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Effects of Fish Meal Substitution with Poultry By-product Meal on Growth Performance, Nutrients Utilization and Blood Contents of Juvenile Nile Tilapia (*Oreochromis niloticus*)

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Abstract

Four isonitrogenous and isocaloric diets (30.22 ± 0.02% CP and 19.007 ± 0.015 MJ kg^{-1} diet) were formulated to represent four dietary treatments. The first treatment (control) without poultry by-product (PM0), while the second, third and fourth diets formulated with substitution of fish meal by poultry by-product meal as 50, 75 and 100%, respectively. Each diet was fed to 100 juvenile tilapia (1.5 ± 0.05 g), in triplicate cement ponds of 2 m^3. Fish were fed diets at a rate of 3% of its biomass daily divided into two equal portions. The highest (P<0.05) growth performance parameters (finial weight, weight gain, daily gain and specific growth rate) and best nutrient utilization (feed conversion ratio, protein efficiency ratio and net protein utilization) were recorded with PBM0 and PBM100% groups. The applied treatments showed insignificant effects on nutrients digestibility coefficient among dietary groups for Dry matter, Energy, CP, Fat and nitrogen free extract. No significant effects on dry matter, crude protein, fat and ash contents were recorded between dietary treatments. Juvenile tilapia fed the experimental diets showed insignificant different in blood contents (P<0.05) between groups. The present study recommended substitution of 100% fish meal with poultry by-product meal in juvenile Nile tilapia diets.

Keywords: *Oreochromis niloticus*; Poultry by-product meal; Digestibility coefficient; Growth performance; Blood contents

Introduction

The global production of tilapia drastically increased from 124 thousand metric tons (Mt) in 1997 to 2.5 million Mt in 2010 [1]. This trend suggests that there will be even greater increases in the future. Among the cichlid species, it is the Nile tilapia (*Oreochromis niloticus*) that has dominated in different culture system. The tilapia market has expanded from a subsistence level to meet the protein needs of the middle class because of the year-round supply, delicious flavour and reasonable price of that fish [2]. Golbal tilapia production was recorded 3.500.000 metric tons in 2011, 3.8000.000 metric ton in 2012,4.850.000 metric ton in 2014 and by 2015, world production tilapia is forecast to reach 4.6-5.0 million metric ton [3,4].

Traditionally, fish meal has provided a major part of protein sources in formulated feeds because of its suitable protein quality. Science the recent scarcity and uncertain consistency of supply encourage its replacement by alternative protein sources that are of high quality, but less expensive has been investigated in many fish species. The limitations on the world's food supply provide additional motivation [5,6]. Therefore, numerous studies have undertaken to examine the effects of replacing fish meal by another source of protein such as animal by-product or plant-based protein in diets that can be fed to tilapia [7,8].

Animal by-products such as meat meal, bone meal and poultry by-product meal have considerable potential as feed ingredients in fish production system [9-12] and comparatively less expensive than fish meal [13]. These animal protein ingredients are good sources of amino acids with high protein content, total digestible dry matter and energy similar to fish meal [9]. Therefore, poultry by-product meal is considered a probable replacement for fish meal [14-21]. Many studies have also shown that animal protein ingredients can be useful for fish feed formulation and they are comparatively much less expensive than fish meal [13,22,23].

Some studies have shown that poultry by-product meal cannot replace more than 50% of fish meal in fish diets [24], but other studies have shown that with the recent improvement of the quality of poultry by-product meal it could replace 75% or 100% of fish meal without significant decrease in fish growth [25-28].

The present study aims to evaluate the effects of use poultry by-product meal as a alternative protein source to replace fish meal on growth performance, nutrient utilization, digestibility coefficient and some blood constituents of Nile tilapia (*Oreochromis niloticus*) reared in cement tanks.

Materials and Methods

Fish culture and experimental diets

The present study was conducted using the research facilities of the experimental station at Shakshouk, Fayoum Governorate, National Institute of Oceanography and Fisheries (NIOF). The system contained two water pumps and upstream sandy filter units at a point between the water source and tanks. Each pump was drowning the water from the lake Qaroun to collection cement pond and forced it through storage units and then to the rearing tanks in open system. Physicochemical characteristics of water tanks were examined every week, (Table 1) according to APHA [29].

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Diets				
Parameters	**PBM0**	**PBM50**	**PBM75**	**PBM100**
Temperature °C	27.0 ± 0.12	27.1 ± 0.12	27.4 ± 0.1	27.1 ± 0.15
pH	7.6 ± 0.1	7.8 ± 0.12	7.8 ± 0.11	7.6 ± 0.1
Dissolved oxygen (mg l^{-1})	6.3 ± 0.11	6.2 ± 0.12	6.2 ± 0.11	6.1 ± 0.12
Salinity (g l^{-1})	2.22 ± 0.1	2.23 ± 0.1	2.22 ± 0.1	2.22 ± 0.1
Unionized ammonia (mg l^{-1})	0.03 ± 0.01	0.028 ± 0.01	0.026 ± 0.002	0.028 ± 0.001

Table 1: Averages of water physicochemical characteristics parameters during experimental period.

Ingredientes	DM	CP	EE	NFE	CF	ASH
Fish meal	916	700	128	-	-	172
Poultry by-product meal	921	560	134	128	24	154
Soybean meal	915	480	44	368	42	66
Gluten meal	906	350	48	532	22	48
Wheat bran	904	144	34	666	92	68
Yellow maize	896	90	24	760	72	54

DM: Dry Matter, CP: Crude Protein, EE: Ether Extract, NFE: Nitrogen Free Extract, CF: Crude Fiber.

Table 2: Proximate composition of feed ingredients (g kg^{-1} d.m) n=3.

The fry of Nile tilapia (*Oreochromis niloticus*) used in the present study were obtained after brood stock hatching in the station. The fry were acclimatized for two weeks in rearing tanks and fed on prepared powder diet contain 30% crude protein, formulated from the same ingredients use in the growth trial. Juvenile tilapia with an initial average weight of 1.5 ± 0.05 g were randomly distributed and stocked at 100 juvenile per tank in 12 cement tanks, each with a water volume of (2 m^3) and the treatments were performed in triplicates. The diets were given at 3% of live body weight (BW) and offered in two equal portions at 10.00 a.m and 16.00 p.m. The experiment lasted 120 days after start.

Four isonitrogenous diets were formulated to contain an average of 30.11 ± 0.07% crude protein for meeting the recommended nutritional requirements of tilapia [30]. The ingredients were obtained from Zoocontrol fish Co at 6 October city, Egypt. Ingredients, diets formulation and chemical composition analysis are presented in Tables 2 and 3. The first diet was formulated without poultry by-product meal and considered as a control diet (PM0), Diets 2 (PM50), 3 (PM75) and 4 (PM100) were formulated to be comprised with partial and total inclusion levels of 50, 75 and 100% poultry by-product meal, respectively. All diets were processed into dry sinking pellet form, using California pelleting machine with 1 mm diameter.

Apparent digestibility coefficient

The experimental test diets with addition of 0.5% chromic oxide (Cr_2O_3) were fed to fishes after the growth trial lasted for a period of two week in order to study the apparent digestibility coefficient (ADC %) of nutrients. Any uneaten and fecal residues were siphoned out from the tank bottom after two hours of first feeding (10.00 a.m.) and discarded. Fish fecal samples were collected every afternoon before the second feeding, new fecal materials were carefully siphoned and collected using the filtration system developed by Choubert et al. [31]. After freeze-drying of 20 g fecal samples in each replicate tank, the feces were analyzed. Dry matter was calculated by gravimetric analysis at 105°C for 24 hrs. Chromic oxide levels were determined spectrometry (Spectra, AA220FSNarin) based on the method described by Bolin et al. [32].

The apparent digestibility coefficients ADC for test diets were calculated according to the equation described by Cho [33].

$$ADC\ (n)=100-\{100\ (\%Cr_2O_3d)/\%Cr_2O_3f) \times (\%\ Nf/\%\ Nd)\}.$$

Where ADC (n)=apparent digestibility coefficients of a nutrient in the test diets; Cr_2O_3d=% chromic oxide of the diet; Cr_2O_3f=% chromic oxide of the feces; *N*d=nutrient in the test diet; *N*f=nutrients in feces.

Chemical analysis

The chemical composition of the experimental diets, feces and whole fish samples was performed via proximate composition analysis according to standard methods [34]. Briefly dry matter was determined gravimetrically in an oven dried samples at 105°C for 24h, protein (N×6.25) content was determined using Kjeldhal method and crude fat by chloroform-methanol extraction (2:1) using Soxhlet system. Ash was determined by incinerating samples in a Germany muffle furnace at 550°C for 18h. Nitrogen free extract (NFE) was calculated by the difference.

Diets samples were hydrolyzed in 6 N (HCL) at 106°C over 24 h in nitrogen-flushed glass vials before amino acid analysis. Total amino acids were analysed by high pressure liquid chromatography (HPLC) in a Pico-Tag amino acid analysis system (Water, Bedford, MA, (USA), using norleucine as internal standard and according to the procedure described by Cohen et al. [35].

Gross energy (MJ Kg^{-1} diet) was calculated according to Schulz et al. [36] using the following calorific values: 23.9, 39.8 and 17.6 MJ g^{-1} diet for protein, ether extract and nitrogen free extract, respectively. The metabolizable energy contents of the experimental diets were calculated as 18.9, 35.7 and 14.7 MJ g^{-1} diet for protein, lipid and nitrogen free extract, respectively according to Jobling [37].

Diets				
Ingredients	**PBM0**	**PBM50**	**PBM75**	**PBM100**
Fish meal	200	100	50	-
Poultry by-product meal	-	150	200	260
Soybean meal	150	130	150	150
Corn gluten meal	110	100	100	100
Wheat brain	200	200	200	2100
Yellow corn maize	260	240	220	200
Fish oil	30	30	30	30
Sunflower oil	30	30	30	30
Vitamin and mineral mix[1]	15	15	15	15
Chromic oxide	5	5	5	5
Proximate analysis (*n=3*)				
Dry matter	928	922	924	926
Crude protein	301.9	301	301.8	300
Ether extract	110.4	115.9	116.6	118.8
Nitrogen free extract	453.9	451.3	449.2	447.8
Crude fiber	45.8	46.8	50.4	49.4
Crude ash	88	85	82	84
Gross energy (MJ kg^{-1} diet)[2]	19.58	19.74	19.75	19.77
ME (MJ kg^{-1}diet)[3]	16.31	16.44	16.46	16.49

[1]Vitamin-mineral premix, mg Kg^{-1} dry diets: vitamin A (as acetate), 7500 Iu kg^{-1} dry diet, Vitamin D3 (as cholecalcipherol); 6000IU kg^{-1} dry diet, vitamine E (as DL-L-tocopheryl-acetate); 150 IU kg^{-1} dry diet, vitamin k (as menadione Na-bisulphate); 0.06 ascorbic acid (as ascorbyle polyphosphate), 150 D-biotin, 42 choline (as chloride) 3000; folic acid, 3 niacin (as nicotinic acid), 30 pantothenic acid, 60 pyridoxine, 15; ribflavine, 0.06; manganese sulphate, 0.18; potassium iodide, 0.02 zinc sulphate.

[2] Schulz et al. [36].

[3]Jobling [37].

Table 3: Formulation and approximate composition of experimental diets (g kg^{-1}).

Growth performance and feed utilization

Standard formulae were used to assess growth-feed utilization and other relevant parameters during the growth trial and these included, initial average weight, final average weight, total feed consumed, weight gain (g), average daily gain (g fish day^{-1}), specific growth rate (SGR% day^{-1}), feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU%).

Blood assays

Blood samples were collected using heparinized syringes from the caudal vein of the experimental fish at the end of the growth trial. Blood samples were centrifuged at 3000 rpm ×15 min at 4°C to allow separation of plasma which use to determine the blood parameters. Total plasma protein were carried out using Colorimeteric method, (Roch Diagnostics, GmbH, Monnheim, Germany) as recorded by Ruane et al. [38]. Creatinine was determined according to Pincus [39]. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were carried out using Colorimeteric method, Roch Diagnostics, GmbH, Monnheim, Germany kits according to Reitman and Frankel [40].

Statistical analysis

One way Analysis of Variance (ANOVA) was applied to test the effects of partially and totally replacement of poultry-by product meal on various growth parameters, chemical composition, blood constituents and apparent digestibility coefficients according to Snedecore and Cochran [41]. Duncan Multiple Range test was used to detect the significant differences between the means of treatments [42]. All analysis were performed using SAS (version 9.1 2004 SAS Institute, Cary, NC, USA) [43]. The level of significance was chosen at $p \leq 0.05$ and the results are presented as a group means (n=3 per tanks in each treatments ±S.E.M.).

Results

Physicochemical characteristics

Water physicochemical characteristics (Table 1) revealed that temperature, pH, dissolved oxygen, salinity and unionized ammonia are within the optimum ranges for rearing Nile tilapia according to Wangead et al., El-Shafai et al. and Ferreira et al. [44-46]. Similar physicochemical conditions were found in all tanks.

Chemical composition of diets

As can be seen in Table 3, the four experimental diets were almost similar in protein content (30.0-30.19%) and gross energy (19.58-19.77 MJ kg^{-1} diets). However, they differed in their amino acids contents (Table 4). The all essential amino acids met the requirements of this species as recommended by NRC.

Growth performance

As presented in Table 5 averages of initial weights ranged between 1.5 to 1.6 g/fish with insignificant differences among the dietary groups indicating the random distribution of the experimental fish among treatment groups. Concerning finial weights the fish fed the tested treatments recorded an insignificant values ($P<0.05$) between each other. The same trend was observed with total gain in weight, the daily gain and specific growth rate of fish fed the different inclusion levels (50, 75 and 100% PM) of poultry by-product meal.

As shown in Table 5 average amounts of feed intake were found to be 70.0, 71.0, 71.0 and 71.0 g for the PBMO, PBM50, PBM75 and PBM100% groups, respectively. On the other hand, the best FCR (lowest) values were obtained by the PBMO group without significant differences ($P<0.05$) were registered among treatments. As presented in the same table the PER and NPU values showed an insignificant values between the different groups.

Diets					
Amino acids	**RE***	**PBM0**	**PBM50**	**PBM75**	**PBM100**
Essential amino acids					
Arginine	11.8	16.8	17.4	18.2	18.3
Hisitdine	8.4	9.2	8.8	8.9	9.1
Lysine	14.3	14.6	14.8	14.5	14.8
Leucine	9.5	20.4	21.6	22	22.4
Isoleucine	8.7	10.8	11.3	11.7	12
Valine	7.8	13.8	14.8	15.3	15.8
Methionine +Cystine	9	9.8	9.6	10.4	12.1
Threonine	10.5	10.3	11.1	11.6	11.9
Phenyl alinine+Tyrosine	15.5	20.7	20.4	24.9	24.5
Tryptophan	2.8	2.9	3.1	3	3.1
Non essential amino acids					
Glutamic acid	-	43	42.9	43.8	43.9
Aspartic acid	-	24.3	24.2	24.9	24.5
Glycine	-	18.8	19.2	18.9	17.9
Serine	-	11.7	15	16.4	17.7
Proline	-	18.4	20.4	21.1	21.8

*Requirements according to, NRC (2011).

Table 4: Amino acids contents of the experimental diets (g kg^{-1}) n=3.

Diets				
Parameters	**PBM0**	**PBM50**	**PBM75**	**PBM100**
Initial average weight (g fish^{-1})	1.5 ± 0.05	1.6 ± 0.06	1.6 ± 0.05	1.5 ± 0.06
Final average weight (g fish^{-1})	54.6 ± 2.5	53.8 ± 1.8	54.1 ± 2.0	54.3 ± 1.5
Gain in weight (g fish^{-1})	53.1^{a} ± 1.1	52.2^{a} ± 1.4	52.5^{a} ± 1.5	52.8^{a} ± 1.6
Average daily gain (g fish day^{-1})	0.44 ± 0.15	0.43 ± 0.2	0.43 ± 0.3	0.44 ± 0.1
Specific growth rate (% day^{-1})[1]	3.0 ± 0.5	2.92 ± 0.4	2.93 ± 0.2	2.99 ± 0.3
Feed consumed (g fish^{-1})	70.0 ± 3.0	71.0 ± 2.0	71.0 ± 4.0	71.0 ± 3.0
Feed conversion ratio[2]	1.31 ± 0.15	1.36 ± 0.2	1.35 ± 0.2	1.34 ± 0.1
Protein efficiency ratio[3]	2.51 ± 0.2	2.44 ± 0.15	2.45 ± 0.18	2.46 ± 0.16
Net Protein Utilization (NPU%)[4]	39.75 ± 4.0	37.10 ± 3.0	37.98 ± 3.0	38.51 ± 4.0

Values are the mean ± S.E. of triplicate groups of each treatment.

[1]Specific growth rate = 100 × (Ln final weight- Ln initial weight)/ 120 day.

[2]Feed conversion= (feed given per fish)/ (weight gain per fish).

[3]Protein efficiency ratio = (weight gain per fish)/ (protein intake per fish).

[4]Net protein utilization (%) = (final body protein - initial body protein)/(protein intake) ×100.

Table 5: Growth performance of Nile tilapia fed the experimental diets.

Apparent digestibility coefficient

Data on apparent digestibility coefficients in present study for dry matter (DM), energy (E), crude protein (CP), fat and nitrogen free extract (NFE) are presented in Table 6. Results revealed that apparent digestibility coefficients DM, E, CP, fat and NFE were not significantly affected with the inclusion levels of poultry by-product meal.

Blood characteristics

Blood characteristics revealed that insignificant differences

Diets				
Nutrients	**PBM0**	**PBM50**	**PBM75**	**PBM100**
Dry matter	85.8 ± 3.0	85.5 ± 2.0	85.6 ± 1.0	85.4 ± 2.0
Energy	90.4 ± 4.0	89.8 ± 2.0	89.6 ± 3.0	90.2 ± 1.0
Protein	94.4 ± 3.0	94.5 ± 4.0	94.3 ± 2.0	94.6 ± 3.0
Fat	95.5 ± 2.0	95.3 ± 2.0	95.4 ± 3.0	95.6 ± 3.0
Nitrogen free extract	71.8 ± 1.0	71.9 ± 3.0	71.6 ± 2.0	71.5 ± 4.0

Table 6: Apparent digestibility coefficients (%) for the experimental diets (Mean ± S.E *n=3*).

Diets				
Parameters	**PBM0**	**PBM50**	**PBM75**	**PBM100**
Total protein g/dl	6.4 ± 1.11	6.4 ± 0.8	6.42 ± 0.9	6.3 ± 0.6
Albumin g/dl	3.22 ± 0.33	3.24 ± 0.22	3.3 ± 0.32	3.26 ± 0.2
Urea mg/dl	7.2 ± 0.14	7.13 ± 0.12	7.3 ± 0.16	7.5 ± 0.15
Creatinine	1.12 ± 0.3	1.14 ± 0.34	1.16 ± 0.2	1.18 ± 0.26
Ast[1]	116.0 ± 0.65	117.0 ± 0.48	124.0 ± 0.56	126.0 ± 0.52
Alt[2]	44.0 ± 0.41	44.0 ± 0.32	45.0 ± 0.22	44.5 ± 0.38

Values are the mean ± S.E. of triplicate groups of 20 fishes

1-Aspartate aminotransferase 2-Alanine aminotransferase

Table 7: An average of blood characteristics parameters of tilapia fed the experimental diets.

Diets					
Items	**Initial**	**PBM0**	**PBM50**	**PBM75**	**PBM100**
Dry matter	242 ± 1.6	271 ± 3.18	270 ± 3.6	276 ± 3.14	274 ± 1.16
Crude protein	152 ± 1.8	158 ± 2.16	152 ± 2.2	156 ± 2.2	154 ± 2.11
Crude lipid	32 ± 1.5	55 ± 1.8	62 ± 1.6	61 ± 1.4	58 ± 1.16
Crude ash	58 ± 1.2	58 ± 1.4	56 ± 1.6	59 ± 1.2	54 ± 1. 6

Table 8: Carcass analysis of Nile tilapia fed the experimental diets (g kg^{-1} wet basis) mean ± S.E. n=3.

(P<0.05) were detected between the experimental groups (Table 7) for total protein, albumin, creatinine, Aspartate aminotransferase (Ast) and Alanine aminotransferase (Alt) of tilapia, which indicate that substitution of fish meal by poultry by-product had no hazardous effects on blood parameters tested.

Carcass analysis

Results of whole fish body composition in terms of wet weight, were not significantly affected (P<0.05) by the increment of PBM levels. The chemical analysis showed that the applied dietary treatments had no significant effects (P<0.05) on whole body dry mater, crude protein, fat and ash content between groups (Table 8).

Discussion

The replacement of dietary FM in aqua feeds with readily available and more economical alternatives sources, such as poultry by-product meal is an important aim for each aquaculture industry and feed-manufacture company. This by-product of poultry processing industry is high in protein, low price and contains a favorable profile of indispensable amino acids for fish production.

The results of the present study indicated that PBM is a suitable replacement of fish meal in practical formulation diets for juvenile tilapia.

The growth performance (final weight gain, daily gain and specific growth rate) and nutrient utilization (feed conversion ratio, protein efficiency ratio and net protein utilization) of tilapia has shown enhancement for dietary PBM without significant reduction when the replacement level of fish meal up to 100%. These findings are in agreement with other studies in tilapia [47]. However, PBM can be used to replace 75% of the fish meal in diets without amino acid supplementation for gilthead sea bream [26] up to 100%, red sea bream [27], sunshine bass [48]; grouper [49] and gible carp [50]. In contrast, fish meal could only be replaced with PBM at a level which did not exceed 50% for some marine fish species [51-53]. In the present study the growth performance and feed utilization recorded comparable results with fish meal diets. In contrast, Rawles et al. [16], recorded that PBM had lower growth than fish fed the control diet with FM in sunshine bass. They attributed that imbalance of some limiting amino acid content may have caused reduced growth performance in sunshine bass. They also speculated that reduced growth observed in this species may be due to reduced palatability for PBM. However, in the current study the balance in limiting amino acid, all diets were consumed similarly and different species may be enhance the growth performance in juvenile tilapia.

As can be seen from Table 5, the FCR, PER and NPU were not significantly differed (P<0.05) between groups and the best value was recorded by PBM0. Similar and comparable results of FCR, PER and NPU were recorded with tilapia [47,54,55].

Amongst the experimental diets in the present trial, fish receiving the PM0 and PM100 diets showed high growth performance and net protein utilization. This result is in agreement with the other several warm water finfish species obtained by Hernandez et al. and Pine et al. [47,56]. They recorded that poultry by-product has been shown to enhanced growth performance of Nile tilapia and sunshine bass.

The major reasons for different results may be due to the different fish species and the varying quality of tested PBM, which are significantly influenced by their processing methods [49].

The experimental diets in the present study showed a good digestibility coefficient for the tested diets. These results are in agreement with the results of Hernandez et al. [47]. Similar and comparable ADC values of feed dry matter, protein, lipid and energy were also observed by several authors in digestibility studies with tilapia fed the conventional commercial ingredient [55,57-60]. Similar results were recorded in rainbow trout by Burea et al. [9,23].

The carcass proximate composition of tilapia indicated that the dry matter, protein, lipid and ash were not affected by incorporation of 100% poultry by-product meal. Similar results have been reported for tilapia by Hernandez et al. [47] and sunshine bass [56], grouper [53] and gibel carp [50].

Measures of blood parameters not significantly different between treatments, where the total protein, albumin, urea, creatinine Ast and Alt are comparable with the control diet. These results are in agreement with the previous study in tilapia recorded by Metwalli, Yue et al. [61,62] and other species such as sturgeon [63] and sunshine bass [48].

Results of the present study suggest that potential replacing 100% of fish meal with poultry by-product meal in the feed of tilapia *Oreochromis niloticcus*, without compromising growth performance, nutrients utilization and some blood contents. It is important to establish that alternative dietary sources to fish meal are not only supplied in the correct quantities and balance for optimal growth and feed efficiency, but can maintain optimal whole body composition and blood contents.

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