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RESEARCH ARTICLE

Effects of Partial Substitution of Fish Meal with Tuna Liver Meal on the Fatty Acid Profile of Nile Tilapia Fry, *Oreochromis niloticus*

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Summary

This study was designed to evaluate the effect of diets containing different levels of fish meal (FM) and tuna liver meal (TLM) on the growth performance and fatty acid profiles of Nile tilapia fry, Oreochromis niloticus (initial mean weight: 2.29±0.07 g fish^{-1}). Each diet replacing 0, 10, 20, 30, 40, and 50% FM by TLM was randomly allocated to triplicate groups of fish in aquaria for 13 weeks. Results demonstrated that final weight, specific growth rate and protein efficiency ratio of fish fed diets replaced up to 30% TLM were not significantly ($P>0.05$) different from fish fed the control diet, except for TLM levels of ≥40. There were no significant differences in protein and ash contents in whole-body, while dry matter and lipid contents were significantly affected ($P<0.05$) by dietary treatments. Increasing level of TLM resulted in a gradual increase in the average level of saturated fatty acid in fish flesh as compared to the control group. This increase was characterized by a significant ($P<0.05$) increment in the level of 16:0, which is the most common fatty acid. Diets containing TLM up to 30% showed a significant increase ($P<0.05$) in monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid from the n-6 group, mainly due to 18:2n-6. Levels of MUFA and PUFA in fish flesh decreased with increasing dietary TLM levels of more than 40%. Level of n-3 in fish flesh was not significant different ($P>0.05$) between experimental groups. Furthermore, the increase of dietary TLM did not significantly affect the n-3: n-6 ratio in fish flesh.

Keywords: *Oreochromis niloticus, Fish meal, Tuna liver meal, Fatty acids*

Balık Ununun bir Kısmı Yerine Kullanılan Orkinos Karaciğer Ununun Nil Tilapia Yavrularında, *Oreochromis niloticus,* Yağ Asidi Profili Üzerine Etkileri

Özet

Bu çalışma, balık unu ve orkinos karaciğer ununu farklı oranlarda içeren yemlerin Nil tilapia (Oreochromis niloticus, başlangıç ağırlık: 2.29±0.07 g balık^{-1}) yavrusunun büyüme performansı ve yağ asidi profili üzerine etkisini belirlemek için gerçekleştirilmiştir. Balık ununun %0, 10, 20, 30, 40 ve 50'si oranında orkinos karaciğer unu ilave edilen yemlerle akvaryumdaki balıklar üç tekrarlı olarak 13 hafta süreyle beslenmiştir. Elde edilen sonuçlara göre %30'a kadar orkinos karaciğer unu içeren yemlerle beslenen balıkların son ağırlık, spesifik büyüme ve protein etkinlik oranı kontrol grubuna göre istatistiksel olarak önemsiz, ancak diğer gruplara göre önemli bulunmuştur ($P<0.05$). Yemlerdeki orkinos karaciğer unundaki artış kontrol grubu ile karşılaştırıldığında balık etindeki doymuş yağ asidi miktarının artışına neden olduğu belirlenmiştir. Bu durum 16:0'ın düzeyindeki önemli artıştan kaynaklanmaktadır ($P<0.05$). Yüzde 30'a kadar orkinos karaciğer unu içeren yemler ile beslenen balıkların etinde tekli doymamış yağ asidi (MUFA) ile çoklu doymamış yağ asidi (PUFA) olan n-6 grubunun artışına neden olmuştur. n-6 grubunun artışına 18:2n-6 önemli katkı sağlamıştır ($P<0.05$). Balık etindeki MUFA ve PUFA düzeyleri, yemdeki orkinos karaciğer ununun %40'ın üzerine çıktığında azalma göstermiştir. Deneme gruplarında n-3 düzeyi istatistiksel olarak önemsiz bulunmuştur. Ayrıca n-3: n-6 oranı yemlerdeki orkinos karaciğer ununun artışıyla önemli bir değişikliğe uğramamıştır.

Anahtar sözcükler: *Oreochromis niloticus, Balık unu, Orkinos karaciğer unu, Yağ asidi*

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INTRODUCTION

The main protein source in aquafeeds is fish meal (FM). However, not only its rising price but chancing supply is an important restrictive for the expansion of aquaculture industry [1]. Thus, finding alternative protein sources for replacing partial or complete of the fish meal in fish feeds is crucial in order to sustain aquaculture industry. Many studies have been made to use alternative protein sources such as plant proteins [2-6] and animal proteins [7-12] for reducing fish meal in aquafeeds. As animal or plant protein sources are used to substitute fish meal in fish feeds, it will become increasingly important to ensure that these diets are balanced in view of amino acid and fatty acid compositions. This will ensure not only that the diet satisfies the fish's requirement for these essential highly unsaturated fatty acids (HUFAs) but also that these fatty acids are retained in sufficient amounts in the fish flesh. However, there concerns that extensive substitute of fish meal in fish feeds could adversely affect the marketability of the fish because of consumer perception of altered taste of reduced health benefit due to changed HUFAs content [13-16].

One of the encouraging animal protein sources is tuna liver meal (TLM) which is a by-product of the Tuna fish rearing industry. Generally, TLM is cheaper than FM because of its reputation as a by- product. Therefore, it may still be considered as an ingredient for use in aquaculture larvae diets. TLM has been tested in carp fry *(Cyprinus carpio)* by Gümüş [11], and the use of TLM to substitute FM as protein sources has supplied good growth and feeding efficiency value in fish. However, as far as we know, there is not much information concerning the effects of different levels of dietary TLM on the growth performance and fatty acid profile of the fish, when TLM has been tested in Nile tilapia fry *(Oreochromis niloticus)* in a similar study. The purpose of this study was to evaluate the growth performance and fatty acid profile changes in Nile tilapia fry when partially replacing FM with TLM as an alternative protein source.

MATERIAL and METHODS

The experiment was carried out in the Laboratory of Fisheries Programme of Ortaca Vocational School, Muğla, Turkey, from 15 October 2008 to 15 January 2009. Experimental fish were obtained from the same Laboratory. Prior to start of the experiment, the fry were fed with control diet twice a day for 2 weeks to acclimate to the experimental conditions. At the start of the experiment, 18 glass aquaria (65-L) were each randomly stocked with 20 fry with an average weight of 2.29±0.07 g $fish^{-1}$. The aquaria were filled with dechlorinated tap water throughout the study and two-third of the aquarium water was changed daily. Each aquarium was supplied with compressed air by air stones from a central compressor. Each diet was assigned to triplicate groups of fish. Fish were fed to apparent satiation twice daily for 13 weeks.

Tuna liver meal (TLM) and other ingredients were from the same sources and experimental diets were prepared as described by Gümüş [11]. Six isonitrogenous (crude protein 42%, on dry matter basis), isolipidic (16%, on dry matter basis) and isoenergetic (18 kJ g^{-1}) diets replacing 0 (control diet), 10, 20, 30, 40 and 50% of dietary FM by TLM were formulated *(Table 1)*. The digestible energy (DE) content of the diet was estimated using the DE values 20.9 kJ g^{-1} protein, 37.7 kJ g^{-1} lipid and 14.6 kJ g^{-1} carbohydrate [17]; fibre was not included in the calculation. Diets were made based upon the results of the chemical composition of the ingredients. Prior to experimental diet preparation, all dry ingredients were ground in a hammer mill and passed through a 0.5 mm mesh sieve and thoroughly mixed and blended thereafter with additional 100 ml of water per kg diet to make a paste. The resulting mixture was then pelleted with a meat grinder to obtain a 2-mm die. The pellets were dried about 24 h in an oven at 70°C. After drying, the diets were broken up and sieved into proper pellet size (0.8-1 mm diameter) and stored at -20°C until use.

During the experimental period, the water temperature ranged from 24°C to 26°C, dissolved oxygen from 4.8 to 5.5 mgL^{-1}, pH from 7.8 to 8.4. Water quality parameters remained within the acceptable ranges for Nile tilapia fry growth during the experimental period.

At the beginning and at the end of the experiment, all fry in each aquarium were anaesthetized under moderate anaesthesia (ethanol solution of clove oil, 20% v:v) and then weighed. A sample of 10 fish at the beginning of feeding experiment and 10 fish at termination were collected and stored frozen (-20°C) for proximate carcass composition and fatty acid analysis.

Proximate composition analyses of diets and fish body were performed following standard methods of AOAC [18]. Samples of diets and fish were dried in an oven at 105°C for 24 h to determine dry matter. Protein was determined by measuring nitrogen (Nx6.25) using the Kjeldahl method; lipid by ether extraction using the soxhlet method; ash by combustion at 550°C in a muffle furnace for 24 h; crude cellulose after an alkali and acid digestion, and nitrogen-free extract (NFE) by the difference [NFE=100-(moisture+protein+lipid+ash+ fibre)].

Table 1. *Ingredient composition of the experimental diets*
Tablo 1. *Deneme yemlerindeki hammadde komposizyonu*

Ingredients (%)	%TLM Replacing FM in Diets					
	0 (Control)	10	20	30	40	50
FM [1]	17.14	15.42	13.71	11.99	10.28	8.57
TLM [2]	0	4.04	8.09	12.13	16.18	20.23
Corn meal [3]	5.74	5.74	5.74	5.74	5.74	5.74
Corn gluten [4]	7.23	7.23	7.23	7.23	7.23	7.23
Soybean meal [5]	46.9	46.9	46.9	46.9	46.9	46.9
Starch [6]	10.27	10.12	10.06	9.93	9.90	9.83
Fish oil [7]	11.12	8.95	6.7	4.52	2.24	0
Vitamin premix [8]	0.2	0.2	0.2	0.2	0.2	0.2
Mineral premix [9]	0.3	0.3	0.3	0.3	0.3	0.3
L-methionine [10]	0.5	0.48	0.46	0.44	0.42	0.4
Iodized salt	0.1	0.1	0.1	0.1	0.1	0.1
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100

[1] ***FM:*** *fish meal,*
[2] ***TLM:*** *tuna liver meal. Tuna liver was obtained from Tuna Cage Farm, Ak-Tuna, Gazipaşa, Antalya, Turkey and TLM was prepared at the laboratory of fisheries faculty of Akdeniz University*
[1,3-10] *obtained from Kilic feed manufacture factory Co. Ltd.*
[8] *Vitamin premix (mg or IU kg^{-1} of diet): Vit-A 7000 IU; Vit-D$_3$, 1000 IU; Vit-E, 100; Vit-K$_3$, 4.8; Vit-B$_1$, 12; Vit-B$_2$, 20; Vit-B$_6$, 12; Vit-B$_{12}$, 0.04; Vit-C, 200; Niacin, 120; Folic acid, 3.2; Calcium D- Pantothenate, 30; Biotin, 0.4; Inositol, 200; Endox D Dry, 100*
[9] *Mineral premix (mg kg^{-1} of diet): Iron, 18; Copper, 3.6; Manganese, 36; Cobalt, 3; Zinc, 45; Iodine, 1.2; Selenium, 0.24; Magnesium, 90*

The contents of fatty acids in experimental diets and fish body were determined by Gas Chromatography (GC). For this purpose, total lipids were extracted by using a modified Bligh and Dyer method [19], and then saponified and methylated for fatty acid quantification [20]. The fatty acid was extracted with *n*-hexane and converted to methyl esters before injecting to gas chromatograph for analysis. Fatty acid methyl esters (FAMEs) were analyzed by Gas Chromatography (GC). Gas chromatography was performed on a QP 5050 Pelkin Elmer Aut System XLGC (GCndFID) using column CP SIL 88 FOR FAME (50 m x0.32 mm inner diameter; 0.25 µm film), FID detector at 250°C, automatically injector at 240°C and helium as the carrier gas. Column oven temperature conditions were as follows: initial temperature of 60°C was held for 4 min and then increased 13°C min^{-1} to 175°C, then held constant for 27 min and finally increased 4 °C min^{-1} to 250°C, where it was held constant for 15 min. A quantity of 1 µL of the samples was injected on the column, at a split rate of 1:70. Authentic fatty acid methyl esters were used for peak identification. The results of the individual fatty acids were expressed as percentages of total identified FAMEs.

The results are presented as mean±SD. Data analysis was performed by one-way analysis of variance (ANOVA) using SPSS 15.0 (SPSS INC. Chicago, IL, USA). Differences among the means were compared using Duncan's multiple range test at a 5% probability level [21].

RESULTS

The proximate composition and fatty acid profiles of TLM and each of the main plant and animal protein sources are given in *Table 2*. The crude lipid content of TLM was higher (65.23%) than that of FM (9.76%), whereas the level of crude protein (30.10%) was lower than that of FM (73.79%). The fatty acid composition of the ingredients indicated that TLM contained highest of monounsaturated fatty acid (MUFA; 40.9%) but contained moderate of saturated fatty acid (SFA; 33.81 %). Amount of polyunsaturated fatty acid (PUFA; 22.4%) in TLM was lower than that of FM and SBM containing considerable amounts of PUFA (39.19 % and 46.32, respectively). On the other hand, SBM did not contain EPA (20:5n-3) or DHA (22:6n-3). TLM contained large amount of n-6, but its n-3 was lower than those of FM. The highest level of n-6 (45.55%) was measured in SBM.

The proximate composition and fatty acid profiles of experimental diets containing different levels of TLM are given in *Table 3*. The crude protein, crude lipid and digestible energy of all experimental diets were 42%, 16% and 18 kJ g^{-1}, respectively. The fatty acid composition SFA, MUFA and PUFA of the experimental diets ranged from 26.84 to 33.47, 31.75 to 34.11 and 26.63 to 37.65, respectively. Total n-3 of experimental diets followed the similar values as total n-6 fatty acids and ranged from 12.83 to 19.01 and 13.80 to 18.64, respectively. The n-3/n-6 ratio also ranged from 0.85 to 1.12.

Table 2. *Proximate composition (% on dry matter basis) and fatty acid profiles (% of total fatty acids) of the main protein sources used in the experimental diets*

Tablo 2. *Deneme yemlerinde kullanılan temel protein kaynaklarının ham değerleri (% kuru ağırlık) ve yağ asidi profilleri (% toplam yağ asidi)*

Parameter	TLM[1]	FM[1]	SBM[1]
Crude protein	30.10	73.79	53.58
Crude lipid	65.23	9.76	3.99
Crude fiber	0.79	0.76	4.31
Dry matter	90.94	92.47	90.70
Ash	2.88	14.83	7.71
NFE[2]	1.00	0.86	30.41
Fatty acids [3]			
14:0	2.72	4.48	1.53
15:0	0.55	0.35	-
16:0	21.98	27.48	23.14
17:0	0.32	0.58	-
18:0	8.24	7.28	7.12
16:1n-7	4.03	4.41	1.04
18:1n-7	4.11	4.86	2.56
18:1n-9	22.55	9.28	16.04
20:1n-9	10.21	-	-
18:2n-6	1.63	0.91	40.14
18:3n-6	0.82	0.4	5.41
20:4n-6	5.53	1.34	-
18:3n-3	1.22	1.05	0.77
20:5n-3	10.54	16.02	-
22:5n-3	0.20	0.95	-
22:6n-3	2.66	18.52	-
SFA	33.81	40.17	31.79
MUFA	40.9	18.55	19.64
PUFA	22.4	39.19	46.32
n-3	14.42	36.54	0.77
n-6	7.98	2.65	45.55
n-3/n-6	1.80	13.78	0.01

Values are mean of triplicate analysis. [1] ***TLM:*** *Tuna liver meal;* ***FM:*** *Fish meal;* ***SBM:*** *Soybean meal.* [2] ***NFE:*** *nitrogen-free extract.* [3] ***-,*** *not detected;* ***SFA*** *(saturated fatty acid) included 12:0, 14:0, 15:0, 16:0, 17:0 and 18:0;* ***MUFA*** *(monounsaturated fatty acid) included 16:1, 18:1 and 20:1;* ***PUFA*** *(polyunsaturated fatty acid) included n-3 and n-6; n-3 fatty acid included 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3; n-6 fatty acid included 18:2n-6, 18:3n-6, 20:4n-6*

Nile tilapia fry fed the experimental diets differed significantly (P<0.05) in mean final weight, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) *(Table 4)*. The final weight of fish fed diets including up to 30% TLM was significantly higher (P<0.05) than those of fish fed diets including TLM higher than 30%. This trend was significantly noticeable (P<0.05) for SGR and PER, an obvious decrease was observed as the TLM replacement occurred more than 30%. The FCR value of the fish fed diet with 50%TLM level was poorer than those fed the other diets. No significant differences in condition factor were confirmed on the experimental groups as the level of TLM increased in the diet (P>0.05).

The final proximate composition and fatty acid profiles of the carcass of fish fed the experimental diets are presented in *Table 5*. No significant differences were found in the protein and ash contents of fish fed with diets containing different proportion of TLM (P>0.05), whereas, lipid and dry matter contents were affected significantly by dietary treatments (P<0.05). Fatty acid profiles of the fish carcass varied with dietary treatment. Fish fed the TLM diets showed significantly elevated values for the overall SFA. The total short-chain MUFA values did not change, but 18:1n-9 value increased in carcass of fish, whereas PUFAs decreased in increasing of TLM level in diets, compared with that of fish fed control diet, particularly 18:2n-6 and 20:5n-3, giving a low value for total PUFA. Total n-6 fatty acids were lowest in fish fed the diets containing 20 or 30% of TLM level. On the other hand, the ratio of n-3 to n-6 fatty acids was highest in muscle of fish fed the diet including 50% of TLM level *(Table 5)*.

DISCUSSION

The FM replacement with TLM significantly affected the growth response of Nile tilapia fry (P<0.05). When replacing FM more than 30%, final weight, SGR and PER were significantly lower than those of the other groups, except for control group. However, the FCR value of fish fed TLM level of 50% was poorer than those of fish fed the others. These results indicated that TLM could be used to substitute FM by up to 30% without adverse effects on growth performance and feed utilization for Nile tilapia fry, and higher replacement levels resulted in growth reduction or poor feed utilization. This conformed well to the results of Gümüş [11] reported that no significant differences in growth performance of carp fry *(Cyprinus carpio)*, fed with diets including up to 20% TLM. The results of the present study are in conformity with the previous studies in the literature where the liver meal was shown to be good for growth and survival cyprinids or freshwater prawn post-larvae [22-25].

Partial replacement of FM with TLM did not influence the whole-body crude protein and ash contents of Nile tilapia fry, while lipid and dry matter contents were affected by dietary treatments. Similar results were also obtained in the study realized on carp fry, *Cyprinus carpio* by Gümüş [11]. As different from our findings, Yang [26] in gibel carp, and Gümüş [12] in Nile tilapia found out that no significant changes were observed in whole body lipid and dry matter content resulted from the different replacement of FM with various feed ingredients.

Since animal or plant protein sources become more widely used to replace fish meal in fish diets [1], the lipid content of diet is important as a source of dietary energy,

Table 3. *Proximate composition (% on dry matter basis) and fatty acid profiles of experimental diets (% of total fatty acids)*
Tablo 3. *Deneme yemlerinin ham değerleri (% kuru ağırlık) ve yağ asidi profilleri (% toplam yağ asidi)*

Parameter	%TLM Replacing FM in Diets					
	0 (Control)	10	20	30	40	50
Moisture	5.62	4.89	5.16	5.03	5.68	4.97
Crude protein	42.41	42.06	42.55	42.07	42.44	42.00
Crude lipid	16.18	16.20	16.03	15.95	16.07	16.10
Ash	7.99	7.68	7.06	6.92	6.71	6.64
Crude fiber	2.40	2.39	2.42	2.43	2.47	2.47
NFE [1]	30.99	31.64	31.91	32.61	32.30	32.77
DE(kJ g^{-1}) [2]	18.08	18.09	18.07	18.08	18.06	18.05
Fatty acids [3]						
14:0	5.63	5.86	4.18	4.37	3.61	3.03
15:0	0.30	-	0.30	0.31	0.35	0.37
16:0	17.87	17.20	18.2	21.35	21.73	22.8
17:0	-	0.26	0.32	0.38	0.46	0.51
18:0	3.04	3.76	5.03	5.18	6.29	6.76
16:1n-7	7.58	7.07	5.54	5.39	4.01	3.52
18:1n-7	3.20	3.26	3.59	3.62	3.66	3.77
18:1n-9	11.72	12.7	15.2	16.79	18.93	19.20
20:1n-9	9.25	9.34	9.04	8.65	7.0	6.28
18:2n-6	11.32	9.12	9.12	9.10	9.05	8.59
18:3n-6	1.39	1.32	1.32	1.26	1.15	1.11
20:4n-6	5.93	5.63	5.53	4.57	4.50	3.80
18:3n-3	1.37	1.37	1.21	1.14	1.01	0.80
20:5n-3	11.69	11.65	11.6	9.18	8.14	8.10
22:5n-3	0.68	0.50	0.44	0.40	0.35	0.31
22:6n-3	5.27	4.43	4.34	4.30	4.12	3.42
SFA	26.84	27.08	28.03	27.22	28.83	32.77
MUFA	31.75	32.37	33.37	34.45	33.6	32.47
PUFA	37.65	33.93	33.56	29.95	29.74	26.63
n-3	19.01	17.95	17.59	15.02	13.62	12.63
n-6	18.64	16.07	15.97	14.93	14.7	13.50
n-3/n-6	1.019	1.116	1.101	1.006	0.926	0.935

Values are mean of triplicate analysis. [1] ***NFE:*** *nitrogen-free extract.* [2] *Estimated according to NRC* [17]. [3] ***-,*** *not detected;* ***SFA*** *(saturated fatty acid) included 14:0, 15:0, 16:0, 17:0 and 18:0;* ***MUFA*** *(monounsaturated fatty acid) included 16:1, 18:1 and 20:1;* ***PUFA*** *(polyunsaturated fatty acid) included n-3 and n-6; n-3 fatty acid included 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3; n-6 fatty acid included 18:2n-6, 18:3n-6, 20:4n-6*

Table 4. *Growth performance and feed utilization of Nile tilapia fry (Oreochromis niloticus) fed the experimental diets*
Tablo 4. *Deneme yemleri ile beslenen Nil tilapia yavrularının (Oreochromis niloticus) büyüme performansı ve yem kullanımı*

Parameter	%TLM Replacing FM in Diets					
	0 (Control)	10	20	30	40	50
IBW (g)	2.21±0.06	2.36±0.18	2.24±0.07	2.32±0.02	2.29±0.05	2.28±0.07
FBW (g)	18.64±1.11 [ab]	20.84±2.77 [a]	18.78±3.03 [ab]	20.57±1.18 [ab]	15.95±0.62 [bc]	12.81±0.02 [c]
SGR (%/d)	3.04±0.04 [a]	3.09±0.10 [a]	2.97±0.15 [a]	3.17±0.05 [a]	2.76±0.01 [b]	2.46±0.05 [c]
FCR	1.12±0.13 [a]	1.01±0.04 [a]	1.12±0.27 [a]	1.02±0.08 [a]	1.13±0.04 [a]	1.52±0.03 [b]
PER	1.82±0.28 [ab]	2.10±0.13 [ab]	2.16±0.00 [a]	1.95±0.16 [ab]	1.76±0.05 [b]	1.31±0.02 [c]
CF	1.92±0.005	1.92±0.13	1.86±0.014	1.89±0.37	1.90±0.57	1.90±0.17

[a-c] *Values in the same row with different superscripts are significantly different from each other (P<0.05)*
[1] ***IBW =*** *initial body weight;* ***FBW*** *= final body weight;* ***L****= Body length;* ***SGR*** *(specific growth rate) = (Ln FBW-Ln IBW/time days x 100);* ***FCR*** *(feed conversion ratio) = Feed intake/(FBW-IBW);* ***PER*** *(protein efficiency ratio) = (FBW-IBW)/protein intake);* ***CF*** *(condition factor) = FBW/L^3 x 100*

Table 5. *Body composition (% of wet wt.) and fatty acid profiles (% of total fatty acids) of Nile tilapia fry (Oreochromis niloticus) fed the experimental diets*

Tablo 5. *Deneme yemleri ile beslenen Nil tilapia yavrularının (Oreochromis niloticus) vücut kompozisyonu (yaş ağırlığın %) ve yağ asidi profilleri (toplam yağ asidinin %)*

Parameter	%TLM Replacing FM in Diets					
	0 (Control)	10	20	30	40	50
Protein	19.73±0.23	19.37±0.06	19.27±0.79	19.70±0.48	19.68±0.29	19.86±0.18
Lipid	4.23±0.24 ^{ab}	3.74±0.11 ^{b}	3.53±0.11 ^{b}	4.75±0.46 ^{ab}	3.92±1.02 ^{ab}	5.43±0.82 ^{a}
Ash	2.14±0.02	2.09±0.22	2.03±0.47	1.65±0.01	1.88±0.32	1.90±0.04
Dry matter	26.11±0.50 ^{ab}	25.17±0.47 ^{b}	24.80±0.15 ^{b}	26.38±1.03 ^{ab}	25.39±1.06 ^{ab}	27.12±0.74 ^{a}
Fatty acids [1]						
14:0	0.31±0.29 ^{b}	0.14±0.05 ^{b}	0.11±0.01 ^{b}	2.03±2.69 ^{ab}	3.78±1.27 ^{a}	3.20±0.19 ^{ab}
15:0	0.33±0.02 ^{b}	0.32±0.04 ^{b}	0.17±0.02 ^{c}	0.18±0.02 ^{c}	0.21±0.01 ^{c}	0.47±0.03 ^{a}
16:0	17.56±0.08 ^{b}	18.37±1.11 ^{ab}	18.76±1.88 ^{ab}	17.94±1.63 ^{b}	20.31±0.65 ^{ab}	21.94±2.15 ^{a}
17:0	0.12±0.03 ^{c}	0.14±0.05 ^{c}	0.1±0.0 c	0.2±0.02 ^{bc}	0.26±0.06 ^{ab}	0.34±0.0 ^{a}
18:0	9.12±1.47	7.67±2.72	9.62±0.56	6.94±1.8	6.95±0.26	9.15±2.86
16:1n-7	6.25±1.06	4.87±1.89	3.78±0.39	4.96±1.74	5.33±1.41	3.79±0.65
18:1n-7	4.36±0.69	4.60±0.37	3.44±1.09	4.06±0.31	4.31±0.75	4.73±0.33
18:1n-9	20.43±0.38 ^{b}	19.86±2.65 ^{b}	22.22±1.23 ^{ab}	25.92±1.3 ^{a}	23.81±2.14 ^{ab}	22.38±0.28 ^{ab}
20:1n-9	1.15±0.1	2.27±1.47	0.93±0.07	1.27±0.08	1.67±0.39	1.71±0.0
18:2n-6	10.9±0.83 ^{ab}	12.50±1.4 ^{ab}	14.95±2.53 ^{a}	14.64±3.17 ^{a}	8.13±0.14 ^{b}	8.99±0.85 ^{b}
18:3n-6	0.45±0.09 ^{ab}	0.4±0.16 ^{ab}	0.33±0.07 ^{b}	0.48±0.17 ^{ab}	0.64±0.03 ^{a}	0.67±0.04 ^{a}
20:4n-6	1.22±0.14	1.49±0.05	1.46±0.1	1.2±0.22	1.39±0.33	1.38±0.46
18:3n-3	0.40±0.04	0.26±0.05	0.28±0.0	0.31±0.10	0.37±0.09	0.33±0.04
20:5n-3	5.39±0.82 ^{a}	4.19±0.93 ^{ab}	2.89±0.22 ^{b}	4.15±1.08 ^{ab}	3.94±0.41 ^{ab}	4.41±0.18 ^{ab}
22:5n-3	1.09±0.56	1.62±0.28	1.46±0.29	1.36±0.01	1.28±0.42	1.07±0.24
22:6n-3	3.53±1.49	5.56±0.92	5.73±0.91	3.67±1.38	4.04±2.20	4.89±2.53
SFA	27.44±1.67 ^{b}	26.65±1.65 ^{b}	28.76±1.30 ^{b}	27.31±0.74 ^{b}	31.52±0.43 ^{ab}	35.12±5.24 ^{a}
MUFA	32.2±0.86 ^{ab}	31.61±2.7 ^{ab}	30.38±1.85 ^{b}	36.21±2.82 ^{a}	35.12±3.93 ^{ab}	32.62±0.70 ^{ab}
PUFA	23±2.06 ^{ab}	26.03±1.52 ^{ab}	27.11±1.11 ^{a}	25.82±3.40 ^{ab}	19.8±3.39 ^{b}	21.76±2.30 ^{ab}
n-3	10.42±1.18	11.64±0.22	10.36±1.44	9.49±0.17	11.13±5.06	10.71±2.64
n-6	12.57±0.88 ^{ab}	14.39±1.29 ^{ab}	16.74±2.56 ^{a}	16.33±3.22 ^{a}	9.63±2.94 ^{b}	11.05±0.33 ^{b}
n-3/n-6	0.82±0.03	0.81±0.05	0.63±0.18	0.59±0.10	0.94±0.24	0.97±0.26

Values are mean (±SD) of triplicate analysis. $^{a-c}$ Values in the same row with the different superscripts are significantly different (P<0.05). [1] ***SFA*** *(saturated fatty acid) included 14:0, 15:0, 16:0, 17:0 and 18:0;* ***MUFA*** *(monounsaturated fatty acid) included 16:1, 18:1 and 20:1;* ***PUFA*** *(polyunsaturated fatty acid) included n-3 and n-6; n-3 fatty acid included 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3; n-6 fatty acid included 18:2n-6, 18:3n-6, 20:4n-6*

but is also fundamental for the supply an adequate amount of essential fatty acids (EFA) [27]. This will ensure not only that the diet satisfies the fish's requirement for these essential HUFA but also that these fatty acids are retained in sufficient amounts in the flesh of the fish. Findings suggest that EPA (20:5n-3), DHA (22:6n-3) and probably arachidonic acid (ARA, 20:4n-6) are essential for most marine and fresh water fish during development [28], as they are required for the normal physiological functioning and development of fish [29]. On the other hand, freshwater fish are known to be highly capable of elongating and further desaturating C18 PUFA, in the form of linoleic acid (18:2n-6) and linolenic acid (18:3n-3) to C20 and C22 PUFA in their natural diet [29,30].

Changes in fatty acid profile introduced by diet or feeding history are mirrored in the lipid stores of fish [31-33]. FM is an excellent source of essential amino acids. FM from oily fish species may contain up to 8-10 % of residual fat, which commonly contains from 20 to 35% n-3 HUFA. Hence, if fish meal is included in the diet at a level as low as 30 g/100 g, it will provide from 0.5 to 1% of the dry diet n-3 HUFA, and the general EFA requirements of most commonly farmed finfish species will be fulfilled regardless of the dietary lipid source included in the formulation. If residual fish oil is fully substituted with a complete or partial replacement of the fish meal fraction of the diet, the risk of a net deficiency in EFA is possible, and an appropriate source of n-3 HUFA needs to be included in the diet formulation [27].

FM replacement with TLM was not significantly modifying fatty acid profiles of experimental diets, because residual tuna liver oil was added to the experimental diets in order to counteract the loss of n-3 fatty acids due to FM elimination. However, considerable differences were observed in fatty acid profiles of flesh of fish fed diets progressively replaced of FM with TLM,

and some fatty acids increased or decreased in the flesh fish. FM replacement with different proportions of TLM caused a decline of n-3 and n-6 fatty acid levels of the experimental diets that had to be compensated with other sources rich in this type of fatty acids. This was remarkable in PUFA levels, in terms of negative correlation with the inclusion levels of TLM in the diet. Thus for example, accumulation of PUFA in fish flesh proved considerably higher in experimental groups fed with diets containing TLM up to 20 or 30% than in that of the control group, but lower when replacing FM with TLM more than 40%. Similar result was also obtained with MUFA. On the other hand, with respect to the whole fish fatty acid profile, as TLM increasingly replaces FM, SFA tend to increase significantly, and this effect is mainly due to palmitic and stearic acid included high levels of TLM.

In contrast to marine fish, carp and tilapia have been reported to be able to bio- convert larger quantities of linolenic acid to EPA and DHA [34,35]. This ability for *de novo* synthesis of EFA is consistent with a range of empirically determined requirement data for a range of freshwater species. The HUFA content of cultured fish has often been reported to be lower because of lowered fish meal usage in the diet [13,14]. On the other hand, carp and tilapia are generally regarded as omnivorous fish and are considered to require greater amounts of n-6 fatty acids than n-3 fatty acids in their diet [36]. High levels of n-3 PUFA have been reported to depress the growth of tilapia [37-39] and carp [26]. However, the level of total n-3 not changed in fatty acid profiles of flesh in fish fed TLM diets, suggesting that Nile tilapia fry have an ability to synthesize HUFA from 18- carbon precursors. However, the dietary fatty acid analysis in fish flesh showed that the n-6 decreased with increasing dietary TLM levels more than 40%, which suggested n-6 deficiency at higher TLM levels and resulted in retardation of lipid metabolism [40]. In other words, fish fed diets with low concentrations of total n-6 had low concentrations of total n-6 in fish flesh. These results were supported by some findings that a strong correlation between the fatty acid compositions of each test diet and the composition of the fish flesh was reported by previously for many different fish species [41-46].

The results of the present study indicated that dietary replacement of FM with TLM was significantly modifying Nile tilapia fry fatty acid profile. While FM replacement with TLM more than 40% caused a decline in MUFA and PUFA, up to 30% FM replacement was not deprived of MUFA and PUFA acids in flesh of fish fed experimental diets that have to be compensated with residual tuna liver oil rich in this type of fatty acids were added in order to counteract the loss of n-3 fatty acids due to FM elimination. Further research needs to be conducted using TLM as a FM replacer to determine acceptable fatty acid profiles for larger fish sizes.

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