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Effect of Poultry By-Product Meal on Growth Performance and Fatty Acid Composition of Carp (*Cyprinus carpio*) Fry

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Abstract

The effect of replacement of fish meal (FM) with graded level of poultry by-product meal (PBM) in diets on growth performance and fatty acid composition of carp fry (*Cyprinus carpio*) was examined in the experiment. Five isonitrogenous (34% crude protein), isolipidic (9% crude lipid) and isoenergetic (15.0 MJ/kg DE) experimental diets replacing 0 (control group), 25, 50, 75 and 100% of FM protein by PBM protein were formulated with lysine, methionine and threonine supplementation in order to balance the amino acid profiles of the experimental diets. Each diet was fed to twice daily to apparent satiation of groups of twenty fry (mean weight 0.39 g fish^{-1}) stocked into 65L glass aquaria to maintain three replicates per treatment. At the end of the experiment, body weight gain, specific growth rate, protein efficiency ratio and feed conversion ratio decreased with increased dietary replacement of FM with graded levels of PBM, while condition factor, hepatosomatic indices, viscerosomatic indices and whole body composition did not exhibit significant differences among the experimental groups (P>0.05). There were significant differences in total fatty acid composition of each experimental group. Values of Σn-3 polyunsaturated fatty acids and Σn-3/Σn-6 ratio in the muscle of fish decreased. No significant differences in saturated fatty acids values were observed in fish, while monounsaturated fatty acids values increased as the PBM level increased in the diet.

Keywords: *Cyprinus carpio*, feeding, fishmeal replacement, poultry by-product meal, growth, fatty acids.

Sazan (*Cyprinus carpio*) Yavrusunun Büyüme Performansı ve Yağ Asit Kompozisyonu Üzerine Tavuk Kesim Atıkları Ununun Etkisi

Özet

Bu çalışmada, aynalı sazan (*Cyprinus carpio*) yavru yeminde balık unu proteini yerine tavuk kesim atıkları ununun (TKAU) artan seviyelerde kullanımının balıklarda büyüme performansı ve yağ asidi kompozisyonu üzerine etkisi araştırılmıştır. Protein (%34 ham protein), yağ (%9 ham yağ) ve enerji (15,0 MJ/kg SE) değerleri eşit olmak üzere balık unu proteini yerine TKAU proteini %0 (kontrol grubu), %25, %50, %75 ve %100 oranında ilave edilerek beş farklı deneme yemi hazırlanmıştır. Ayrıca hazırlanan yemlerin amino asit içeriklerini dengelemek için lisin, metiyonin ve tironin ilave edilmiştir. Deneme, 65L'lik 15 cam akvaryumun her birinde 20 balık (ortalama ağırlık 0,39 g) olacak şekilde üç tekrarlı olarak planlanmıştır. Deneme grubu balıkları günde iki kez doyuncaya kadar 13 hafta süre ile beslenmiştir. Deneme sonunda, vücut ağırlık kazancı, spesifik büyüme oranı, protein etkinlik oranı ve yem dönüşüm oranı TKAU'nun artan oranlarını içeren yemlerle beslenen balıklarda azalırken, kondüsyon faktörü, hepatosomatik indeks, visserosomatik indeks ve balık eti kimyasal kompozisyonunda önemli bir farklılık görülmemiştir (P>0,05). Deneme grubu balıkların yağ asidi kompozisyonlarında önemli farklılıklar belirlenmiştir. Deneme yemlerindeki TKAU oranının artışına bağlı olarak deneme grubu balıkların Σn-3 çoklu doymamış yağ asitleri ve Σn-3/Σn-6 değerleri azalmıştır. Doymuş yağ asidi değerlerinde önemli bir farklılık görülmez iken, tekli doymamış yağ asidi değerleri yemlerdeki artan PBM seviyesiyle artış göstermiştir.

Anahtar Kelimeler: *Cyprinus carpio*, besleme, balık unu ikame, tavuk kesim atıkları unu, büyüme, yağ asitleri.

Introduction

Fish meal has traditionally been used in commercial fish feeds as the major source of dietary protein (Hardy, 2010). However, FM is the most expensive and limited availability (Nguyen *et al.*, 2009). Hence, the aquafeed industry has to search for less expensive alternative protein sources to reduce

their dependence of FM (Tacon and Metian, 2009). One of the most promising alternative animal ingredients is poultry by-product meal (PBM) that consists of terrestrial rendered clean parts of the carcass of slaughtered poultry such as necks, feet, intestines and undeveloped eggs (Yu, 2007). The protein level and amino acid profiles of PBM are relatively similar to FM (NRC, 2011) making the ingredient a valuable protein source for many species. High protein content, readily available and lower price of PBM are an ideal candidate for replacing FM in aquafeeds. However, use of PBM in fish diets sometimes results in reduced growth in fish, especially when replaced more than 50% of FM in diets (Fowler, 1991; Steffens, 1994). The reduced performance for fish was because of amino acid imbalances, as well as a lack of n-3 highly unsaturated fatty acids in the diets containing PBM (Nengas *et al*., 1999; Yiğit *et al*., 2006). However, replacing of FM with PBM protein may need supplementation of some essential amino acid such as lysine, methionine and threonine for well growth performance and body composition of fish. Similarly, when used as replacement of FM of PBM with balanced amino acid in the diets has been supplied for carp fry, it is important that how replacement rates of PBM would affect the fatty acid composition of fish muscle, and consequently their fat quality. Thus, the objective of this study was to evaluate the effects of replacement of fish meal protein with PBM protein on growth performance, body composition and fatty acid composition for carp fry.

Materials and Methods

Feed Formulation and Preparation

Fishmeal (FM), poultry by-product meal (PBM) and soybean meal were supplied by the Abalioglu Feed-Soybean and Textile Industries Inc., Denizli, Turkey. The proximate composition of main protein ingredients used in the experimental diets is presented in Table 1. Five isonitrogenous (34% crude protein), isolipidic (9% crude lipid) and isoenergetic (15.0 MJ/kg DE) experimental diets replacing 0% (control), 25%, 50%, 75%, and 100% of FM protein by PBM protein were formulated (Table 2). The essential amino acids compositions of the diets containing PBM were balanced in order to match that of the control diets by lysine, methionine and threonine supplementation. The essential amino acid compositions of feed ingredients and the experimental diets are given in Table 3. All the dry ingredients for the diets were measured, mixed, processed, and pelleted in our lab. Diets were processed using a meat grinder with a 2 mm diameter pellets, dried at room temperature to a moisture content of less than 100 g kg^{-1} diet, crumbled and sieved to appropriate size (0.8-2 mm diameter), sealed in airtight polyethylene bags and stored at -20°C until fed.

Table 1. Proximate (%, on wet wt.) composition of poultry by-product meal (PBM), fish meal (FM) and soybean meal (SBM) used in experimental diets

Parameters	PBM	FM	SBM
Dry matter	96.85±0.51	88.43±0.75	86.89±0.39
Crude	62.84±0.28	65.08±0.81	46.70±0.46
Crude lipid	20.28±0.21	10.18±1.62	1.01±0.37
Crude fiber	0.98±0.17	0.40±0.22	3.90±0.15
Crude ash	12.31±0.26	11.52±0.13	6.84±0.23

Values are mean (±SD) of triplicate analysis.

Rearing Conditions of Fish and Feeding

This study was conducted at the Laboratory of the Fisheries Faculty of Akdeniz University, Antalya, Turkey, from July 21, 2009 to October 20, 2009. Carp fry were obtained from a local fish hatchery (Mediterranean Fisheries Research Production and Training Institute, Antalya, Turkey). Fish were acclimated to the system and fed with control diet (PBM0) for 2 weeks before the trial. At the beginning of the experiment, each 15 glass aquaria (65L) were randomly stocked with 20 fry with an average weight of 0.39±0.08 g $fish^{-1}$ with three replicate tanks for each dietary treatment (5 treatments x 3 replicates). Fish were hand-fed to apparent satiation twice a day ($09:^{00}$ and $16:^{00}$ h) for 13 weeks. Fish weights and amount of feed consumed were measured weekly. Any uneaten feed was collected 1 h after each feeding, dried to constant weight at 70°C and reweighed. The aquaria were siphoned daily to remove feces materials and two-third of the aquarium water was changed daily. Aeration was supplied to each tank 24 h day^{-1} with compressed air by air stones from a central compressor.

Water temperature was also maintained constant with a 100 W automatic heater set. Water temperature and dissolved oxygen were recorded daily using a Model WTW Oxi 330i multi-oxygen meter (WTW Wissenschaftlich-Weilheim, Germany). The water temperature ranged from 24 to 26°C, dissolved oxygen from 4.8 to 5.5 mg/L, pH from 7.8 to 8.4. All parameters remained within the acceptable ranges required for normal growth of carp fry during the course of the study (Horvath *et al*., 2002). During the feeding trials, Fish were subjected to a constant photoperiod of 12 h light/12 h darkness under fluorescent lighting.

Chemical Analysis

A sample of 15 random fish per aquarium at the termination were collected and stored frozen (-20°C) for determination of proximate and fatty acid composition. Proximate composition of the feed ingredients, experimental diets and fish carcass were

Table 2. Formulation and proximate composition of the experimental diets

	Experimental diets				
Ingredients (%)	PBM0	PBM25	PBM50	PBM75	PBM100
Fish meal	42.30	31.50	20.7	10.30	0
Corn gluten	27.90	27.90	27.90	27.90	27.90
Soybean meal	15.80	15.80	15.80	15.80	15.80
PBM[1]	0	10.75	21.40	31.72	41.80
Corn oil	2	3.20	2.25	1.30	0.50
Fish oil	2.25	0	0	0	0
Cellulose	2.15	2.81	3.48	4.16	4.75
Vitamin premix[2]	2	2	2	2	2
Mineral premix[3]	3	3	3	3	3
Methionine	0	0.10	0.20	0.25	0.30
Lysine	0	0	0.15	0.30	0.50
Threonine	0	0.34	0.52	0.67	0.85
Sodium chloride (NaCl)	0.1	0.1	0.1	0.1	0.1
$CaHPO_42H_2O$[4]	1	1	1	1	1
Carboxymethyl cellulose	1	1	1	1	1
Chromium oxide (Cr_2O_3)	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100
Proximate composition (% wet wt.)					
Moisture	8.63±0.11	8.21±0.07	7.61±0.10	8.46±0.05	7.63±0.08
Crude protein	34.13±0.30	34.04±0.17	34.06±0.20	33.80±0.49	33.62±0.25
Crude lipid	8.61±0.21	9.37±0.14	9.12±0.10	8.89±0.15	8.76±0.13
Crude ash	11.37±0.14	11.42±0.85	11.23±0.78	11.42±0.23	11.34±0.33
Crude fiber	3.96±1.53	4.36±1.45	5.11±1.87	6.27±0.97	6.79±1.05
Nitrogen-free extract	33.30±0.51	32.60±0.41	32.87±0.37	31.16±0.42	31.86±0.47
Dietary energy (MJ kg^{-1})	15.02	14.97	14.95	14.92	14.93

Values are mean of triplicate analysis.
[1] Poultry by-product meal
[2,3] Aydın and Gümüş (2013)
[4] Calcium hydrogen phosphate

Table 3. Essential amino acid composition (g 100 g^{-1} dry diet) of main protein ingredients and experimental diets, and essential amino acid requirements for carp fry (% of dry feed)

				Experimental diets					
Parameters	PBM	FM	SBM	PBM0	PBM25	PBM50	PBM75	PBM100	Req.[1]
Histidine	2.33±0.03	1.94±0.06	1.28±0.12	0.47±0.05	0.59±0.10	0.80±0.13	1.20±0.08	0.73±0.09	0.8
Isoleucine	2.58±0.09	3.70±0.17	1.95±0.29	1.76±0.10	1.86±0.02	1.65±0.05	1.59±0.19	1.57±0.02	1.1
Leucine	4.13±0.21	5.76±0.02	3.08±0.06	2.73±0.02	2.98±0.08	2.60±0.07	2.20±0.02	2.33±0.05	1.5
Lysine	5.97±0.42	7.60±0.17	3.10±0.03	1.10±0.04	2.58±0.17	3.22±0.20	3.98±0.17	2.12±0.06	2.4
Threonine	2.20±0.12	3.25±0.03	1.70±0.09	1.47±0.06	2.17±0.05	1.97±0.07	1.74±0.25	2.21±0.04	1.1
Valine	2.89±0.26	5.49±0.08	2.08±0.07	2.01±0.19	2.21±0.04	1.90±0.04	1.86±0.03	1.85±0.11	1.2
Methionine	0.94±0.11	1.49±0.04	3.59±0.06	0.63±0.05	0.70±0.11	0.51±0.01	0.64±0.02	0.64±0.06	0.7
Phenylalanine	2.30±0.39	2.61±0.22	1.82±0.13	1.26±0.12	1.49±0.04	1.35±0.04	1.26±0.05	1.26±0.12	0.9

Values are mean (±SD) of two analysis.
[1]Required, NRC (2011)

analyzed according to standard procedures following AOAC (1995): Dry matter after drying in an oven at 105°C to constant weight; ash content by incineration in a muffle furnace at 550°C for 24 h; crude protein (Nx6.25) by the Kjeldahl method after acid digestion; lipid by ethylether extraction in a Soxlet method; crude fiber content by alkali and acid digestion and nitrogen-free extract (NFE) by difference [NFE= 100 - (%moisture + %protein + %lipid + %ash + %fiber)]. Amino acids were analyzed following acid hydrolysis using a phenomenex EZ fast GC-FID hydrolyzed amino acid analysis kit (Varian GC, CP-3800 GC). Fatty acids were determined by Gas Chromatography (GC). For this purpose, total lipids were extracted by using a modified method of Bligh and Dyer (1959), and then saponified and methylated for fatty acid quantification (Morrison and Smith, 1964).

Growth Studies and Sample Collection

At the end of the study, all fry in each aquarium were collected, anesthetized with clove oil, individually weight and measured. Five fish from each aquarium were dissected, and the liver and viscera weight for the determination hepatosomatic index and viscerosomatic index. Fish growth parameters and feed utilization were calculated as

follows:

Specific growth rate (SGR, %day^{-1}) = 100 x [Ln final body weight (g) - Ln initial body weight (g)]/days; Feed conversion ratio (FCR) = feed intake (g) / [final body weight (g) - initial body weight (g)]; Protein efficiency rate (PER) = [final body weight (g) - initial body weight (g)] / protein intake (g); Condition factor (CF) = 100 x [final body weight (g) / (total body length (cm))^3]; Hepatosomatic index (HSI, %) = 100 x [wet liver weight (g) / final body weight (g)]; Viscerosomatic index (VSI, %) = 100 x [visceral weight (g) / final body weight (g)]; Survival rate (SR, %) = 100 x [final fish number / initial fish number].

Statistical Analysis

The results are presented as mean ±SD. All the data were subjected to one-way ANOVA in SPSS version 15.0 (SPSS INC. Chicago, IL, USA). Differences among the means were compared using Duncan's multiple range tests at a 5% probability level.

Results

The essential fatty acid compositions of feed ingredients and the experimental diets are given in Table 4. Concerning the fatty acid composition of the ingredients, total monounsaturated fatty acid (MUFA) content was the highest in PBM (43.8%), but total saturated fatty acid (SFA) was moderate in comparison with FM (39.89%). On the other hand, amount of total polyunsaturated fatty acid (PUFA) (12.39%) in PBM was distinctly lower than that of FM containing considerable amounts of ΣPUFA (32.94%) mainly composed of eicosapentaenoic acid (EPA, C20:5n-3) (11.9%) and docosahexaenoic (DHA, C22:6n-3) (17.53%). These two acids were absent in PBM and SBM. The highest level of Σn-6 (53.56%) was measured in SBM. PBM contained large amount of Σn-6, but Σn-3 values were lower than those of FM. However, the fatty acid composition ΣSFA, ΣMUFA and ΣPUFA of the experimental diets ranged from 30.99 to 38.17%, 21.04 to 41.28% and 16.88 to 30.26%, respectively. Σn-3 and Σn-6 fatty acid values also ranged from 1.17 to 26.0% and 4.26 to 16.11%, respectively.

Initial weight of fish stocked in the growth trial did not significantly differ among dietary treatments (Table 5). Fish fed the diets with replacement of PBM demonstrated significantly less final weight than that of the control diet. There was a progressive decrease in growth performance as dietary inclusion levels of PBM increased. The values for SGR, FCR, PER of fish fed the experimental diets generally followed the same pattern as that of final weight. No significant differences were detected in CF, HSI and VSI among treatments. The survival rate was 100% in all

Table 4. The fatty acid (%, on total fatty acids) composition of experimental diets, poultry by-product meal (PBM), fish meal (FM) and soybean meal (SBM)

				Experimental diets				
Fatty acids	PBM	FM	SBM	PBM0	PBM25	PBM50	PBM75	PBM100
C14:0	0.77	5.5	-	6.44	5.41	4.26	2.87	1.34
C15:0	-	0.5	-	0.47	0.41	0.33	0.23	0.12
C16:0	28.89	21.75	13.13	19.12	20.0	21.16	23.80	27.25
C17:0	0.27	-	-	-	0.86	0.68	0.37	0.31
C18:0	9.83	4.03	4.22	4.47	5.15	5.95	7.26	8.99
C20:0	0.13	0.23	0.29	0.49	0.42	0.35	0.27	0.16
ΣSFA[1]	39.89	32.01	17.64	30.99	32.25	32.73	34.80	38.17
C16:1n-7	3.48	5.12	-	6.23	5.68	5.05	4.42	3.74
C18:1n-9	40.08	11.04	16.68	12.13	16.37	21.38	29.06	37.13
C20:1n-9	0.24	2.69	-	2.68	2.28	1.77	1.16	0.41
ΣMUFA[2]	43.80	18.85	16.68	21.04	24.33	28.20	34.64	41.28
C18:3n-3	0.24	0.72	9.94	0.98	1.08	1.19	1.13	0.63
C20:5n-3	-	-	-	13.88	11.40	8.44	4.05	0.35
C22:5n-3	-	11.90	-	-	-	-	-	-
C22:6n-3	-	17.53	-	11.14	9.10	6.58	3.01	0.19
Σn-3 PUFA[3]	0.24	30.15	9.94	26.0	21.58	16.21	8.19	1.17
C18:2n-6	12.15	1.43	53.56	3.15	6.87	11.29	15.70	15.71
C18:3n-6	-	-	-	0.14	0.11	0.08	-	-
C20:2n-6	-	0.19	-	0.16	0.16	0.15	0.12	-
C20:4n-6	-	1.17	-	0.81	0.69	0.53	0.29	-
Σn-6 PUFA[4]	12.15	2.79	53.56	4.26	7.83	12.05	16.11	15.71
ΣPUFA[5]	12.39	32.94	63.50	30.26	29.41	28.26	24.30	16.88
Σn-3/Σn-6	0.02	10.81	0.19	6.10	2.76	1.35	0.51	0.07

Fatty acids composition values are mean (±SD) of two analysis. - not detected.

[1] Total saturated fatty acids included C14:0, C15:0, C16:0, C17:0, C18:0, and C20:0.

[2] Total monounsaturated fatty acids included C16:1n-7, C18:1n-9, and C20:1n-9.

[3] Total n-3 polyunsaturated fatty acids included C18:3n-3, C20:5n-3, C22:5n-3, and C22:6n-3.

[4] Total n-6 polyunsaturated fatty acids included C18:2n-6, C18:3n-6, C20:2n-6, and C20:4n-6.

[5] Total polyunsaturated fatty acids included C18:2n-6, C18:3n-3, C18:3n-6, C20:4n-6, C20:5n-3, C22:5n-3, and C22:6n-3.

treatments.

The final proximate and fatty acid compositions of fish fed the experimental diets containing different levels of PBM are shown in Table 6. No significant differences were determined for dry matter, protein, lipid and ash in the muscle of carp fry fed with dietary inclusion levels of PBM (P>0.05).

The ΣSFA, ΣMUFA and ΣPUFA in fish muscle ranged from 26.99 to 28.16%, 29.81 to 51.36% and 10.98 to 25.66%, respectively. In addition, Σn-3 and Σn-6 fatty acids ranged from 1.10 to 18.08% and 6.74 to 16.99%, respectively. Fatty acid analysis showed that ΣSFA in fish muscle was not changed with the dietary inclusion levels of PBM. When ΣMUFA significantly increased with increasing dietary PBM content, ΣPUFA decreased (P<0.05). However, while Σn-6 PUFA increased in muscle of fish, except PBM100, Σn-3 PUFA decreased with increasing dietary PBM content. The ratio of Σn-3 PUFA to Σn-6 PUFA was found to be lowest in muscle of fish fed the experimental diets containing graded levels of PBM, except control diet.

Discussion

Poultry by-products meal has a very good source of dietary protein for fish culture. However, it is vary widely in quality and some are deficient in one or more essential amino acids (Davies *et al*., 1991; Nengas *et al*., 1999; Yiğit *et al*., 2006). Therefore, in the present study, some essential amino acids were adjusted to equate that of the control diets by lysine, methionine and threonine supplementation. Supplemented amino acid values in the diets were in the acceptable value for carp fry when compared to be suggested by NRC (2011). In the present study, however, the results show that final weight and SGR values of experimental fish were negatively affected by the experimental diets containing inclusion levels of PBM protein (Table 5). On the contrary, Nengas *et al*. (1999) reported that PBM can be used without amino acid supplementation to replace 50% of the FM in diets for gilthead seabream. Aydın and Gümüş (2013) also found PBM was able to replace 50% of the protein provided by fishmeal in diets for Nile tilapia fry. Similar results have also been reported in rainbow trout (Steffens, 1994), African catfish (Abdel-Warith *et al*., 2001), gibel carp (Yang *et al*., 2004), Australian snapper (Rawles *et al*., 2006), Blacksea turbot (Yigit *et al*., 2006) and tilapia (Yıldırım *et al*., 2009) without affecting growth. Turker *et al*. (2005) indicated that up to 25% of the fishmeal protein can be replaced by poultry by-product meal with no negative effects in black sea turbot performance. On the other hand, Emre *et al*. (2003) observed drastic reductions in growth and nutrient retention of carp fingerlings fed diets containing as little as 33% PBM. These differences reflect the fact that PBM was mainly due to differences in processing method and technology, different amounts of constituents such as bone, offal, meat, connective tissue, and skin contents (Bureau *et al*., 1999; Shapawi *et al*., 2007; Aydın and Gümüş, 2013).

The values for FI, FCR, PER of fish fed the PBM-based experimental diets generally followed the same pattern as that of growth performance. This finding is similar to those of Emre *et al*. (2003) on mirror carp, Sevgili and Ertürk (2004) on rainbow trout, Rawles *et al*. (2006) on hybrid striped bass, and Hu *et al*. (2008) on gibel carp.

There were no significant differences among the experimental diets in CF, HSI, or VSI values. This result is in accordance with the findings of Webster *et al*. (2000), Emre *et al*. (2003), Sevgili and Ertürk (2004), Shapawi *et al*. (2007), Hu *et al*. (2008), and Aydın and Gümüş (2013), in contrast to results announced by Steffens (1994), Takagi *et al*. (2000), Rawles *et al*. (2006), and Yang *et al*. (2006).

Whole-body moisture, protein, lipid and ash content of of carp fry fed diets containing different levels of PBM were not significantly differed in the present study (Table 6)(P>0.05). These findings are in agreement with the values reported by Nengas *et al*. (1999), Takagi *et al*. (2000), Webster *et al*. (2000), and Emre *et al*. (2003). Conversely, the results were not similar with the findings indicated by Yang *et al*. (2006) and Rawles *et al*. (2006), who reported

Table 5. Growth parameters in carp fry (*Cyprinus carpio*) fed with different experimental diets[1]

	Experimental diets				
Parameters[2]	PBM0	PBM25	PBM50	PBM75	PBM100
IBW (g)	0.39±0.01	0.40±0.01	0.40±0.00	0.39±0.00	0.39±0.00
FBW (g)	12.08±0.16^{a}	9.04±0.02^{b}	9.56±0.50^{b}	8.73±0.84^{b}	6.55±0.18^{c}
SGR (% day^{-1})	3.81±0.01^{a}	3.47±0.02^{b}	3.53±0.05^{b}	3.44±0.09^{b}	3.14±0.03^{c}
FI (g fish^{-1})	18.12±0.46	15.86±0.78	16.30±2.37	17.56±0.60	15.63±0.60
FCR	1.56±0.06^{a}	1.84±0.09^{ab}	1.78±0.16^{ab}	2.11±0.28^{b}	2.54±0.16^{c}
PER	1.89±0.06^{a}	1.60±0.10^{ab}	1.66±0.12^{ab}	1.41±0.19^{bc}	1.17±0.08^{c}
CF	1.29±0.10	1.32±0.05	1.33±0.01	1.42±0.11	1.39±0.04
HSI (%)	1.89±0.11	1.50±0.39	1.79±0.09	1.71±0.67	1.88±0.33
VSI (%)	10.15±0.67	9.63±0.54	9.55±0.18	10.39±1.21	11.46±1.01
SR (%)	100	100	100	100	100

[1]Values are mean (±SD) of triplicate analysis; [a-c]Values in the same row with different superscripts are significantly different from each other (P<0.05). [2]IBW: Initial body weight; FBW: Final body weight; SGR: Specific growth rate; FI: Feed intake; FCR: Feed conversion ratio; PER: Protein efficiency ratio; CF: Condition factor; HSI: Hepatosomatic index; VSI: Viscerosomatic index; SR: Survival rate.

Table 6. The fatty acid (%, on total fatty acids) and proximate (% wet wt.) composition of carp fry (*Cyprinus carpio*) fed the experimental diets

	Experimental diets				
Parameters	PBM0	PBM25	PBM50	PBM75	PBM100
Dry matter	23.48±0.17	23.87±0.24	24.83±0.18	24.28±1.39	23.71±1.14
Crude protein	17.65±0.69	17.42±0.23	17.61±0.90	17.47±0.34	17.04±0.68
Crude lipid	2.38±0.04	2.33±0.24	2.26±0.33	2.37±0.02	2.98±0.04
Crude ash	1.86±0.08	2.35±0.25	2.31±0.09	2.20±0.42	2.28±0.25
Fatty acids					
C14:0	5.20±0.23^{a}	4.79±0.48^{a}	3.40±0.21^{b}	2.12±0.02^{c}	1.40±0.19^{d}
C15:0	0.51±0.01^{a}	0.48±0.01^{a}	0.41±0.02^{b}	0.36±0.01^{c}	0.32±0.00^{d}
C16:0	18.09±0.71^{b}	18.37±0.26^{b}	18.84±0.47^{b}	18.87±0.39^{b}	20.41±0.84^{a}
C17:0	0.43±0.02^{a}	0.47±0.04^{a}	0.33±0.11^{ab}	0.28±0.01^{b}	0.20±0.01^{b}
C18:0	2.98±0.01^{c}	3.60±0.65^{bc}	4.63±0.09^{ab}	5.21±0.27^{a}	5.69±0.74^{a}
C20:0	0.17±00	0.18±0.02	0.17±0.02	0.15±0.01	0.16±0.01
ΣSFA[1]	27.37±0.98	27.90±0.40	27.78±0.69	26.99±0.63	28.16±1.79
C14:1	0.16±0.01	0.13±0.01	0.07±0.09	0.06±0.09	0.12±0.01
C16:1	7.06±0.04^{a}	6.44±0.27^{b}	5.58±0.16^{cd}	5.26±0.13^{d}	5.81±0.23^{c}
C18:1n-9	19.27±1.32^{d}	21.28±3.47^{d}	26.96±0.06^{c}	35.52±0.17^{b}	43.33±0.23^{a}
C20:1n-9	2.61±0.15^{a}	1.89±0.69^{d}	1.99±0.18^{c}	2.03±0.17^{b}	1.96±0.08^{c}
C24:1	0.72±0.03	0.80±0.06	0.27±0.38	0.53±0.44	0.14±0.00
ΣMUFA[2]	29.81±1.55^{d}	30.52±2.44^{d}	34.86±0.51^{c}	43.40±0.22^{b}	51.36±0.56^{a}
C18:3n-3	1.09±0.03^{b}	1.17±0.07^{ab}	1.23±0.04^{a}	0.89±0.00^{c}	0.29±0.05^{d}
C20:3n-3	0.13±0.00	0.14±0.02	0.18±0.03	0.30±0.01	0.28±0.01
C20:5n-3	6.06±1.24^{a}	6.58±1.52^{a}	3.55±0.53^{b}	0.82±0.04^{c}	0.19±0.12^{c}
C22:6n-3	10.81±0.07^{a}	8.45±1.60^{b}	4.54±0.01^{c}	0.19±0.07^{d}	0.34±0.18^{d}
Σn-3 PUFA[3]	18.08±1.34^{a}	16.34±3.03^{a}	9.50±0.54^{b}	2.20±1.12^{c}	1.10±0.36^{c}
C18:2n-6	5.73±0.46^{b}	8.48±2.68^{b}	14.49±0.29^{a}	15.69±0.34^{a}	8.95±0.38^{b}
C18:3n-6	0.13±0.01^{b}	0.05±0.07^{b}	0.07±0.09^{b}	0.16±0.02^{ab}	0.28±0.03^{a}
C20:2n-6	0.28±0.01	0.29±0.04	0.34±0.03	0.65±0.52	0.15±0.01
C22:2n-6	0.60±0.01	0.50±0.12	0.67±0.42	0.49±0.01	0.50±0.01
Σn-6 PUFA[4]	6.74±0.45^{c}	9.32±2.60^{bc}	15.57±0.20^{a}	16.99±0.36^{a}	9.88±0.41^{b}
ΣPUFA[5]	24.82±0.89^{a}	25.66±0.42^{a}	25.07±0.34^{a}	19.19±0.48^{b}	10.98±0.77^{c}
Σn-3/Σn-6	2.68±0.47^{a}	1.75±0.98^{a}	0.61±0.05^{b}	0.13±0.00^{b}	0.11±0.03^{b}

∗ $^{a-d}$Values in the same row with different superscripts are significantly different (P<0.05).
Proximate composition values are mean (±SD) of triplicate analysis.
Fatty acids composition values are mean (±SD) of two analysis.
[1] Total saturated fatty acids included C14:0, C15:0, C16:0, C17:0, C18:0, and C20:0.
[2] Total monounsaturated fatty acids included C14:1, C16:1, C18:1n-9, C20:1n-9, and C24:1.
[3] Total n-3 polyunsaturated fatty acids included C18:3n-3, C20:5n-3, C22:5n-3, and C22:6n-3.
[4] Total n-6 polyunsaturated fatty acids included C18:2n-6, C18:3n-6, C20:2n-6, and C22:2n-6.
[5] Total polyunsaturated fatty acids included C18:2n-6, C18:3n-3, C18:3n-6, C20:2n-6, C20:3n-3, C20:5n-3, C22:2n-6, and C22:6n-3.

significant decrease in the protein contents of gibel carp fed increasing level of PBM.

Numerous studies have shown that fatty acid profiles of fish tissue reflect the dietary fatty acid profiles (Francis *et al*., 2006; Xue *et al*., 2006; Bahurmiz and Neg, 2007; Gümüş and Erdogan, 2010; Hu *et al*., 2013). In the present study, considerable differences were, nevertheless, observed in fatty acid profiles of muscle of fish fed diets progressively replaced of FM with PBM, and some fatty acids inclined to increase or decrease in the muscle of fish (Table 6). ΣMUFA was significantly higher with the increased dietary PBM and this effect is mainly due to C18:1n-9 in fish muscle increased with higher inclusion of this fatty acid in the diets. However, progressively higher PBM levels in diets resulted in lower ΣPUFA levels in the fish muscle. This was due to a decreasingly lower total content of eicsopentoenoic acid (EPA, C20:5n-3) and decosohexoenoicacid (DHA, C22:6n-3) in the muscle of the fish fed diets including graded levels of PBM, low content of EPA and DHA, than that of the control diet. Gümüş (2011) also reported to change of the ΣMUFA and Σn-6 PUFA in muscle of fish was significantly higher with the increased dietary sand smelt meal and this effect is mainly due to oleic acid and linoleic acid included high levels of sand smelt meal. Similarly, progressively higher levels of tuna liver meals in the diets were influenced the fatty acids composition of tilapia fry muscle (Gümüş and Erdogan, 2010). According to Maina *et al*. (2003) and Bahurmiz and Ng (2007), the fatty acid composition of many fish resembles that of their food. This was also proved in many studies in which FM or fish oil was replaced by non-conventional sources of protein or lipid (Bell *et al*., 2002; Cabellaro *et al*., 2002; Williams *et al*., 2003; Francis *et al*., 2006; Xue *et al*., 2006; Gümüş and Erdogan, 2010; Hu *et al*., 2013).

Our results show that the Σn-3:Σn-6 ratio in the muscle of fish progressively decreased as the PBM

levels increased in the diet because of low level of Σn-3 fatty acid of PBM, thus reflecting what occurs in the diet itself. Similar results were reported in the mirror carp fed diets with graded levels of sand smelt meal (Gümüş, 2011). The Σn-3 content of cultured fish has often been reported to be lower because of lowered fish meal usage in the diet (Pigott, 1989).

In conclusion, the present study has demonstrated that carp fry is not unable to grow fairly effectively with high replacement of the fishmeal protein of the diet with PBM protein with amino acid supplementation. Growth and feed utilization was much lower than the control diet group of fish. On the other hand, one positive effect of the diets based on PBM is the increase in n-6 level in the muscle of fish. It is thought as an important factor that affected the nutritional quality of fish muscle. So, future investigation should focused to use of PBM as a replacement of fish meal with supplementation highly amount of amino acids to improve the growth performance and acceptability fatty acid profiles especially n-3 for fish.

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