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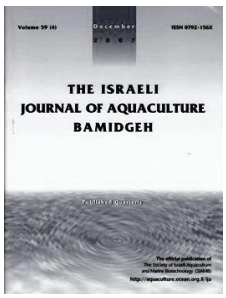

Growth, Fatty Acids, and Protein Profiles of Carp (*Cyprinus carpio* L. 1758) Fed Diets with Incremental Levels of Sunflower Seed Meal

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Key words: *Cyprinus carpio*, sunflower seed meal, growth, fatty acids, protein profile

Abstract

This study evaluates the growth performance and nutritive value of carp fed diets containing moderate levels of sunflower seed meal in partial replacement of fishmeal. Four isocaloric and isonitrogenous diets were formulated with 0% (control), 15%, 30%, or 45% sunflower seed meal and fed to triplicate groups of carp (993.82±11.68 g). Growth was assessed using a range of biometric parameters and nutrient utilization was determined by muscle content, fatty acids, and electrophoretic protein profiles of tissues. Condition factor and survival were unaffected by the sunflower seed level but weight gain, protein efficiency rate, and absolute and specific growth rates significantly declined as the inclusion level rose. Feed conversion ratio negatively correlated with SGR and reflected feed intake. There were no significant effects on protein, lipid, or pH content of fish, but there were slight alterations in dry matter and ash values. Total saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) dropped as the incorporation of sunflower seed meal increased, contrary to total polyunsaturated fatty acids (PUFA) of both diets and flesh. Minor differences existed in the fractions of sarcoplasmic and myofibrillar proteins, independent of the dietary treatment. In conclusion, sunflower seed inclusion beyond 15% can lead to changes in growth performance and fatty acid composition.

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Introduction

Due to the scarcity and cost of fishmeal, development of inexpensive protein sources for aquafeeds is a major necessity for the sustainability of aquaculture. Plant feedstuffs can be partial or total substitutions for fishmeal but antinutritional factors, biologically active compounds such as non-starch polysaccharides and non-digestible carbohydates, and deficiencies in essential amino acids and minerals (NRC, 1993) can accompany plant protein sources and limit their use in fish diets.

Sunflower seed is an important and, following rapeseed, the biggest oilseed crop, with an annual global production of 32.39 million tons. Turkey was the eighth rank producer in 2009, with 0.88 million tons (FAO, 2011). Sunflower seed is used almost exclusively as a feedstuff for ruminants and poultry due to its high fiber and linoleic acid content. Further, the presence of anti-nutritional factors (excluding phenolic constituents such as tannins) and phytic acid is less in sunflower seeds than in other oilseeds (Francis et al., 2001; Gill et al., 2006). Sunflower products have been used as protein sources with good results in diets for tilapia (Sintayehu et al., 1996; Olvera-Novoa et al., 2002; Maina et al., 2003), Atlantic salmon (Gill et al., 2006), rainbow trout (Tacon et al., 1984; Martinez, 1986; Cardenete et al., 1993; Sanz et al., 1994; Stickney et al., 1996), and European eel (García-Gallego et al., 1998).

Our study monitored the influence of incremental incorporation of sunflower seed meal at moderate (≤45%) levels on the growth performance and fatty acid/protein profile of carp, *Cyprinus carpio* L. 1758.

Materials and Methods

Diets. Fishmeal (65% crude protein) was supplied by Çamlı Yem, Pınarbaşı, Izmir, Turkey. Sunflower seed meal (25% crude protein) and all other ingredients were obtained from Bil-Yem Co. Yenikent, Ankara, Turkey, where four isonitrogenous and isocaloric experimental diets were formulated and prepared (Table 1). Sunflower seed meal was included as a partial substitution of fishmeal at inclusion levels of 0% (control), 15%, 30%, and 45%. The diets were stored at 4°C during the experiment.

Experimental design. The feeding trial was conducted for 12 weeks at the Cifteler Sakaryabasi Aquaculture and Research Station of Ankara University. Each diet was provided to three 500-l tanks randomly stocked with eight carp (993.82±11.68 g, 37.66±0.28 cm) each. Fish were acclimatized to indoor tanks for 2 weeks prior to the start of the experiment during which they were fed a commercial carp diet (35% crude protein). The fish were fed manually to apparent satiation three times a day, which amounted to an average of 1.0% of the total biomass, adjusted according to biweekly weighings. Mean water temperature was 19.29°C, pH 7.57, and dissolved oxygen 6.49 ppm, monitored daily with a YSI Professional Plus oxygenmeter.

Nutrient composition analysis. Diets and flesh samples were analyzed for dry matter, crude ash, crude protein, crude fat, and crude fiber according to standard procedures following AOAC (1995). Briefly, dry matter was computed after drying at 105°C for 24 h, crude ash after combustion at 550°C for 4 h in a muffle furnace, crude protein (N × 6.25) by Kjeldahl distillation (Kjeltec System, Tecator, Hoganas, Sweden), crude fat after extraction with petroleum ether by the Soxhlet method (Soxtec System HT, Tecator, Hoganas, Sweden), and crude fiber in a Fibertec TM 1020 hot extractor and Fibertec System 1021 cold extractor (Tecator TM Technology, Sweden). pH was determined in a 10-g sample of minced and homogenized flesh using a Consort NV P901 model pH-meter (Turnhout, Belgium) as per Varlık et al. (1993).

Fatty acid analysis. Total lipid was extracted by the cold extraction method (Bligh and Dyer, 1959) and fatty acids of the diets and flesh samples were analyzed after methylation by gas chromatograph (Shimadzu GC 2010), equipped with a flame ionization detector (IUPAC, 1987). In methylation, 0.4 g of the lipid sample was dissolved with 0.2 ml of 2 N methanolic KOH and the mixture was shaken until clear. The mixture was kept in the dark for 6 min and 1-2 drops of methyl-orange were added. Then, 0.45 ml of 1 N HCL was applied and the samples were vigorously mixed. To complete methylation, the samples were kept on a slanted surface for 30 min to separate the

glycerol phase. A fused silica capillary column (30 m x 0.25 mm x 0.25 µm; Omegawax 250, Supelco, Bellefonte, PA, USA) and auto injector (0.5 µl per sample) were used. The temperatures of the injector, column, and detector were 250°C, 205°C, and 260°C, respectively. The split ratio was 1:90 and helium was used as the carrier gas at 1.25 µl/min. Fatty acids were identified with a standard (PUFA-1 Marine Source, Supelco, Bellefonte, PA, USA) and the relative amount of each fatty acid in the respective pattern was calculated as the percent of the total content.

Extraction of sarcoplasmic and myofibrillar proteins. Sarcoplasmic and myofibrillar proteins were extracted as described in Candoğan (2000). Samples of 10 g flesh were homogenized with 40 ml of 0.03 M potassium phosphate buffer, pH 7.4, for 2 min and the homogenate was centrifuged at 4°C and 10,000 x *g* for 20 min. After centrifugation, the supernatant containing sarcoplasmic proteins was obtained by filtering it through glass wool twice. Supernatants were pooled and kept at 80°C until analysis. The remaining pellet after the second wash was used to extract myofibrillar proteins. The pellet was dissolved in 40 ml of 8 M urea containing 1% β-mercaptoethanol and centrifuged at 4°C and 10,000 x *g* for 20 min. The filtered supernatant was kept at the same storage conditions as for the sarcoplasmic proteins. The protein concentrations of the sarcoplasmic and myofibrillar extracts were estimated by the spectrophotometric method of Bradford (1976) with Bradford reagent and bovine serum albumin as standards.

Table 1. Formulation and proximate composition of carp diets containing sunflower seed meal in replacement of fishmeal.

	Diet (% sunflower seed meal)			
	Control	*15*	*30*	*45*
Ingredient (g/kg)				
Wheat	415.96	440.63	354.11	194.08
Fishmeal	373.04	345.98	313.80	281.96
Sunflower seed meal	-	150.0	300.0	450.0
Bran	200.0	50.0	-	-
Vegetable oil	-	2.39	22.09	63.96
Methionine	5.0	5.0	5.0	5.0
NaCl	2.0	2.0	1.0	1.0
Lysine	1.0	1.0	1.0	1.0
DCP	1.0	1.0	1.0	1.0
Vitamin premix[1]	1.0	1.0	1.0	1.0
Mineral premix[2]	1.0	1.0	1.0	1.0
Proximate composition (% dry matter)				
Dry matter	91.96	92.37	92.56	91.89
Crude protein	32.38	33.63	33.53	32.64
Crude lipid	5.45	4.94	6.94	9.89
Crude fiber	5.17	6.20	9.88	13.69
Crude ash	8.50	8.62	8.63	8.13
NFE[3]	53.67	52.81	50.90	49.34
Gross energy (MJ/kg)[4]	19.36	19.30	19.73	20.42

[1] includes (IU/kg): retinol 15,000,000; chelocalciferol 5,000,000; tocopherol 50,000; menadione 10,000; thiamine 2000; riboflavin 4000; pyridoxine 5000; cobalamin 25; biotin 200; ascorbic acid 75,000; niacin 50,000; pantothenic acid 10,000; folic acid 1500 mg; choline 300,000

[2] includes (mg/kg): Mn 70; Zn 105; Fe 70; Cu 14; I_2 1.05; Co 0.35; Se 0.14; Mo 0.70; Mg 35

[3] Nitrogen free extract including crude fiber = 100 - (crude protein + crude lipid + crude ash)

[4] Calculated by: crude protein = 23.9 MJ/kg, crude lipid = 39.8 MJ/kg, NFE = 17.6 MJ/kg

SDS-PAGE analysis. The sarcoplasmic and myofibrillar extracts were separated by SDS-PAGE according to Laemmli (1970) with a Mini Protean III and Power Pack Basic System (Bio-Rad, CA, USA) for 2.5 h at 32 mA. The extracts were dilluted to 1.5 mg/ml with Laemmli sample buffer and incubated at 95°C for 5 min prior to electrophoresis; 25 µl of each sample was placed in 12% polyacrilamide gradient gels. The molecular weight marker loaded in each gel was SDS-PAGE Pre-Stained Standard (Bio-Rad, CA, USA). After electrophoretic separation, gels were stained with Commassie Brillant Blue R-250 staining solution (Bio-Rad, CA, USA) for 45 min and, using a shaker, destained with Destain Commassie R-250 solution (Bio-Rad, CA, USA) for 12 h. Densitometry of gels was performed using Gel Logic 200 Imaging System (Kodak) to determine the relative molecular masses of the separated bands.

Statistics. Data are presented as means±standard deviation of three replicates. One way analysis of variance (ANOVA) was applied using statistical software SPSS 11.5 (SPSS Inc., 2003) to verify the presence of significant differences among dietary groups. Duncan's multiple comparison test with a confidence level of 95% was used to compare means.

Results

Growth, feed efficiency, fatty acid composition. Growth and feed parameters decreased as the level of sunflower seed meal increased but there were no significant differences between treatments in condition factor or survival (Table 2). Dry matter and crude ash contents of the fish body differed among treatments, but there were no significant differences in crude protein, lipid, or pH. The fatty acid compositions of the diets significantly differed (Table 3). Total saturated fatty acids (SFA) decreased as the level of

Table 2. Growth and body composition of carp fed diets containing different levels of sunflower seed meal in replacement of fishmeal (means±SD, n = 3).

	Diet (% sunflower seed meal)			
	Control	*15*	*30*	*45*
Initial wt (g)	1001.25±47.19	993.19±28.60	972.71±29.05	1008.13±63.65
Final wt (g)	1696.50±13.31^{a}	1615.31±70.88^{ab}	1505.83±47.83^{c}	1533.29±66.07^{bc}
Wt gain (g)[1]	695.25±33.95^{a}	622.12±48.30^{a}	533.13±59.52^{b}	525.17±19.29^{b}
Absolute growth rate (g/d)[2]	8.28±0.40^{a}	7.41±0.58^{a}	6.35±0.71^{b}	6.25±0.23^{b}
SGR (%/d)[3]	0.61±0.02^{a}	0.61±0.04^{a}	0.54±0.05^{b}	0.52±0.02^{b}
FCR[4]	1.56±0.11^{b}	1.72±0.04^{ab}	1.89±0.19^{a}	1.96±0.12^{a}
PER[5]	1.87±0.09^{a}	1.25±0.10^{b}	0.87±0.10^{c}	0.72±0.03^{c}
Condition factor[6]	2.00±0.05	2.01±0.10	1.89±0.03	1.99±0.08
Survival (%)	100.00±0.00	95.83±7.22	100.00±0.00	100.00±0.00
Fillet proximate composition (%)				
Dry matter	31.65±0.54^{b}	36.77±2.60^{a}	33.66±1.85^{ab}	31.38±2.71^{b}
Crude ash	1.05±0.05^{ab}	0.99±0.06^{b}	1.10±0.01^{a}	0.99±0.03^{b}
Crude protein	18.02±1.87	18.86±1.09	18.30±0.88	18.62±0.14
Crude lipid	9.51±2.33	9.86±2.16	9.47±2.36	10.19±3.65
pH	6.46±0.04^{b}	6.41±0.00^{b}	6.41±0.02^{b}	6.57±0.02^{a}

Means in the same row with different superscripts differ significantly (p<0.05).
[1] Weight gain = final wt - initial wt
[2] Absolute growth rate = wt gain/days of growth
[3] Specific growth rate = 100(ln $_{\text{Final wt}}$ - ln $_{\text{Initial wt}}$)/days of growth
[4] Feed conversion ratio = wt of feed given/wt gain
[5] Protein efficiency rate = wt gain/wt of dietary crude protein supply
[6] Condition factor = 100(final wt/final body length)

Table 3. Fatty acid profiles of experimental diets containing different inclusion levels of sunflower seed meal (means±SD, n = 3).

Fatty acid	*Diet (% sunflower seed meal)*			
	Control	*15*	*30*	*45*
C14:0	3.54±0.01^{a}	3.71±0.50^{a}	3.12±0.01^{a}	1.63±0.01^{b}
C16:0 (palmitic)	20.25±0.28^{a}	19.32±0.16^{b}	16.67±0.03^{d}	17.16±0.01^{c}
C18:0	1.05±0.08^{a}	1.01±0.06^{a}	0.82±0.00^{b}	0.41±0.03^{c}
Total saturated (SFA)	24.83±0.18^{a}	24.03±0.40^{b}	20.60±0.01^{c}	19.20±0.02^{d}
C14:1	0.26±0.02^{a}	0.25±0.01^{a}	0.18±0.00^{b}	0.14±0.01^{c}
C16:1	3.79±0.19^{a}	4.18±0.23^{a}	3.17±0.12^{b}	1.99±0.05^{c}
C18:1 n-9 (oleic)	13.88±0.45^{b}	13.22±1.70^{b}	18.27±0.07^{a}	17.03±0.04^{a}
C18:1 n-7	2.78±0.02^{b}	2.92±0.10^{a}	1.30±0.00^{c}	0.69±0.01^{d}
C20:1 n-9	0.45±0.02^{a}	0.44±0.08^{a}	0.45±0.01^{a}	0.26±0.03^{b}
C22:1 n-11	0.24±0.01^{b}	0.35±0.01^{a}	0.23±0.01^{b}	0.14±0.03^{c}
C22:1 n-9	0.13±0.06^{ab}	0.16±0.02^{a}	0.18±0.03^{a}	0.06±0.03^{b}
Total monounsaturated (MUFA)	21.52±0.62^{ab}	21.50±2.09^{ab}	23.80±0.50^{a}	20.31±0.22^{b}
C18:2 n-6 (linoleic)	22.55±0.08^{c}	22.29±1.90^{c}	38.1±0.07^{b}	43.94±0.05^{a}
C18:4 n-3	1.06±0.07^{a}	1.01±0.11^{ab}	0.84±0.04^{b}	0.41±0.01^{c}
C20:5 n-3 (eicosapentaenic)	11.40±0.03^{a}	11.44±0.14^{a}	8.30±0.01^{b}	5.46±0.02^{c}
C22:5 n-3	2.38±0.04^{a}	2.63±0.38^{a}	1.12±0.01^{b}	1.23±0.02^{b}
C22:6 n-3 (docosahexaenic)	16.27±0.58^{a}	17.09±3.97^{a}	7.13±0.46^{b}	9.45±0.08^{b}
Total polyunsaturated (PUFA)	53.66±0.42^{b}	54.47±2.49^{b}	55.59±0.06^{b}	60.49±0.71^{a}
Total n-3	31.11±0.51^{a}	32.18±4.38^{a}	17.39±0.20^{b}	16.55±0.45^{b}
Total n-6	22.55±0.08^{c}	22.29±1.90^{c}	38.21±0.09^{b}	43.94±0.12^{a}
n-3/n-6	1.38±0.03^{a}	1.46±0.32^{a}	0.46±0.01^{b}	0.38±0.01^{b}

Means in the same row with different superscripts differ significantly (p□ 0.05).

sunflower seed meal increased; the acid with the highest value was palmitic acid (C16:0) in all diets. Similarly, total monounsaturated fatty acids (MUFA) decreased except in the 30% diet; oleic acid (C18:1 n-9) was the most abundant in all diets. Total polyunsaturated acids (PUFA) increased and the n-3/n-6 ratio decreased according to the linoleic acid (C18:2 n-6) content. While eicosapentaenic acid (EPA; C20:5 n-3) and docosahexaenic acid (DHA; C22:6 n-3) in the diets dropped remarkably as the sunflower seed meal content rose, the drops in the carp flesh were insignificant (Table 4).

Table 4. Fatty acid profiles of carp flesh fed diets containing different inclusion levels of sunflower seed meal (means±SD, n = 3).

Fatty acid	*Diet*			
	Control	*15%*	*30%*	*45%*
C14:0	2.74±0.04^{a}	2.39±0.33^{ab}	2.37±0.06^{ab}	2.10±0.01^{b}
C16:0 (palmitic)	18.01±0.86	17.98±1.29	17.00±0.06	17.62±0.62
C18:0	0.65±0.11	0.57±0.03	0.52±0.02	0.48±0.04
Total saturated (SFA)	21.29±0.72^{a}	20.91±1.56^{b}	19.89±0.98^{b}	20.20±0.56^{b}
C14:1	0.29±0.01	0.24±0.03	0.24±0.04	0.22±0.01
C16:1	8.28±0.42^{ab}	9.64±0.99^{a}	7.55±0.90^{b}	7.61±0.23^{b}
C18:1 n-9 (oleic)	29.80±0.40	32.56±3.03	33.17±5.78	31.66±2.23
C18:1 n-7	3.48±0.91	3.31±0.05	2.87±0.56	2.55±0.16
C20:1 n-9	2.84±0.11	2.63±0.35	3.07±0.15	3.12±0.04
C22:1 n-11	0.56±0.01	0.42±0.20	0.51±0.02	0.53±0.01
C22:1 n-9	0.14±0.01^{a}	0.08±0.04^{b}	0.12±0.02^{ab}	0.09±0.00^{ab}
Total monounsaturated (MUFA)	45.38±0.04	48.87±1.38	47.50±6.22	45.77±2.32
C18:2 n-6 (linoleic)	18.84±1.38	16.24±2.90	20.66±5.22	20.41±1.22
C18:4 n-3	0.53±0.03	0.52±0.13	0.49±0.07	0.43±0.04
C20:5 n-3 (eicosapentaenic)	4.79±0.13	4.65±0.51	4.08±0.36	3.98±0.08
C22:5 n-3	1.37±0.16	1.26±0.35	1.16±0.18	1.23±0.07
C22:6 n-3 (docosahexaenic)	7.70±0.66	7.54±1.64	6.24±1.36	7.09±0.18
Total polyunsaturated (PUFA)	33.22±0.76	30.21±0.26	32.62±7.20	33.13±1.59
Total n-3	14.39±0.62	13.97±2.64	11.96±1.98	12.72±0.37
Total n-6	18.84±1.38	16.24±2.90	20.66±5.22	20.41±1.22
n-3/n-6	0.77±0.09	0.89±0.33	0.59±0.05	0.63±0.02

Means in the same row with different superscripts differ significantly (p<0.05).

Electrophoretic protein profiles. In the electrophoretic profile of sarcoplasmic proteins, a group of bands with molecular weights of approximately 47-49 and 42-44 kDa were distinguished between the ovalbumin (54 kDa) and carbonic anhydrase (37 kDa) in all dietary groups, but more intensely in the control and 15% groups (Fig. 1). Two additional

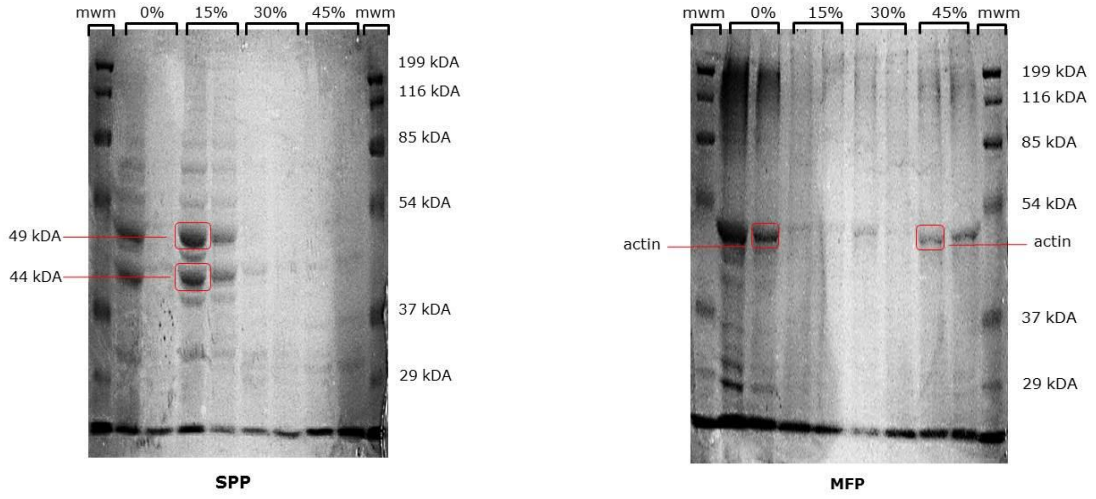

Fig. 1. Electrophoretic profiles of sarcoplasmic (SPP) and myofibrillar proteins (MFP) of carp fed diets with increasing levels of sunflower seed meal (mwm: molecular weight marker).

bands with estimated weights of 80 and 54 kDa were noticed only in the control and 15% diets. In the electrophoretic profile of the myofibrillar proteins, the 47-49 kDa band, assumed as actin, was potently revealed in the control and 45% groups.

Discussion

The sunflower seed meal considerably depressed average weight gain, specific growth rate, protein efficiency rate, and feed conversion ratio in all dietary treatments, possibly as a result of the high non-digestible fiber content of sunflower seed meal that is associated with substantial levels of methionine and lysine. Likewise, growth and feed efficiency were reduced in tilapia fed diets in which the sunflower seed meal replacement exceeded 20% (Olvera-Novoa et al., 2002), probably because of deficient dietary amino acids or high fiber content as found by El-Sayed (1990) and supported by our results. Both tilapia and carp are herbivorous and more adaptable to diets rich in plant-derived ingredients than carnivorous species. However, growth results in Atlantic post-smolts fed fiber-reduced and high-temperature extruded sunflower seed meal were controversial (Gill et al., 2006). Similarly, when dietary digestible energy was low due to indigestible carbohydrates, the growth performance of rainbow trout was similar to those fed a solely fishmeal-based diet (Sanz et al., 1994).

The dry matter and ash contents of the fish fluctuated and, therefore, it is difficult to conclude that this was a result of the inclusion of sunflower seed meal in the diets. Despite the observable growth reduction, even high inclusion levels of sunflower seed meal had no adverse effects on the composition of the fish flesh, specifically protein, lipid, and pH.

Lipid sources influence the fatty acid composition of the neutral and phospholipid fractions in trout (Bayır et al., 2011). However, the fatty acid profile of the flesh did not reflect the fatty acid profile of diets in rainbow trout (Beyter, 2008). In our study, the reduced saturated fatty acid contents in fish as well as diets indicate an influence of dietary treatment. Palmitic acid, the dominant saturated fatty acid, decreased in the diets as the amount of sunflower seed meal increased, but there were no significant differences in the flesh. The preponderance of palmitic acid is specific for fish and found in almost all fish tissue (Aras et al., 2003; Rinchard et al., 2007). In contrast, the monounsaturated fatty acid levels rose, mainly in oleic acid, similar to the effect of canola oil in rainbow trout (Dernekbasi et al., 2011).

Fish are unable to synthesize polyunsaturated fatty acids including linoleic and linolenic acids. Generally, freshwater species need to receive n-3 and n-6 fatty acids in their diets (Schulz et al., 2005; Beyter 2008). Some freshwater fish possess enzymes that elongate and desaturate fatty acids of the n-3 and n-6 series, particularly linolenic acid, to obtain sufficient levels of PUFA for membrane function and fluidity (Maina et al., 2003; Dernekbasi et al., 2011). Linoleic acid was the most abundant fatty acid in the sunflower seed meal diets in our study and the proportion of linoleic acid rose with the inclusion level. Nevertheless, EPA and DHA declined with the increasing level of sunflower seed meal and vegetable oil, as shown in tilapia (Maina et al., 2003) and Murray cod (Francis et al., 2007).

As revealed by SDS-PAGE, there were slight differences in the electrophoretic fractions of the sarcoplasmic and myofibrillar proteins, suggesting that minimal proteolysis emanated but seems unrelated to the sunflower seed meal inclusion level. In corroboration of our findings, the proteolytic processes that soften muscles in fish after slaughter were not significantly influenced by diet composition in dentex fed diets in which protein was partially replaced by lipid and carbohydrate (Suárez et al., 2009).

In general, the main adverse effect of sunflower seed meal was growth deficiency which occurred from even the 15% inclusion level. Together with this, there was no evidence of deleterious effects on the proximate composition of the carp body. The fatty acid composition of the flesh closely resembled the dietary fatty acid composition of the diet, however, electrophoretic patterns of sarcoplasmic and myofibrillar proteins did not demonstrate an explicit relationship to the diet composition.

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