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Effects of Dietary Fish Oil Replacement by Unrefined Peanut Oil on the Growth, Serum Biochemical and Hematological Parameters of Mozambique Tilapia Juveniles (*Oreochromis mossambicus*)

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Abstract

This research aimed to evaluate the effects of partial or total replacement of fish oil (FO) with unrefined peanut oil (PO) on growth and some physiological parameters of Mozambique tilapia juveniles for 60 days. Three triplicate groups (initial weight 6.36±0.19 g) were fed with isoenergetic and isonitrogenous diets in which FO was replaced with PO in graded increments of 50% (PO_0, PO_{50}, PO_{100}). The results showed that weight gain and feed efficiency of fish fed with PO_{50} diet were statistically higher than in the other two groups ($P<0.05$). No significant effects were observed on whole body proximate composition of fish fed with the experimental diets ($P>0.05$). The partial or total replacement of FO with PO did not affect on the hematological and immunological parameters of fish. Serum glucose, total cholesterol and triglyceride were lower in fish fed with PO_{50} diet ($P<0.05$). The present study revealed that PO can be used as an alternate source of dietary lipid up to 50% in tilapia feeds without adverse effects on growth performance, hemato-immunological and serum biochemical parameters

Keywords: *Oreochoromis mossambicus*, growth performance, hematological parameters, serum biochemical, vegetable oil source.

Yemlerde Kullanılan Rafine Edilmemiş Yerfıstığı Yağının Yavru Mozambik Tilapiya Balıklarında (*Oreochromis mossambicus*) Büyüme Performansı, Serum Biyokimyasal ve Hematolojik Parametrelere Etkileri

Özet

Bu çalışmada, rafine edilmemiş yerfıstığı yağının (PO) kısmen veya tamamen balık yağı yerine (BO) yemlerde kullanılmasının 60 günlük besleme sonunda Mozambik tilapiya jüvenillerinin büyüme performansı ve bazı fizyolojik parametreleri üzerine etkileri araştırılmıştır. Çalışma üç farklı izonitrojenik ve izolipidik yemlerle (PO_0, PO_{50}, PO_{100}) üç tekerrürlü olacak şekilde (başlangıç ağırlığı 6,36 ± 0,19 g) yürütülmüştür. Çalışmada PO_{50} grubu yemlerle beslenen balıklarda büyüme diğer gruplara göre yüksek bulunmuştur ($P<0,05$). Deneme grupları arasında balıkların kimyasal vücut kompozisyonlarında hiçbir fark bulunmamıştır ($P>0,05$). Yemlerde balık yağının kısmen veya tamamen PO kullanılması balıkların hematolojik ve immünolojik özelliklerinde farklılık meydana getirmemiştir. Serum glukoz, kolesterol ve trigliserit bulguları PO_{50} grubunda diğer gruplara göre düşük bulunmuştur ($P<0,05$). Bu çalışma rafine edilmemiş yerfıstığı yağının yavru tilapiya balığı yemlerinde %50 oranında büyüme performansı, serum biyokimsayal ve hemato-immunolojik parametrelere hiçbir negatif etkisi olmadan kullanılabileceğini göstermiştir.

Anahtar Kelimeler: *Oreochoromis mossambicus,* büyüme performansı, hematolojik parametreler, serum biyokimyası, bitkisel yağ kaynağı.

Introduction

Tilapia culture has showed a rapid growing throughout the world in the past two decades, particularly in Asia, Africa and America. Tacon and Metian (2008) estimated that the global production of tilapia will increase to 8.89 million MT by the year 2020. This rapidly increasing trend of tilapia production caused raising dependence by formulated feed (El-Sayed, 2006). The profitability of tilapia farm relies on proper feed. Currently, tilapia feeding costs more than 60% of the total production cost. Fish oil (FO) is still preferred as a main energy source for fish diets. The increase in the global demand for fish oil as a human and animal usage has created the necessity to use alternative oils to replace FO in fish

diets. On the contrary, the global production of vegetable oil has steadily increased. Several studies suggested that dietary vegetable oil can be used as a partial or total replacer for FO in fish diets (Montero *et al*., 2003; Lin and Shiau, 2007; Yıldız *et al*., 2013; Yıldırım *et al*., 2013). One potential substitute for FO in aqua feeds is peanut oil (PO). It is currently the fourth largest oilseed crop and is cultivated in more than 60 countries with an annual global production reaching some 35 million tones (Liu *et al*., 2012). In 2013 the world PO production was 5.58 million tones (USDA, 2014) and the actual PO price is 1000 USD/tones against the 2000 USD/tones of FO. PO contains high level of polyunsaturated fatty acid and it is cholesterol free. The replacement of FO with PO was already examined with satisfied result in the diet of common carp *Cyprinus carpio* (Yıldırım *et al*., 2013). Blood is a good indicator to determine the health of an organism. Differences in hematological parameters, immune response and serum biochemical variables as a function of dietary vegetable lipid sources have been reported for catfish (Ochang *et al*., 2007), atlantic salmon (Balfry *et al*., 2006), largemouth bass (Subhadra *et al*., 2006) and carp (Yıldırım *et al*., 2013). However, the influence of alternative vegetable lipid source on *Oreochoromis mossambicus* hematological and serum biochemical variables are very limited.

The main aim of this study was to determine growth performance, nutrient utilization and physiological response of Mozambique tilapia (*O. mossambicus*) juveniles fed with partial or total replacement of FO with PO diets.

Materials and Methods

Experimental Conditions and Diets

Unrefined peanut oil was obtained from a local factory (Başpınar Fıstık, Osmaniye, Turkey). The experiment was held in triplicate groups. A total of 180 fish were obtained from Kastamonu University, Turkey, randomly allocated into 80 L glass aquarium (20 fish per aquarium) and acclimated for 15 days. The specimens of tilapia, *O. mossambicus* (average weight 6.36±0.19 g). Fish were kept under a constant photoperiod (12 h light / 12 h dark). The aeration of aquariums was facilitated with an air pump. During the experiment, water was exchanged daily at a rate of ~10% of the total volume. Water quality parameters were periodically measured throughout the study, and the following parameters were recorded: temperature 24.2±0.5°C, pH 8.24±0.2, pH 8.27±0.3, dissolved oxygen, 7.38±0.27 mg L^{-1}, nitrite 0.06±0.02 mg L^{-1} and total ammonia 0.3±0.1 mg L^{-1}.

The fish oil substitution levels were 0%, 50% and 100%. The experimental diets used were isonitrogenous and isolipidic, with 34.5% protein, 12.8% lipid (Table 1). The dry ingredients were carefully mixed with a laboratory food mixer. The mixtures were primed with water to yield a suitable pulp. Wet diets were assembled into 2 mm pellet size and dried at 40°C in a drying cabinet and stored at -20°C until used. Fish were hand fed handed *ad libitum* twice a day ($10:^{00}$–$17:^{00}$ h) for 60 days. Proximate analyses of the dietsand fish fillets (12 fish/group) were performed by standard methods (AOAC, 1998). Growth performance and feed utilization were calculated with following formulas:

FCR (Feed conversion ratio)= feed consumed / weight gain

RGR (Relatively growth rate %)= [(final wet weight - initial wet weight) / initial wet weight] × 100

SGR (Specific growth rate %/day)= [(ln final wet weight – lninitial wet weight) / days] × 100

HSI (Hepatosomatic index %)= (100 × liver weight ×BW^{-1})

VSI (Viscerasomatic index %)=(100×carcass weight×BW^{-1})

Table1. Formulation and analytical composition of the experimental diets (% dry matter)

Ingredients	PO_0	PO_{50}	PO_{100}
Fish meal	22	22	22
Soybean meal	40	40	40
Wheat meal	24	24	24
Fishoil	10	5	
Peanutoil		5	10
Vitamin mix[1]	2	2	2
Mineral mix[2]	2	2	2
Analytical composition (% dry matter)			
Protein	34.3	34.7	34.5
Lipids	12.8	12.8	12.8
Ash	6.86	7.26	6.66
NFE[3]	38.04	38.04	38.04
Energy (kj/g)[4]	19.62	19.53	19.67

[1]Vitamin Mix (IU/kg or g kg^{-1} or mg kg^{-1}): Vit. A, 18000 IU; Vit. D3, 2500 IU ; Vit. E, 250 mg/kg; Vit. K3, 12 mg/kg; Vit. B1, 25 mg; Vit. B2, 50mg; Vit. B3, 270 mg; Vit. B6, 20 mg; Vit. B12, 0.06 mg; Vit. C, 200 mg; Folic acid, 10 mg; Calcium d–pantothenate, 50 mg; Biotin, 1 mg; Inositol, 120 mg; Choline chloride, 2000 mg.
[2]Mineral Mix (IU/kg or g kg^{-1} or mg kg^{-1})Fe, 75.3 mg; Cu, 12.2 mg; Mn, 206 mg; Zn, 85 mg; I, 3 mg; Se, 0.350 mg; Co, 1 mg.
[3]Nitrogen free extracts = dry matter- (crude lipid + crude ash + crude protein)
[4]Energy calculated according to 23.6 kJ/g protein, 39.5 kJ/g lipid and 17.0 kJ/g NFE.

Blood Collection

After day 60, four fish from each aquarium were anesthetized with clove oil at 20 mg/L. Blood samples from all fish (12 fish/group) were collected from the caudal vein with a syringe, added to the tubes containing EDTA (BD Microtainer®, UK) and then preserved for hematological analysis. Blood serum was separated by centrifugation (4000 × g, 10 min) in plastic biochemistry tubes (Kima-vacutest®, Italy) and stored at -20°C until biochemical analysis (Bricknell *et al*., 1999).

Hematological Analyses

Hematocrit (Hct, %) and hemoglobin (Hb, g dl^{-1}) were determined by using the method of Blaxhall and Daisley (1973). Red Blood Cell (RBC) count was obtained with a Thoma hemocytometer using Dacie's diluting fluid. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated by using the following formula (Bain *et al*., 2006):

$MCV(\mu m^3) = [(Hct, \%) \times 10]/(RBC, \times 10^6 \text{ per } mm^3)$,
$MCH(pg) = [(Hb, g/dL) \times 10]/(RBC, \times 10^6 \text{ per } mm^3)$,
and
$MCHC(\%) = [(Hb, g/dL) \times 100]/(Hct, \%)$.

Biochemical Analysis

Biochemical indices, including glucose (GLU), total protein (TPROT), albumin (ALB) triglyceride (TRI) and cholesterol (COL) in serum were analyzed using bioanalytic test kits (Bioanalytic Diagnostic Industry, Co) and measured by a Shimadzu spectrophotometer (PG Instruments, UK).

Lysozyme Activity

Serum lysozyme was assessed using the turbidometric assay (Ellis, 1990). A suspension of 875 µl of *Micrococcus lysodeikticus* (Sigma, ATCC 4698)at a concentration of 0.2 mg/ml (in PBS) was added to 25 µl of serum samples. Then the samples were measured with a spectrophotometer at 530 nm after 0.5 and 4.5 minutes at 25°C. A unit of lysozyme activity was defined as the amount of serum producing a reduction in absorbance of 0.001 min^{-1}.

Statistical Analyses

Values of all measured variables are expressed as (Mean±SD). Statistical significance was determined by one-way ANOVA; when differences between treatment found, Tukey's test (in SPSS version 17.0) was used to compare means. Differences were considered significant at *P*-values less than 0.05.

Results

All diets were accepted by the fish very well. Weight gain and SGR of fish fed enriched with PO50 diet were statistically higher than in the other group ($P<0.05$) (Table 2). Moisture, crude protein, crude lipid and ash content of the whole body of fish did not differ significantly among the groups. GLU, TRIG and CHOL levels were significantly reduced in fish fed with PO50 diet ($P<0.05$) (Table 3). TPROT and ALB level in serum increased by increasing PO level in diets. Partial or total replacement of FO with PO

Table2. Growth parameters of *Oreochromis mossambicus* juveniles fed diets containing alternative lipid sources for 60 days

Diets	Initial weight (g)	Final weight (g)	RGR (%)	SGR (% body weight day^{-1})	FCR	HSI (%)	VSI (%)
PO0	6.37±0.20	13.04±0.41^a	104.59±0.57^a	1.19±0.01^a	2.36±0.04^b	0.91±0.08^a	8.70±0.48^a
PO50	6.36±0.18	16.20±1.44^c	153.15±1.38^c	1.55±0.01^b	1.59±0.02^a	0.93±0.07^a	8.51±0.29^a
PO100	6.35±0.20	13.59±0.87^b	114.165±1.56^b	1.27±0.08^a	2.19±017^b	0.90±0.14^a	8.34±1.17^a

Mean with common superscripts in the same column are not significantly different (P>0.05).
RGR (%) = 100 (final fish weight – initial fish weight) / initial fish weight
Specific growth rate (SGR) = 100 (ln final fish weight) – (lninitial fish weight) / experimental days
Feed conversion ratio (FCR) = feed intake / weight gain
HSI, % = (100 × liver weight $\times BW^{-1}$)
VSI, %= (100 × carcass weight $\times BW^{-1}$)

Table3. Body composition of *Oreochoromis mossambicus* juveniles fed diets containing alternative lipid sources for 60 days (% of wet weight±SD)

Parameters	Moisture	Protein	Lipid	Ash
PO0	78.32±0.16^a	16.13±0.11^a	2.03±0.4^a	1.86±0.18^a
PO50	77.86±0.30^a	16.02±0.08^a	2.09±0.32^a	1.81±0.09^a
PO100	78.02±0.10^a	16.08±0.09^a	1.81±0.09^a	1.82±0.62^a

Values are mean (n = 12) ± SD. Mean with common superscripts in the same column are not significantly different (P>0.05).

did not affect the serum lysozyme activity among the groups. Hematocrit, hemoglobin, MCHC and MCV did not differ significantly among the groups (Table 4). MCH values increased by increasing of PO level in diets (P<0.05) (Table 4).

Discussion

The present study clearly demonstrated that partial or total replacement of FO with PO did not affect negativelyon the growth of tilapia. The results of the present study are similar to earlier studies on freshwater species such as Murray cod (*Maccullochella peelii*), black carp (*Mylopharyngodon piceus*) and common carp (*Cyprinus carpio*) (Francis *et al*., 2007; Sun *et al*., 2011; Yıldırım *et al*., 2013). Similar results were also reported for marine species such as gilthead seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) (Izquerdo *et al*., 2003; Figuerdo-Silva *et al*., 2005). The present study showed as partial or total replacement of FO with PO did not affect the ash, moisture, lipid, protein and HSI and VSI values in whole body of fish. Similar results were obtained in common carp (*C. carpio*) when PO was used as a replacement for FO in practical diets (Yıldırım *et al*., 2013).

Increased GLU level is a well-known stress indicator in fish (Morgan and Iwama 1997) and nutritional status is a factor on glucose response. In this study, the GLU level was lower in serum of fish fed with PO50 and PO100 diets. Similar results were reported for Caspian brown trout (*Salmo trutta caspius*) and common carp (*C. carpio*) when vegetable oil was used as a replacer of FO in the diets (Kenari *et al*., 2011; Yıldırım *et al*., 2013). Serum proteins are the other useful indicator for fish immune system. In this study, TPROT level increased with the PO supplementation. This result could be typical sign of increasement level of Immunity system. Similarly, vegetable oil did not lead to immune suppression in grouper (Lin and Shiau, 2007). The present study suggested that dietary PO has no adverse effects on the immune system of tilapia. Serum TRIG and CHOL levels were found significantly lower in PO50 diet. Fish plasma containing ALB was verified by electrophoretic mobility, fatty-acid-binding features and molecular mass (Davidson *et al*., 1988). The ALB was not influenced by the diets. This shows that the lipid sources do not lead to osmoregulatory dysfunction or damage tissues surrounding blood vessels of *O. mossambicus*. TRIG level was higher in fish fed with PO_{100} diet, indicating that PO stimulates triglyceride production while fish oil reduced (Vegusdal *et al*., 2005). The benefic herbs effects on cholesterol are already known (Lin *et al*., 2007). In this study, serum cholesterol level decreased with increasing of vegetable oil in the diets as already observed in other studies testing FO replacement with vegetable oil (Peng *et al*., 2008; Yıldırım *et al*., 2013).

Fish hematologyis important in fish culture to monitor the fish health status (Hrubec *et al*., 2000). In this study, the survival rate was 100% in all experimental groups. Hct values obtained in present study showed no significant differences. Adversely, Ochang *et al*. (2007) reported increasing Hct value while increasing dietary palm oil level in diet for *O. niloticus*. The Hb content of fish in the present study was found higher than those obtained by Subhadra *et al*. (2006) for largemouth bass (*Micropteruss almoides*) fed with diets containing canola oil, chicken oil and menhaden fish oil. This shows that the oxygen carrying abilities of the blood of *O. mossambicus* is higher and could be attributed to the utilization ability of n-6 fatty acids in vegetable oil (Babalola *et al*., 2009). However, no differences in lysozyme activity were found in the present study among the groups which was also reported in other studies on fish fed vegetable oil (Montero *et al*., 2003; Mourente *et al*., 2007). Blood parameters such as MCV, MCH and MCHC are important indices for the symptom of anemia in most animals (Coles, 1986).

Table 4. Serum biochemical and hemoto-immunological parameters of *Oreochromis mossambicus* juveniles fed diets containing alternative lipid sources for 60 days

Parameters	PO_0	PO_{50}	PO_{100}
GLU (mg/dl)	68.50±8.54^b	44.35±4.87^a	59.61±10.22^a
TRIG (mg/dl)	55.91±5.48^b	48.35±8.56^a	69.46±3.01^c
CHOL (mg/dl)	67.73±5.99^c	45.15±4.40^a	5372±8.70^b
TPROT (g/dl)	3.51±0.02^a	5.23±0.91^b	6.10±0.57^c
ALB (g/dl)	1.60±0.46^a	1.87±0.47^a	2.34±0.87^a
Hct(%)	36.4±0.30^a	37.27±0.38^a	36.83±0.45^a
Hb (g/dl)	7.59±0.09^a	7.84±0.06^a	7.81±0.17^a
RBC ($\times 10^6$per mm^3)	2.43±0.47^a	2.41±0.36^a	2.31±0.27^a
MCV (μm^3)	179.04±2.94^a	176.10±2.33^a	180.07±1.85^a
MCH (pg $cell^{-1}$)	26.08±0.50^a	28.54±0.48^b	30.10±0.09^c
MCHC (%)	21.37±0.55^a	21.04±1.05^a	22.17±0.85^a
Lysozymeactivity (mg ml^{-1})	358.21±2.21^a	363.27±2.08^a	361.81±3.07^a

Values are mean (n = 12). (Mean±SD) with common superscripts in the same line are not significantly different (P>0.05).
GLU, glucose; Trıg, triglyceride; CHOL, cholesterol; TPROT, total protein; ALB, albumin; Hct, hematocrit; Hb, hemoglobin; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration.

The results of the present study did not show any symptom of anemia in specimens of tilapia fed with PO replacements.

In conclusion, results of the present study suggest that potential exist for replacing FO with PO in Mozambique tilapia diets up to 50% without compromising growth, non-specific immune function and serum biochemical parameters. Moreover the results obtained will further contribute to reduce the feed costs by replacing fish oil with unrefined peanut oil for tilapia diets in aqua feed industry.

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