyprinus carpio L.), gümüş sazanı (Hypophthalmichthys molitrix), ot sazanı/(Ctenopharyngodon idella), yayın (Silurus glanis), sudak balığı (Stizostedion lucioperca) ve kadife balığı (Tinca tinca)] nın biyolojik verimliliği ve et kalitesi karşılaştırılmıştır. Protein düzeyi %15.74 (kadife) ile %19.26 (sudak) arasında bulundu. Sudak, kadife, yayın balığı, gümüş sazanı ve ot sazanı filetlerindeki yağ düzeyi, sırasıyla, %0.38, %0.79, %2.19, %2.42, %3.82 ve %5.24 olarak belirlendi. En yüksek toplam kolesterol düzeyi gümüş sazan filetinde (62.32 mg/100 g), en düşük toplam kolesterol ise yayın balığı etinde (34.34 mg/100 g) ölçüldü. Toplam doymuş yağ asitleri en fazla gümüş sazan etinde (%33.05), en düşük ise yayın balığı etinde (%24.97) ölçüldü. Toplam çoklu-doymamış yaş asitleri ise en fazla yeşil sazan etinde (%44.05) ve en düşük gümüş sazan etinde (%29.95) ölçüldü. Pullu sazan balığı etinde en düşük n-3:n-6 oranına sahip incelenen balık türü idi. Yağ asidi bileşenleri ve n-3 yağ asitleri miktarı balık türüne göre oldukça değişkenlik gösterdi. Özetle, hayvan gübresi ve zirai kireç taşı katılmış polikültürlerde doğal gıdalar ile beslenerek yapılan balık üretimi mümkün olabilmekte ve balıkların biyolojik verimliliği ile et besin madde bileşimi balık türlerine göre değişiklik göstermektedir.

Anahtar sözcükler: Verimlilik, Besin madde bileşenleri, Yağ asiti profili, Balık türleri, Hayvan gübresi, Polikültür

INTRODUCTION

Fish farming in Serbia in pondpolyculture consisted mainly of carp with silver carp, bighead carp, grass carp, catfish and zander. Additional fish species are farmed in carp ponds to increase total yield per unit area. Polyculture enables

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 \bowtie ljubojevic.ljubojevicd.dragana@gmail.com better utilization of natural food resources, saving artificial food or using low cost food as well as improvement in pond environmental conditions, feeding terms for fish, and control of aquatic weeds and oxygen concentration in ponds.

Cyprinids, omnivore fish, are the most widely cultured fish in the world and Serbia. It effectively uses natural foods (debris, detritus, and zooplankton), tolerates ambient conditions, have resistance to diseases ¹. The "Chinese carps" (grass carp, silver carp, bighead carp) were introduced into the European waters (Danube Basin) in 1960s to increase total ichthyoproduction at the expense of food resources available in the plankton form ², and now account for 20-30% share in polyculture ³. These herbivore fish contribute to ecological potential of fish ponds and make production more economical. The silver carp is a filter feeder. In general, they rely largely on phytoplankton as well as zooplankton and detritus. Thus, they are considered "water quality controller", especially for cyanobacteria. The grass carp, freshwater fish species is a herbivorous and consumes higher aquatic plants and submerged terrestrial vegetation. Despite presence in our market for 40 years, biological guality of Chinese carp hasnot been investigated yet. Due to its palatable meat and high attractiveness for sport anglers, the tench is likely to have a great potential for future, either as a supplementary species for pond aquaculture or for stocking open waters. The tench diet under natural conditions is comprised primarily of zooplankton (Cladocera, Copepoda) and bottom sediments ¹. Catfish and zander are carnivores fishes, whose participation in polyculture has a task to control undesirable fish which enter carp ponds as well as to select fish that poorly grows and causes adverse health conditions. European catfish is characterized by a fast growth rate, high feed utilization efficiency, high carcass yield, no scales and tasty white flesh devoid of bones. Zander is a carnivorous fish, it generally feeds on other fish species rich in poly fatty acid ⁴. Carnivorous fish gains 1 kg weight by eating 15 kg of other fish³, suggesting that they can be grown in small operations using undesirable fish. Indeed, catfish species are recommended as component in policulture with carp, tench, and herbivorous fish ⁵. Growth rate and feed utilization effectiveness obtained by European catfish cultivated in polyculture are more advantageous than those in monoculture ⁶.

Fish meat lipid content varies by breed, season, and diet ⁷. Fish meat fat is highly variable and consist of 15-36% saturated fatty acids (SFA) ^{8,9} and 58-85% unsaturated fatty acids (UFA) ^{10,11} as well as cholesterol (49-92 mg/100 g) that is not correlated with total fat level ¹². Fish meat contains 13-25% protein ¹³⁻¹⁶ with high biological value ¹⁷. There are no data about meat quality of fish rearing in pondpolyculture on natural food. This study was conducted to compare productivity and meat quality of carp (*Cyprinus carpio L.; Hypophthalmichthys molitrix, Ctenopharyngodon idella*), catfish (*Silurus glanis*), zander (*Stizostedion lucioperca*) and tench (*Tinca tinca*) that were grown in policulture on natural food in pond fertilised with livestock manure.

MATERIAL and METHODS

Pond Management and Fish Samples

Fish were grown in earthen pond with a surface area of 1 ha and average depth of 1 m in the polyculture on natural food. The fishpond was stocked in April 2011 and harvested in October 2011. Samples of two years old carp, two years old silver carp, grass carp, catfish and zander and three years old tench were taken during the harvesting. The stocked tench was older in comparison with the other species to avoid the possibility that catfish and zander eat up two years old tench.

Freshwater fish species were reared under variable natural atmospheric conditions and fed natural food. The production of natural food was based on the natural production of benthic and planktonic organisms that were increased by application of agrotechnical measures such as drying of fishponds during winter, soil treatment, fertilization and adding lime. Livestock manure (2000 kg/ha) was applied to the bottom of empty pond and later biweekly over the water surface (4000 kg/ha during growing season). Agricultural limestone was applied to the bottom of empty pond and over the water surface. Aeration of fish pond was provided.

After weighing, eight samples from each breed were stored at a temperature of –18°C. Before examination, the fish were left at room temperature, in order to partially defrost and remove skin easily, and separate head, tail, and viscera. Fish fillets were blended (Braun CombiMax 600). To examine fatty acid content and total cholesterol samples were stored in dark plastic bags at temperatures of –18°C. The meat from dorsal muscles was used for chemical analyzes.

Chemical Analysis

Chemical composition of fish muscle tissue was determined using standard SRPS ISO methods. Protein content was determined by Kjeldahl method (Kjeltec Auto 1030 Analyzer, Tecator, Sweeden). Water content was determined by drying at 103±2°C to constant weight. For determination of total fat, the samples were hydrolyzed with 4M hydrochloric acid and extracted with petroleum ether by Soxhlet apparatus. Ash content was determined by combustion at 550±25°C for 18 h.

Extraction of Lipids by ASE

Total lipids were extracted from fish muscle tissues by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA). Homogenate of sample mixed with diatomaceous earth was extracted with a mixture of n-hexane and iso-propanol (60:40 v/v) in 33 ml extraction cell at 100°C and nitrogen pressure of 10.3 MPa ^{14,15}. Then, the solvent was removed under stream of nitrogen in Dionex Solvent Evaporator 500 at 50°C until dryness. The fat extract was further used for fatty acids determination.

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Fatty Acid Analysis by Capillary Gas Chromatography (CGC)

Fatty acid methyl esters (FAME) were prepared by transesterification using trimethylsulfonium hydroxide (SRPS EN ISO 5509:2007 procedure). The GC instrument Shimadzu 2010 (Kyoto, Japan) used for FAME determination was equipped with a split/splitless injector, fused silica cianopropyl HP-88 column (length 100 m, i.d. 0.25 mm, film thickness 0.20 µm, J&W Scientific, USA), and flame ionization detector and workstation (Shimadzu GC Solution ver. 2.3). The column temperature was programmed as follows: initial temperature 125°C, rate 10°C min⁻¹ to 175°C, hold 10 min, rate 5°C min⁻¹ to 210°C, hold 5 min, rate 2°C min⁻¹to final temperature of 230°C. The column temperature was programmed, injector temperature of 250°C and detector temperature of 280°C. The carrier gas was nitrogen at a flow rate of 1.33 ml/min and injector split ratio of 1:50. Injected volume was 1 µl and total analysis time was 50.5 min. The chromatographic peaks in the samples were identified by comparing relative retention times of FAME peaks with peaks in a Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA)¹⁴. Quantification was done by using heneicosanoic acid methyl ester as internal standard. The response factors were calculated by the ratios between the peak area of the individual fatty acid methyl esters and that of internal standard (heneicosanoic acid methyl ester). Relative amount of each fatty acid methyl ester was expressed as a percentage of the total amount of fatty acids in the analysed sample.

Cholesterol Determination

Cholesterol in carp fillets (from direct saponification) was measured using HPLC/PDA system (Waters 2695 Separation module/Waters photodiode array detector, USA), on a Phenomenex Luna C18 (2) reverse phase column, 150 mm x 3.0 mm, 5µm particle size, with C18 analytical guard column, 4.0 x 2.0 mm as described by Maraschiello et al.¹⁸. The injected volume was 10 µL. The mobile phase was consisted of isopropanol-acetonitrile (20:80, v/v) at a flow rate of 1.2 mL/min. Detection was performed at 210 nm and total analysis time lasted 10 min.

Statistical Analysis

The data were subjected to one-way ANOVA at P<0.01.

Intergroup differences were determined (P<0.01) using the Tukey HSD test (Statistica 10, StatSoft Inc., Tulsa, USA). The data were presented as means \pm SE.

RESULTS

The mean stocking weight was the highest common carp (250 g) and the lowest in zender (30 g) and harvesting weight was the highest in silver carp (1450 g) and the lowest in tench (200 g) and the survival rate was the highest in catfish (90%) and the lowest in tench (40%) (*Table 1*). Total stocking density was 274 kg/ha and harvesting density was 820 kg/ha.

Table 2 summarizes chemical composition and cholesterol content of fish samples. The amount of protein was the highest in the fillets of zander (19.3%) and the lowest in grass carp and tench fillets (15.8%). Fat ranged from 0.38% in the muscles of zander to 5.24% in the muscles grass carp. The total cholesterol content was highly variable, being the highest in the silver carp fillets (62 mg/100 g) and the lowest in catfish (34 mg/100 g).

Fatty acid composition of samples are presented in *Table 3*. The total amount of saturated fatty acids (SFA) was the highest in silver carp (33%) (*Fig. 1*) and the lowest in catfish (25%). Individual SFA's were variable by species. Palmitic acid (C_{16:0}) was predominant, whereas lauric acid (C_{12:0}) was the least SFA in samples (*Table 3*). The most abundant MUFA was oleic acid (C_{18:1, n:9}), ranging from 22% in silver carp to 18% in tench, followed by palmitoleic aic (C_{16:1, n:7}) (*Table 3*). The MUFA proportion was the lowest in tench (28%) and greatest in silver carp (37%). Tench had the highest (44%), silver carp had the lowest (30%) PUFA proportions (*Table 3*).

Samples were also highly variable in terms of n-3 and n-6 contents (*Table 3*), for instance the n-3:n-6 ratio was the lowest in common carp 0.92, whereas it was the greatest in grass carp (2.28). Similarly (*Table 3*), the PUFA:SFA, an indicator of the quality of lipids, was the least favorable in silver carp (0.91) and the most favourable in tench (1.63).

DISCUSSION

Meat quality of carp is highly variable, and changes depending on age, breeding system, and diet. Although fat

Table 1. Fish stock composition, weight gain and survival rate							
Tablo 1. Balık stok kompozisyonu, büyüme ve hayatta kalma oranı							
Variable	Species						
	Common carp	Silver carp	Grass carp	Catfish	Zander	Tench	
Individual/ha	1000	100	50	30	20	100	
Weight at stocking (g)	250±16.04ª	50±11.95 ^d	130±13.89 ^b	50±11.95 ^d	30±3.29 ^d	100±6.78°	
Weight at harvesting (g)	910±10.86°	1450±15.12ª	500±17.06 ^d	1320±16.8 ^b	400±13.09 ^e	200±8.96 ^f	
Survival rate (%)	70	80	75	90	60	40	
Data are means \pm SE (n=8). Different superscripts within the same rows differ (P<0.01)							

Table 2. Proxiamate composition of fish reared in polyculture Table 3. Polykültürde vetistirilen belyketlerinin besin isseriği							
Chemical Composition Carp Silver carp Grass carp Wels catfish Zander Tench							
Moisture content (%)	80.36±0.24 ^b	76.93±0.15 ^f	78.03±0.16 ^e	78.76±0.16 ^d	79.32±0.05°	82.41±0.25 ^a	
Protein content (%)	16.21±0.12 ^c	18.1±0.09 ^b	15.76±0.07 ^d	18.16±0.06 ^b	19.26±0.04ª	15.74±0.15 ^d	
Fat content (%)	2.42±0.17°	3.82±0.19 ^b	5.24±0.12ª	2.19±0.12 ^c	0.38±0.04 ^e	0.79±0.34 ^d	
Ash content (%)	1.02±0.02 ^b	1.15±0.09ª	0.98±0.04 [⊾]	0.88±0.03 ^c	1.03±0.01 ^b	1.06±0.02 ^b	
Total cholesterol (mg/100 g)	55.81±0.11°	62.32±0.37ª	60.06±0.11 ^b	34.34±0.33 ^f	42.91±0.06 ^d	35.87±0.54 ^e	
Data are means±SE (n=8). Different superscripts within the same rows differ (P<0.01)							

Table 3. Fatty acid composition of fish raised in poly-culture

Tablo 3. Polikültürde yetiştirilen balıkların yağ asitleri kompozisyonu

Fatty Acids, %	Species							
	Carp	Silver carp	Grass carp	Wels catfish	Zander	Tench		
C _{12:0}	0.06±0.01 ^d	0.43±0.02ª	0.40±0.03ª	0.24±0.02 ^b	0.14±0.02 ^c	0.07±0.02 ^d		
C _{14:0}	1.53±0.25°	2.83±0.02ª	2.80±0.07ª	2.32±0.03 ^b	0.96±0.02 ^d	1.14±0.07 ^d		
C _{15:0}	1.11±0.08ª	1.02±0.01 ^{ab}	1.01±0.03 ^b	0.84±0.02 ^c	0.29±0.06 ^e	0.63±0.08 ^d		
C _{16:0}	18.35±0.28 ^b	22.12±0.06 ^a	22.17±0.04 ^a	16.02±0.07 ^c	22.22±0.28ª	18.23±0.17 ^b		
C _{17:0}	1.32±0.09ª	1.37±0.01ª	1.34±0.03ª	1.25±0.02ª	0.45±0.03 ^c	0.80±0.15 ^b		
C _{18:0}	4.51±0.12 ^d	5.03±0.03°	4.93±0.06°	4.08±0.05 ^e	6.46±0.12ª	6.09±0.13 ^b		
C _{20:0}	0.26±0.03 ^{bcd}	0.26±0.01 ^{bcd}	0.20±0.02 ^c	0.22±0.02 ^{bcd}	0.26±0.05 ^b	0.37±0.04ª		
Total SFA	27.15±0.38°	33.05±0.09 ^a	32.84±0.06ª	24.97±0.06 ^d	30.79±0.26 ^b	27.34±0.34 ^c		
C _{16:1, ω-9}	5.73±0.28°	9.32±0.02ª	9.15±0.06ª	9.35±0.19ª	6.12±0.06 ^b	5.85±0.11 ^{bc}		
С _{18:1сі5-9. ш-9}	19.39±0.21°	21.56±0.01ª	21.08±0.03 ^b	18.22±0.06 ^d	19.57±0.08°	17.80±0.29 ^e		
C _{18:1cis-11.ω-7}	2.33±0.27 ^f	4.89±05 ^b	4.57±0.23°	6.19±0.08ª	3.40±0.14 ^e	3.98±0.05 ^d		
C _{20:1. ω-9}	1.35±0.12 ^{ab}	1.27±0.01 ^{bc}	1.23±0.03°	1.85±0.04ª	0.95±0.03 ^d	0.55±0.09 ^e		
Total MUFA	28.79±0.48 ^d	37.04±0.07 ^a	36.03±0.19 ^b	35.61±0.12 ^b	30.05±0.2 ^c	28.18±0.21°		
C _{18:2.ω-6}	10.29±0.11ª	5.87±0.16 ^e	5.78±0.09 ^e	6.08±0.05 ^d	7.10±0.15 ^c	9.88±0.07 ^b		
С _{18:3.ω-6}	5.22±0.06ª	0.24±0.01 ^d	0.24±0.02 ^d	0.7±0.03°	3.30±0.09 ^b	0.61±0.1°		
C _{18:3, ω-3}	5.96±0.14 ^b	6.24±0.01ª	6.37±0.06ª	5.22±0.03°	1.34±0.03 ^e	4.28±0.31 ^d		
C _{20:2, ω-6}	0.33±0.07 ^c	0.36±0.01°	0.36±0.02°	1.51±0.03ª	1.61±0.14ª	0.86±0.07 ^b		
C _{20:3, ω-6}	0.91±0.06 ^b	0.46±0.01°	0.44±0.02°	0.85±0.02 ^b	1.99±0.06ª	0.85±0.08 ^b		
C _{20:3, ω-3}	0.89±0.05°	0.60±0.01 ^d	0.7±0.03 ^d	4.60±0.03ª	0.36±0.04 ^e	1.66±0.13 ^b		
C _{20:4, ω-6}	6.21±0.11ª	2.75±0.01°	2.71±0.02 ^e	3.05±0.03 ^d	3.78±0.27℃	5.09±0.13 ^b		
C _{20:5, ω-3}	4.05±0.08 ^b	3.45±0.06 ^c	3.64±0.07°	5.34±0.19ª	1.26±0.04 ^d	3.52±0.21°		
C _{22:5, ω-3}	4.47±0.24 ^b	5.26±0.23ª	5.46±0.05ª	2.84±0.12 ^d	5.46±0.12ª	3.25±0.14 ^c		
C _{22:6, ω-3}	5.75±48 ^d	4.72±0.12 ^e	5.52±0.16 ^d	9.24±0.31°	12.99±0.51 ^ь	14.50±0.27ª		
Total PUFA	44.08±0.55ª	29.95±0.15 ^d	31.23±0.22 ^c	39.44±0.17 ^b	39.19±0.26 ^b	44.50±0.21ª		
ω-6	22.96±0.20ª	9.68±0.15°	9.53±0.10 ^e	12.20±0.07 ^d	17.78±0.31 ^ь	17.29±0.27°		
ω-3	21.12±0.48 ^b	20.26±0.21°	21.69±0.15 ^b	27.24±0.19ª	21.41±0.52 ^b	27.21±0.36ª		
ω-3/ω-6	0.92±0.02 ^e	2.09±0.05 ^b	2.28±0.02ª	2.23±0.02ª	1.21±0.05 ^d	1.57±0.04 ^c		
ω-6/ω-3	1.09±0.02ª	0.48±0.01 ^d	0.44±0.01 ^e	0.45±0.01 ^{de}	0.83±0.04 ^b	0.64±0.02 ^c		
PUFA/SFA	1.62±0.04ª	0.91±0.01 ^d	0.95±0.01 ^e	1.58±0.01 ^b	1.27±0.02 ^c	1.63±0.03ª		
UFA/SFA	2.68±0.05 ^b	2.03±0.01 ^d	2.05±0.01 ^d	3.01±0.01ª	2.25±0.03 ^c	2.66±0.05 ^b		
PUFA/MUFA	1.53±0.04 ^b	0.81±0.01 ^f	0.87±0.01°	1.11±0.01 ^d	1.30±0.02 ^c	1.58±0.01ª		
Data are means±SE (n=8). Different superscripts within the same rows differ (P<0.01), ¹ UFA - unsaturated fatty acids, SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids								

content in carp ranges from 2.3 to 16.8%, protein content lightly varies between 14 and 18% ^{13,15,16}. Domaizon et al.¹¹ reported that lipid content in fillets was in the range of

4.51 to 6.7% in one-year and three-years old silver carp. Fat content in catfish grown in pond on natural food was reported to be 2.33% ¹⁹. Protein and fat data for zander are higher than



Fig 1. Fatty acid chromatogram of silver carp **Şekil 1.** Gümüş sazanı yağ asidi kromatogramı

a study by Celik et al.⁴ who reported 18.1 and 18.8% protein and 0.1 and 0.12% in cold and warm lake. Cholesterol content varies by season. Trbović et al.¹⁵ reported 48.9 mg/100 g fat in one-year old carp in April and 54.3 mg/100 g fat in the same age samples harvested in June. In the present study, cholesterol level was 55.8 mg/100 g fat in 2-year old carp. In agreement literature, cholesterol content carp varies considerably from 47 to 120 mg/100 g, depending on fish breed and age, husbandry system, and harvest season ^{20,21}. This variability was very notable in silver carp, grass carp, tench, and catfish ²¹. Terrestrial animal fat contains also variable amount of total cholesterol. According to Williams²² cholesterol content in meat of beef, veal, lamb and mutton was 50, 51, 66 and 66 mg/100g, respectively, while Swize et al.²³ and Scherz and Senser ²⁴ reported that beef contain 58.3-83.4 mg/100 g, lamb 63-75.4 mg/100 g and and mutton 70 mg/100 g. Pork meat has been reported to contain 44-98 mg/100 g ²⁵. Further, the average fat content in beef, sheep and pig meat is 3.8-17.3; 6.3-35.0 and 1.6-11.5%, respectively 13.

The total value of SFA in catfish reared traditionally on natural food and processed feed was 25 and 26% ¹⁹. Of this, palmitic acid was reported to be predominant when fish was on natural and artificial food (16%). Our SFA and UFA (about 25 and 75%, respectively) data are comparable with literature. In the study of Jankowska et al.¹⁹, the amount of MUFA and PUFA and the n3:n6 ratios were 40%, 35%, and 2.31, respectively. According to Bieniarz et al.²⁶, the meat of catfish cultivated in a polyculture with common carp had 22% PUFA with the n-3:n-6 ratio of 2.39. However, in the study of Fullner and Wirth²⁷, this ratio was reported to be 1.7. In agreement with present data, in natural habitat, zander meat contained 30.5-32.9% SFA ⁴. Palmitic acid was the primary saturated fatty acid in lipids of zander, contributing approximately 72.2% to the total SFA in the present study. In natural habitat, palmitic acid was also noted as predominant SFA in zander, constituting 66% of the total SFA ^{4,28}. Oleic acid was the dominant MUFA. Among the n-3 series, zander was a good source of eicosapentaenoic acid (EPA, 1.26%),

(DPA, 5.46%), and docosahexaenoic acids (DHA, 13.0%).

Silver carp and grass carp fed phytoplankton, zooplankton, and macrophytes are rich in n-3 PUFA, especially EPA and DHA ^{11,29}. The proportion of total n-3 fatty acids varies between 20 and 30% and the n-3:n-6 ratio is about 2 to 3²⁹. As age increased, the n-3:n-6 ratio in fillets of silver carp increased from 1.19 to 1.9 at 1 and 3 years old ¹¹. Diet is also a contributing factor for different fatty acid profile. Zooplankton appears as the major contributor to the diet of one year old silver carp (90% of ingested biomass), whereas three years old silver carp exhibited a more evenly balanced food spectrum between zooplankton (45% of ingested biomass) and phytoplankton (55% of ingested biomass) ¹¹. Shapiro ³⁰ also showed that mature carp tended to consume more phytoplankton than one year old silver carp. However, in the study of Domaizon et al.¹¹, the impact of the phytoplankton on the fatty acid composition of the digesta was not pronounced in either one year or three years old silver carp, probably because it was masked by the influence of the zooplankton. Thus, DHA level was 2.56% in oneyear old silver carp and 7.76% in three-year old silver carp, while in our studies in fillets of silver carp contained 4.72% DHA.

Fatty acid composition in tench varies. The n-3:n-6 ratio is reported to range between 1.0 and 2.2²⁹ and between 1.93 and 3.6³¹. Ćirković et al.¹⁶ reported the n-3:n-6 ratio of 1.05 in tench. In the present study, the n-3:n-6 ratio was 1.57, and the percentage of SFA, MUFA, and PUFA 27, 28, and 44%, respectively.

In common carp, fatty acid composition is also variable. The n-3:n-6 ratio varies between 0.8 and 2.4²⁹. Other studies reported a smaller ratio, for example 0.54¹⁶, 0.50³², 0.26¹⁵ and it was 0.92 in this study. This could be attributed to the synergistic interaction between carp and silver carp which increases food sources available to the other species. The fecal pellets of the silver carp, which are rich in partially digested phytoplankton, make this food source available to the carp that otherwise could not utilize the phytoplankton.

Freshwater fish contain high levels of PUFA, which are very important in human nutrition ¹³. Essential fatty acids affect the fluidity, flexibility, and permeability of membranes as well as involves in cholesterol transport and metabolism²⁹. Arachidonic acid ($C_{20:4}$), a precursors of the eicosanoids, was measured in high percentages in all sample groups. Since there are several biochemical interactions between the n-6 and n-3 series, a balanced proportion of these fatty acids in the diet is important for the functioning of human and animal life. Technological advancements reduces residues of antibiotic in meat of fish ³³. Organic fertilization with livestock manure is a very cheap and effective method of increasing practically all nutrient components in fish pond ecosystems. Indeed, rearing of fish in polyculture on natural food with fertilization results in satisfactory fish production with a preferrable fatty acid composition as well as enhances utilization of natural food resources.

In conclusion, rearing fish in poly-culture on natural food with the use of agricultural limestone and livestock manure was achieved satisfactory results in terms of the final weight of two years old fish and their nutritive composition. It is necessary to take into account the stocking density of fish to grow in pond-polyculture, as well as on the combination of appropriate species. In conclusion, nutrient composition varies widely among fish breed. Especially, lipid profile seems related more to the fish species, their consumption habits (herbivorous, omnivorous or carnivorous).

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