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Defatted Peanut Meal (*Arachis hypogea*) as a Complementary Protein Source in Diets for Mozambique Tilapia (*Oreochromis mossambicus*) Fry and Effect on Fatty Acid Composition

Önder Yıldırım* and Ümit Acar
Department of Aquaculture, Faculty of Fisheries, Muğla Sıtkı Koçman University, Kötekli, Muğla, Turkey

ABSTRACT

This study was conducted to investigate the partial replacement of fish meal with defatted peanut meal (DPNM) in diets of Mozambique tilapia, *Oreochromis mossambicus* and to evaluate its effect on body fatty acids composition. Four levels of DPNM inclusion (0, 10%, 20%, 30% and 40%) were used and one control group was included. Fish diets were isonitrogenous (crude protein 36%) and isoenergetic (20 kJ/kg). One hundred and fifty tilapia fry (average weight of ~ 0.85 g) were fed during a 45-day feeding trial conducted in a recirculation system aquarium. Polyunsaturated fatty acids (PUFAs) in fish fillet showed increasing with increase of DPNM level. The present results suggest that defatted peanut meal is a promising alternative feedstuff and that it could be safely used in Mozambique tilapia diets up to 10% without adverse effects on fillet fatty acid composition.

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Authors' Contributions
UA designed the experiment. OY performed the experiment and analyzed the data. Both the authors wrote the article.

Key words
Plant protein, defatted peanut meal, Tilapia, growth performance, fatty acids profiles.

INTRODUCTION

Over the last 30 years a global rapid growth in the aquaculture sector has been registered (FAO, 2012). Sustainable growth in this industry will depend upon the use of marine protein and lipids in feeds for farmed fish. Protein is the most important content for good growth performance and fish health. Fish meal (FM) is used frequently as a source of protein in fish feeds due to its high protein content, palatability and well balanced aminoacids profile. However, a fishery production also makes it is an expensive raw material. In order to sustain the aquaculture industry, farmers need to find cheap alternative protein sources to replace the more expensive marine protein sources. Aquaculture researchers aim to reduce the cost of feed. Vegetable protein sources constitute an optimistic advance in the quest for fish meal replacement because of their increased annual production, high availability and greater economic value (Garduno-Lugo and Olvera-Novoa, 2008). Some vegetable protein sources such as soybean, corn gluten meal, sunflower meal and rapeseed meal and their by-products has already been used as alternative sources of protein for several freshwater or marine fish species (Garduno-Lugo and Olvera-Novoa, 2008; Yıldırım *et al*., 2009; Yiğit *et al*., 2012).

Peanut is the fourth largest oilseed crop and it is cultivated in more than 100 countries with an annual global production reaching about 35.5 million tonnes (FAOSTAT, 2015). Peanut by-products, which remain after the extraction of peanut oil, can be used in animal feeds and contain lower levels of lysine and higher levels of arginine compared with soybean meal (Batal *et al*., 2005). Defatted peanut meal (DPNM) has been used in aquatic animal feeds (Liu *et al*., 2012; Garduno-Lugo and Olvera-Novoa, 2008). Peanut oil industry by-product can be used as an alternative protein source. Nevertheless, evidence of potential use of peanut meal in fish diets is limited to only a few studies. Fish are the main source of omega-3 in human diets (Meyer *et al.*, 2003). Tilapias are important species for fish culture, particularly in Asia and they are chosen due to high growth rate, adequacy to grow and properly reproduce in captivity, and feed on low trophic levels (El-Sayed, 2006; Nguyen *et al.*, 2009). The aim of the present study was to determine the effects of replacing FM with DPNM on growth and fillet fatty acid profile of the Mozambique tilapia *Oreochromis mossambicus*.

MATERIALS AND METHODS

Experimental diets

The peanut pulp was obtained from a local factory (Başpınar Fıstık, Osmaniye, Turkey). The pulp was ground in a hand mill then in an electric mill, screened on a 590 µm mesh and stored in a plastic case at –20°C until use. Then it was added to the feed at different rates: 0%, 10%, 20%, 30% and 40% for diets DPNM–10, DPNM–

* Corresponding author: onderyildirim@mu.edu.tr
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20, DPNM–30 and DPNM–40, respectively. The feed components of the diets are presented in Table I. The ingredients were mixed in a blender. The pellets (2 mm diameter) were made in a mincing machine, dried in a drying cabinet (40°C) until moisture dropped to around 10% then crushed into desirable particle sizes (250-400 µm) and stored at –20°C until use.

Experimental design and feeding trial

The experiment was designed in triplicate for each diet. Fifteen 50 L glass aquaria were stocked with 150 fry (10 fry / aquarium) of Mozambique tilapia with size of ~0.85 g. The fry were fed at satiety three times a day for 45 days. During the experiment, water was exchanged daily at a rate of ~10% of the total volume.

Proximate composition and fatty acid analysis

After day 45, five randomly selected fish from each aquarium were used for fatty acid analysis. Feed samples were analyzed for proximate composition according to (AOAC, 2000). All the samples were frozen at -20°C until analyzed. Dry matter was detected after drying at 105°C until a constant weight was achieved. Ash content was measured in a muffle furnace at 525°C for 12 h. The amount of crude protein was analyzed by the Kjeldahl method and lipid contents were determined by the SOXTEC system.

Fatty acids in the total lipid were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14% boron trifluoride-methanol (AOAC, 2009). Fatty acid methyl esters (FAME) were analyzed using a flame ionization gas chromatograph (Shimadzu GC-2014) equipped with a Omegawax 250 capillary column (30 ml X 0.25 mm internal diameter), a flame ionization detector (FID), and a split injection system with nitrogen carrier gas. The injector port and detector temperatures were maintained at 250°C and 260°C, respectively. The column temperature program was held at 140°C for 5 min, and then increased at a rate of 3°C/min to 200°C. Total run time was 60 min per sample. Fatty acids were identified by comparing their retention times to authentic standard fatty acid standards (Sigma-Aldrich Co., USA).

S*tatistical analysis*

Each value was expressed as mean ± SD for each parameter measured. Statistical significance was determined by one-way analysis of variance (ANOVA). The differences between means were determined by Tukey's multiple comparison test. Differences were considered significant at $P < 0.05$.

RESULTS

The fish developed normally with no sign of malnutrition. The five diets were accepted equally by the fish. The lowest final weight and specific growth rate were observed for tilapia fed the DPNM-40 diet (Fig. 1).

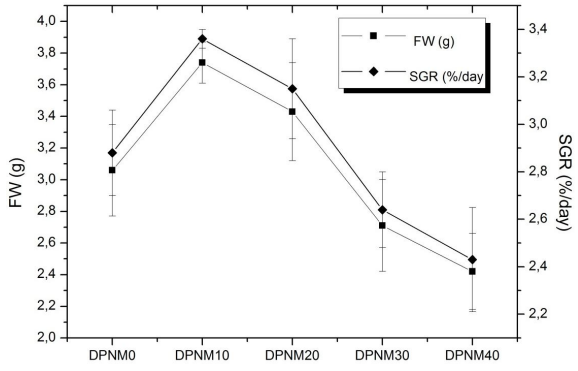

Fig. 1. Mean (±SD) final weight (FW) and specific growth rate (SGR) of *O. mossambicus fry* fed diets with graded levels of fish meal replaced by defatted peanut meal for 45 days.

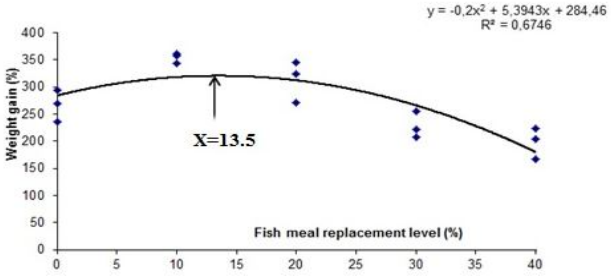

Fig. 2. The relationship between weight gain of *O. mossambicus* fry and dietary fishmeal replacement by peanut meal.

The second–order polynomial regression between dietary DPNM meal levels and each weight gain (Fig. 2) indicated that the most suitable DPNM meal level for maximum growth was 13.5% (Fig. 2). The fatty acid profile of flesh, in the dietary treatments, showed that saturated fatty acids (SFA) were the main group in fillet and followed by monounsaturated fatty acid (MUFA) and polyunsaturated fatty acids (PUFA) (Table II). Saturated fatty acid decreased as DPNM levels in diets were increased. The dosage of n-6 PUFA was significantly higher in the flesh of fish fed the DPNM-30 diet while n-3 ratio was lower in DPNM-40 group. The n-3:n-6 ratio decreased in the fillet of fish fed a diet containing more than 10% DPNM.

Table I.- Proximate composition (%) of the experimental diets containing supplement of different DPNM rate.

	Experimental diets				
	DPNM–0	**DPNM–10**	**DPNM–20**	**DPNM–30**	**DPNM–40**
Ingredients (%)					
Fish meal	30.00	27.00	24.00	21.00	18.00
Soybean meal	33.00	33.00	33.00	33.00	33.00
Fish oil	5	5	5	5	5
Peanut meal	-	6	12.8	19.1	25.3
Corn starch	28	25	21.2	17.9	14.7
Vitamin–mineral mix[1,2]	4.0	4.0	4.0	4.0	4.0
Total	100	100	100	100	100
Chemical analyses (%, DM)					
Protein	36.70	36.60	36.70	36.70	36.60
Fat	8.19	8.51	8.91	9.26	9.60
Ash	6.02	6.02	6.08	6.1	6.12
NFE[3]	47.70	47.50	46.90	46.70	46.30
Gross energy, MJ/kg	19.90	20.07	20.15	20.25	20.30
Cost of diet ($ kg $^{-1}$)[5]	1.02	0.98	0.94	0.90	0.87
Fatty acid composition of diets					
14:00	3,65	3,28	2,91	2,59	2,3
15:00	0,56	0,52	0,51	0,31	0,25
16:00	22,6	18,68	17,67	16,82	16,53
18:00	5,79	5,63	5,48	5,35	5,24
20:00	0,43	0,22	0,53	0,56	0,58
22:00	0,09	0,07	0,05	0,06	0,05
16:1n-7	7,96	7,42	6,86	8,94	7,18
18:1n-9	14,68	20,07	23,52	24,46	27,12
20:1n-9	0,74	0,66	0,88	0,51	0,44
22:1n-9	1,1	1,08	1,07	0,55	1,05
18:2n-6	20,28	19,49	21,65	22,35	23,61
18:3n-3	2,42	3,45	2,61	2,05	2,09
20:3n-3	2,04	3,04	2,03	2,94	2,03
20:5n-3	3,54	4,51	2,61	2,2	1,83
22:2n-6	0,38	0,32	0,27	0,24	0,21
22:6n-3	13,28	11,07	10,96	9,97	8,32
ΣSFA	33,12	28,4	27,15	25,69	24,95
ΣMUFA	24,48	29,23	32,33	34,46	35,79
ΣPUFA	41,94	41,88	40,13	39,75	38,09
n-3	21,28	22,07	18,21	17,16	14,27
n-6	20,66	19,81	21,92	22,59	23,82
n3/n-6	1,03	1,11	0,83	0,7	0,60

[1]Vitamin Mix: Vit. A, 18000 IU; Vit. D3, 2500 IU ; Vit. E, 250 mg/kg; Vit. K3, 12 mg/kg; Vit. B1, 25 mg; Vit. B2, 50 mg; 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: 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 (NFE) = matter – (crude lipid+crude ash+crude protein+crude fiber).

[4]Energy calculated according to 23.6 kJ g^{-1} protein, 39.5 kJ g^{-1} lipid, and 17.0 kJ g^{-1} NFE.

[5]Calculated from following price of ingredients (April 2014): Fish meal=1600 $ kg^{-1}; Peanut meal= 260 $ kg^{-1}; Soybean meal 650 $ kg^{-1}; Corn starch=220 $ kg^{-1}; Fish oil=2000 $ kg^{-1}; Vit-Min mix= 8250 $ kg^{-1}

Table II.- Fatty acid profiles (% of total fatty acids) of tilapia fed on the experimental diets.

Main fatty acids (% total fatty acids)	DPNM0	DPNM10	DPNM20	DPNM30	DPNM40
14:00	4.72±0.01^{c}	4.57±0.00^{b}	4.84±0.00^{d}	4.32±0.00^{a}	4.33±0.00^{a}
15:00	0.81±0.02^{b}	0.76±0.01^{a}	0.88±0.01^{c}	0.74±0.02^{a}	0.93±0.01^{d}
16:00	27.85±0.03^{e}	27.63±0.02^{d}	25.21±0.02^{c}	24.35±0.02^{b}	23.46±0.00^{a}
16:1n-7	8.24±0.02^{e}	8.03±0.01^{d}	7.08±0.01^{c}	6.73±0.00^{b}	6.29±0.00^{a}
18:00	6.44±0.02^{c}	6.5±0.00^{c}	5.86±0.01^{b}	5.64±0.00^{a}	8.89±0.01^{d}
18:1n-9	21.33±0.00^{b}	20.94±0.01^{a}	22.38±0.00^{c}	23.19±0.01^{d}	23.38±0.01^{e}
18:2n-6	5.37±0.01^{a}	5.96±0.02^{b}	8.6±0.00^{d}	8.42±0.01^{c}	10.64±0.00^{e}
20:00	0.18±0.00^{a}	0.2±0.00^{a}	0.4±0.00^{d}	0.31±0.02^{b}	0.35±0.01^{c}
20:1n-9	0.97±0.00^{b}	0.94±0.00^{a}	1.31±0.00^{c}	0.93±0.00^{a}	1.55±0.00^{d}
18:3n-3	2.08±0.00^{c}	1.93±0.00^{b}	2.23±0.00^{c}	1.81±0.00^{a}	1.91±0.00^{b}
22:00	0.25±0.01^{a}	0.30±0.00^{b}	0.53±0.02^{d}	0.36±0.00^{c}	0.38±0.00^{c}
22:1n-9	0.14±0.01^{b}	0.11±0.01^{a}	0.12±0.00^{ba}	0.13±0.00^{a}	0.12±0.02^{a}
20:3n-3	0.76±0.01^{b}	0.80±0.01^{ba}	0.98±0.01^{a}	0.87±0.00^{a}	0.88±0.02^{a}
20:5n-3	3.72±0.01^{c}	3.85±0.01^{d}	3.54±0.01^{a}	3.62±0.02^{b}	3.55±0.01^{a}
22:2n-6	2.23±0.00^{e}	2.14±0.00^{d}	2.04±0.00^{c}	1.89±0.00^{a}	2.12±0.00^{b}
22:6n-3	12.75±0.02^{b}	13.74±0.00^{d}	13.11±0.00^{c}	14.73±0.01^{e}	10.50±0.02^{a}
ΣSFA	40.25	39.96	37.72	35.72	38.34
ΣMUFA	30.68	30.02	30.89	30.98	31.34
ΣPUFA	26,91	28,42	30,5	31,34	29,6
n-3 PUFA	19,31	20,32	19,86	21,03	16,84
n-6 PUFA	7,6	8,1	10,64	10,31	12,76
n3/n6	2,54	2,50	1,86	2,04	1,32

DISCUSSION

Vegetable protein sources successfully replaced fish meal in Mozambique tilapia diets (Olvera-Novoa *et al.,* 1998). Low growth with high levels of DPNM in tilapia fries could be due to a lower lysine and methionine levels in DPNM (Yıldırım *et al.,* 2014). The results showed similarities with (Garduno-Lugo and Olvera-Novoa, 2008), who reported the same level of fish meal replacement with peanut leaf meal in *Oreochoromis niloticus* diets. These results were different to those of Yousif *et al.* (1994), who replaced fish meal with sun-dried alfalfa meal and found a decline in fish growth attributable to antinutritional factors such as trypsin inhibitor. The reduction of growth parameters for several fish species have been reported when vegetable meal replaced fish meal (Olvera-Novoa *et al.,* 1998; Pratoomyot *et al*., 2010). The low growth and feed utilization of tilapia fed with increasing levels of DPNM could be explained by reduced effects of digestive enzymes (Klein *et al.,* 1998).

The protein and lipid levels in all experimental diets were within requirements recommended for tilapia (Jauncey *et al.,* 1982).

The main difference found between prepared feed, given the requirements of tilapia, was the fatty acid profile. Previous studies showed that the fatty acid composition of muscle of most fish species is strongly influenced by the dietary fatty acid content in diets (Güroy *et al.,* 2010). Nutritional quality of fish products is important for human consumption, particularly in terms of flesh fatty acid composition and the content of the beneficial n-3 LC-PUFA, EPA and DHA. In the present research, substituting FM with different levels of DPNM did not reduce levels of EPA and DHA in the flesh below those obtained in fish fed DPNM-0 diet. Indeed, the major bioactive LC-PUFA, EPA and DHA tended to increase in the flesh of fish fed on increased levels of DPNM. PUFA composition in tilapia tissue cannot be explained merely through the dietary fatty acid composition and DHA decreased with increasing DPNM inclusion. Earlier studies showed that levels of n-3 HUFA

in fish tissues could be higher than those found in the feeds (Robin and Skalli, 2007). To obtain a high level of n-3 PUFA in fish is a main objective of producers in order that high nutritional food products can be supplied for human consumption. The present investigate showed clearly that the use of DPNM in diets is effective in producing n-3 PUFA rich products as reported in the muscle of sunshine bass (*Morone chrysops* × *M. saxatilis*) (Lewis and Kohler, 2011). Metabolic effects may include differential oxidation of fatty acids and increased retention of LC-PUFA as vegetable protein sources increased (Pratoomyot *et al.,* 2010). When fatty acids are used at low levels in diets, they tend to be deposited in tissue because fatty acids are the predominant sources of metabolic energy in fish (Sargent *et al.,* 2002). The n-6 PUFA fatty acid values obtained in this study indicated similarities with data reported by (Francesco *et al.,* 2007), who used vegetable meal in diets of seabream (*Sparus aurata*). The n-3 PUFA values showed fluting values, similar results reported by (Dantagnan *et al.,* 2009), who studied macroalg meal (*Macrocytis pyrifera)* as feed ingredients for rainbow trout (*Oncorhynchus mykiss*). Gümüş *et al.* (2010) studied the effects of partial replacement of fish meal with tuna liver meal on the diet of Nile tilapia (*Oreochromis niloticus*) and found a fluctuation of n-3 fatty acid between the groups.

In the current study, the results showed significant differences in the composition of muscle fatty acids. The levels of 18:1n-9 and 18:2n-6 were significantly higher while 20:5n-3 and 22:6n-3 were lower in high level DPNM diets. The fatty acids 18:1n-9 and 18:2n-6 were not accumulated in low vegetable source inclusion in diets; due probably to efficient digestion and metabolism compensating for the lack of long chain n-3 fatty acids (Parés-Sierra *et al*., 2012).

The present experiment exhibits peanut meal up to 10% can be used as a protein source replacement for fish meal in tilapia diets without significant effects fillet fatty acid composition. Such replacement can help lower feed costs in the aquaculture industry. Therefore, further investigations on *O. mossambicus* diets with higher DPNM incorporation levels and addition of enzymes are encouraged.

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