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Effects of Replacing Protein of Fishmeal with Protein of Poultry By-product Meal on Growth Performance, Body Composition, Liver Histological Changes and Selected Serum Parameters of Nile tilapia

Rania E. Mahmoud[1*], Hossam Gadallah[2], Ola A. Orma[1]

[1]Department of Nutrition and Nutritional Deficiency Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt.

[2]Department of Clinical Pathology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt.

***Correspondence**
Corresponding author: Rania E. Mahmoud
E-mail address: ranianutrition@yahoo.com

Abstract

This research evaluated the viability of completely and partially replacing the protein of fishmeal (FM) with the protein of poultry by-product meal (PBM) for juvenile Nile tilapia fish. One hundred and eighty unsexed Nile tilapia fish have been randomly assigned to four treatment groups, with three replicates including 15 Nile tilapia fish in each replicate stocked in 12 glass aquaria for nine weeks. PBM incorporation levels in the diet at 50% (G2), 75% (G3), and 100% (G4) protein content of fish meal substituted for the fish meal-based control diet have been compared (100% fish meal, G1). The final body weight (FBW), body weight gain (BWG), specific growth rate (SGR), and protein efficiency ratio (PER) varied considerably across all treatment groups ($p > 0.05$). FBW, BWG, SGR, and PER were improved for fish fed PBM and were superior in the high level of PBM (G4). However, the morphometric indexes showed no significant differences ($p > 0.05$). Fat & ash % of body composition was higher in all groups containing PBM in particular (G4) as compared with the control one. In conclusion, it can be said that a full replacement PBM protein for FM protein is tolerable in Nile tilapia fish. Several changes in biochemical parameters and antioxidant status markers were observed. The histological exam of the liver in all groups that contained PBM revealed no abnormalities. However, replacing 75% of the protein of FM with PBM's protein is the optimal degree in term of maintaining meat quality and some of the blood parameters.

KEYWORDS
Poultry by product meal, Growth, Liver histology, Serum metabolites, Tilapia fish.

INTRODUCTION

The most vital component in formulated feed is protein representing more than half of the feed's cost (EL-Sayed, 2006). Fishmeal is considered the safest and nutritionally balanced protein supply among all ingredients in the feed because it provides excellent nutritional qualities, excellent profile of amino acid, and other necessary ingredients that may be required to support the nutritional needs of cultivated organisms (Zhu *et al*., 2011). Fishmeal must be substituted with less expensive protein sources due to their high cost and irregular availability (Nathaly *et al*., 2018). The alternative of fishmeal with a local to-be-had and less expensive derivative has proved to be very vital for the region's aquaculture development in the future (Tacon *et al*., 2006).

As stated by Gatlin *et al*. (2007), an ingredient should have the following characteristics to effectively replace fishmeal in aquaculture feeds: a competitive price, full accessibility and simplicity of handling during transportation, preserving, and use in the manufacturing process of feed. Additionally, it must have great nutritional digestibility, a high level of protein, a balanced distribution of amino acids, low starch, non-soluble carbohydrate content, & low fiber content.

Fish diets contain alternative feeds to lower the cost of animal feed and encourage local socioeconomic sustainability (Bicudo *et al*., 2018). The poultry by-product meal, which is a mixture of underdeveloped eggs, necks, heads, toes, gizzards, and intestines from slaughtered hens, is an ideal choice to replace FM in aqua diets (Gümüs and Aydin 2013).

According to Cruz-Suárez *et al*. (2007), a high-quality poultry by-product meal has a higher CP content (58%–65%) and DCP value (87%–91%) as well as important fatty acids, vitamins, minerals, a balanced amino acid profile, and palatability. Poultry by-product meal is one of the most appealing possible feed ingredients for fishmeal in aqua diets because of these qualities. Good Nile tilapia diets today replace fishmeal with poultry by-product meals (Palupi *et al*., 2020).

Compared to fishmeal, animal protein sources are significantly less expensive, provide more protein, are easily obtainable, and free of anti-nutritional components (EL-Sayed, 1999). Consequently, this study was conducted to determine how replacing dietary protein from the fishmeal with protein from poultry by-product meal would affect Nile tilapia fish performance, whole-body chemical composition, liver histopathological alterations, and blood serum analysis.

MATERIALS AND METHODS

Ethical Approval

The Animal Care and Use Committee (MU-ACUC) of Mansou-

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ra University has approved this experimental protocol with the code number (R/100).

Fish, diets, and housing

A total of 180 Nile tilapia with 11 g initial weight were obtained from Kafr Elsheihk, Egypt and kept in 12 glass aquariums, each having a capacity for 15 fish and measuring 80 cm long, 35 cm wide, and 40 cm high. For nine weeks, every diet was given twice daily (from 8:00 to 2:00 hours) to the fish in triplicate at a rate of 4% biomass. The photoperiod regimen for the fish was 12–13 hours of light and 12–11 hours of darkness each day and during the study period, the temperature varied between 24 and 27°C. Every day, dechlorinated tap water (stored for 48 hours) was used to partially replenish the water in each tank as part of the cleaning process. Four isonitrogenous (32%) and isocaloric (3000 Kcal DE/kg) diets have been created to satisfy the nutritional demands of Nile tilapia (O. niloticus) depending on NRC, (2011). For the experimental diets, FM protein was substituted by PBM protein as shown: (G1) basal diet = 100% protein of fishmeal, (G2) replacing 50% from the protein of FM with the protein of PBM. (G3) Replacing 75% of the protein of FM with the protein of PBM. (G4) Replacing 100% of the protein of FM with the protein of PBM. Table 1 contains the ingredients and proximate analyses of the experimental diets. All diets contained 32% protein and 3000 kcal /kg from DE.

Table 1. Ingredients (%) and nutrient composition of experimental diets.

Ingredients	Experimental diets			
	Control	Diet 1	Diet 2	Diet 3
Yellow corn	17.08	15.8	15.5	15
Soybean meal	34.5	35.5	36.14	34.5
Fish meal*	12	5.2	1.8	0
PBM**	0	6.8	10.2	13.6
Corn gluten	3.5	3.5	3.5	3.5
Gelatin	1.5	1.5	1.5	1.5
Oil	0.5	0.75	0.5	0.5
Wheat bran	28.49	28.47	29	29.64
Min., Vit. premixes***	1	1	1	1
Salt	0.3	0.3	0.3	0.3
Vitamin C	0.1	0.1	0.1	0.1
Antioxidant	0.02	0.02	0.02	0.02
Dicaph	0.8	0.8	0.15	0.05
Methionine	0.21	0.26	0.29	0.29
Calculated DE (kcal/kg	3001	3001	3001	3001
Calculated CP%	32.18	32.1	32.1	32.1

*Protein content of FM = 64.5% & ** Protein content of PBM= 57% & ***Trace minerals & vitamins premixes were prepared to cover the levels of the microminerals & vitamins for tilapia fish as recommended by (NRC,1993). Vitamins premix (IU or mg/kg diet); Vit. A 5000, Vit. D3 1000, Vit. E 20, Vit. k3 2, Vit. B1 2, Vit. B2 5, Vit. B6 1.5, Vit. B12 0.02, Pantothenic acid10, Folic acid 1, Biotin 0.15, Niacid 30. Mineral mixture (mg/kg diet); Fe 40, Mn 80, Cu 4, Zn 50, I 0.5, Co 0.2 & Se 0.2.

Growth parameter measurements

At the end of experiment, the weight gain, the daily growth rate, percentage increase in fish weight, the feed conversion ratio, and the survival rate were all calculated for each individual fish in each tank. Various performance parameters were measured utilizing the equations below:

Specific growth rate (SGR %/day) equal Loge final weight (g) – Loge initial weight (g)×100)/t (experimental period in days)

Protein efficiency ratio (PER)= Weight gain (g)/protein intake (g)

Survival rate (%)= Initial fish number– mortality/initial number of fish × 100

Condition factor (K)= (W/L3) × 100

W= weight of fish in grams & L= length of fish body in cm.

Sample collection

The fish samples were taken at the end of the study (9 weeks) and then euthanized by using 100 mg/L of tricaine methane sulfonate (MS.-222). Four fish per tank, twelve fish in each group, blood samples were obtained and collected via caudal venipuncture. Blood samples were centrifuged at 3,000 rpm for 10 min. Serum samples were maintained in vials for further examination at 20°C. Fish sample (4/aquarium were collected preserved in plastic bags and then stored in a deep freezer until preparing for whole-body chemical analysis.

Serum biochemical parameters

Using (Stanbio laboratory) USA kits, albumin (Alb) and total protein (TP) levels in the serum have been assessed (Doumas *et al*., 1972). As stated by (Human Company Germany), ready-made kits were used to measure creatinine (Cr) as described by Numann and Ziegenborn (1977). According to Reitman and Frankel (1957), utilizing kits from Roch Diagnostics, GmbH, Monheim, Germany, and the colorimetric technique was used to assess the aspartate aminotransferase (AST) and alanine aminotransferase activities (ALT).

Lipid peroxidation status assessment

Serum malonaldehyde concentration was measured spectrophotometrically (BM Co. Germany, Photometer 5010, Photometer) (Biodiagnostic, Egypt).

Morphometric indexes

Tricaine methane sulfonate was used to anesthetize the selected fish. Both the fish's weight and length were determined. Then dissected; the viscera were removed to determine the entire carcass yield, and the percentages of visceral fat, and liver somatic index, respectively.

Hepatosomatic index HSI= Liver weight/whole body weight ×100

Splenosomatic index SSI= Spleen weight/ whole body weight ×100

Histopathological examination

At the finish of the trial, five fish from each replication and treatment were randomly selected. After collecting liver samples, they were cut at a thickness of 5 µm, embedded in paraffin, and fixed for 24 hours in a 10% neutral buffered formalin solution. Staining the segments was done with hematoxylin and eosin (Roberts, 2012).

Statistical analysis

Data were subjected to a one-way ANOVA to determine the impact of modifying the diet to substitute fishmeal protein with chicken by-product protein on the growth performance, serum parameters and whole body composition of Nile tilapia. Data were analyzed using statistical SPSS v20 (SPSS Inc, Chicago, Illinois) and expressed as mean ± SE differences between means

were compared using Duncan's multiple range test at the significance of differences (P< 0.05) among dietary treatments.

RESULTS

Growth performance & whole-body composition

Data concerning the growth performance of fish taken dietary treatment diets are displayed in Table 2. Our findings showed that fish fed with PBM had considerably higher FBW, BWG, FCR, and SGR values than those fed with a control diet (P ≤0.05), with the optimum value being at 100% PBM replacement. Additionally, PER increased considerably in fish replaced 75% from protein of FM by PBM & fish replaced FM by100% protein of PBM. Table 3 demonstrated the approximate composition of the whole body fish. The control and experimental groups weren't significantly different in terms of the crude protein and moisture content. However, fish-fed experimental diets containing PBM had considerably higher levels of ash & fat than fish fed on control diet.

Morphometric indexes

Morphometric indices of fish given experimental diets are presented in Table 4. Data obtained revealed that non-significant variability was discovered in K factor, HIS, and SSI among the experimental groups.

Blood serum characteristics and lipid peroxidation

Blood serum parameters revealed non-significant variations (P>0.05) between the experimental groups & control group (Table 5) for albumin & creatinine levels. While in the case of serum total protein all groups of fish that contained PBM in the diet had low levels, especially the group of fish that completely replaced the protein of fish meal with the protein of PBM. Moreover, the substitution of fish meal protein with PBM protein (75 and 100%) showed a significant elevation in ALT activity with a non-significant change in AST and ALT activities in comparison with the control group. Both groups of fish replacing 50% & 75% from

Table 2. Performance parameters of fish fed with the control diet or experimental diets.

Parameters	Level of substitution protein of FM by protein of PBM			
	Control	50%	100%	75%
IW (g)	11.72±0.48	11.89±0.59	11.03±0.41	11.11±0.42
FBW (g)	38.47±1.45 c	44.21±1.20 ab	40.83±2.61 b c	46.07±1.8 a
BWG (g)	26.99±1.66	32.32±1.2	29.8±2.4	34.95±1.9
FI (g)	44.00±0.50 c	51.58±0.71 a	44.66±0.45 c	48.27±0.63 b
FCR	1.84 ±0.11 a	1.64±0.06 a b	1.75 ±0.15 a b	1.5±0.08 b
PER	1.87±0.12 b	1.96±0.07 b	2.07±0.17 a b	2.43±0.13 a
SGR (%/ day)	1.95±0.08 b	2.20±0.08 a b	2.11±0.07 a b	2.29±0.09 a

Values with a different letter superscript in same row indicate significant difference between groups (< 0.05).

Table 3. Body composition of Nile tilapia (on a dry matter basis) fed control diet or experimental diets.

Items	Level of substitution protein of FM by protein of PBM			
	Control	50%	75%	100%
Moisture	7.61±0.48	7.2±0.55	7.83±0.57	7.58±0.7
CP	48.69±0.88 a	47.83±0.57 ab	46.89±0.78 ab	46.26±0.5 ab
Fat	8.15±0.55 b	9.91±0.57 ab	9.88±0.50 ab	10.23±0.48 a
Ash	8.59±0.46 b	9.16±0.5 ab	10.78±0.6 ab	11.09±0.57 a

Table 4. Morphometric indexes of fish fed control diet or experimental diets.

Parameters	Level of substitution protein of FM by protein of PBM			
	Control	50%	75%	100%
K factor	1.63±0.09	1.71±0.01	1.77±0.037	1.55±0.122
HSI	2.73±0.53	3.52±0.09	2.65±0.22	3.36±0.21
SSI	0.20±0.03	0.24±0.02	0.18±0.02	0.28±0.07

Data are displayed as the mean of ten fish±SEM.

Table 5. Serum biochemical analyses of fish fed with the control diet or experimental diets

Parameters	Level of substitution protein of FM by protein of PBM			
	Control	100%	75%	50%
Total protein (g/dl)	2.64±0.28 a	2.06±0.07 a b	2.21±0.17 a b	1.69±0.18 b
Albumin (g/dl)	1.40±0.11	1.13±0.03	1.36±0.14	1.06±0.17
Creatinine (mg/dl)	0.80±0.057	0.83±0.066	0.83±0.033	0.76±0.08
MDA (nmol/ml)	58.66±3.84 b	59.33±4.70 b	55.66±2.72 b	73.66±4.17 a
ALT (U/l)	23.00±1.5 b	24.66±2.4 b	50.66±2.18 a	52.00±5.19 a
AST (U/l)	56.66±3.28 a	41.66±2.33 a b	64.66±3.75 a	55.66±6.69 a

protein of FM by protein of PBM induce a non-significant change in MDA levels.

Histopathological findings

Figure 1 displays histological pictures of fish livers following various treatments. The liver of fish given a control diet (Fig. 1A, B and C) showed diffuse hepatic vacuolation with sinusoidal congestion and syncytial formation resulting from a fusion of numerous hepatic cells. Furthermore, diffuse hepatic necrosis with swollen, hypereosinophilic cells and pyknotic nuclei was also observed. Meanwhile, the group fed on experimental diets showed fewer lesions. In the G2 (Fig. 1D) the liver showed moderate hepatic vacuolation with minimal focal leukocytic aggregations. Although diffuse hepatic vacuolation with multifocal congestion was seen in G3 (Fig. 1E), minimal to mild hepatic vacuolation was detected in G4 (Fig. 1F).

DISCUSSION

The important information from this study includes both the replacement of FM protein in Nile tilapia fish diets and the potential use of PBM as an alternative ingredient. The present investigation discovered that the completely replacing protein of fish meal by the protein of PBM enhanced growth performance in the form of FBW, BWG, FCR, SGR, and PER. The improvement may also result from several factors including higher digestibility, palatability PBM incorporated diets, good quality of PBM, high levels of protein, high concentrations of energy, dry matter, and digestible protein which similar to fish meal, as explained by (Zhu *et al*., 2011; Sathishkumar *et al*., 2021), more favorable EAA balance than a fish meal alone, In addition to meeting the (NRC, 2011) requirements for Nile tilapia, it also has a balanced amino acid profile. Contrary to what we determined, (Felix *et al*., 2020) using of PBM in our experiment appears to be an ideal dietary source of protein for Nile tilapia fish, especially when it is completely replaced by the protein of fish meal and these results support those of (Yang *et al*., 2004), who found that PBM may substitute 150 or 500 g/Kg of fish meal protein in diets for gibel Carp without negatively affecting growth. With the same concept, (Ahmed *et al*., 2020) discovered that the performance of fish was enhanced in the fish fed PBM due to the excellent quality of protein of the PBM, particularly for the fish that ingested the greatest PBM in contrast to the control diet. Sawhney and Gandotra (2010) reported that (FCR) can determine the efficiency of fish and the biological value of protein in feed. As shown in our study, the group of fish that replaced the protein of fish meal by 50% and 100% with the protein of PBM was the better-supplemented level for feed conversion ratio & feed intake in comparison with the other groups. These findings agree with those of Ahmed *et al*. (2020), who claimed that a diet high in PBM exceeded the other treatments in terms of feed utilization and feed conversion ratio.

An essential factor for determining the protein quality and balance of amino acids is the (PER) (Hardy, 2000). The best SGR & PER noticed in the diets containing 100% protein of PBM. Our findings are consistent with Yones and Metwalli (2016) who discovered that juvenile Nile tilapia fish had improved PER & SGR for dietary PBM without a discernible decline when fish meal replacement levels reached 100%. The acceptable values of PER obtained for Nile tilapia fish-fed diets containing 100% protein of PBM are enabled by the non-traditional animal sources of protein that are used PBM which is rich in necessary amino acids.

In the current study, there were no detectable variations in the CP and moisture contents between the control and experimental groups. A fish-fed diet containing PBM at various concentrations showed significantly increased body fat and ash contents when compared to the control group. The current findings support (Mohamad and Javaheri's, 2015) who conclusion that body lipid enhanced with higher FM replacement with PBM in beluga sturgeon fish diets. It can be as a result of the great fat content of the poultry byproducts, skin, and viscera (Kalogeropoulos *et al*., 1992). The ash content of fish fed on diet contains 100% protein of PBM increased. The high ash content indicated the amount of inorganic component of the diets (Abdel-Warith *et al*., 2001).

The condition factor, HSI, and SSI values of the experimental diets did not differ significantly from one other. The highest condition factors recorded in fish fed diet contain 50 & 75% protein of PBM. Ayode (2011) stated that greater condition factors revealed good health with isometric growth, which is preferred for

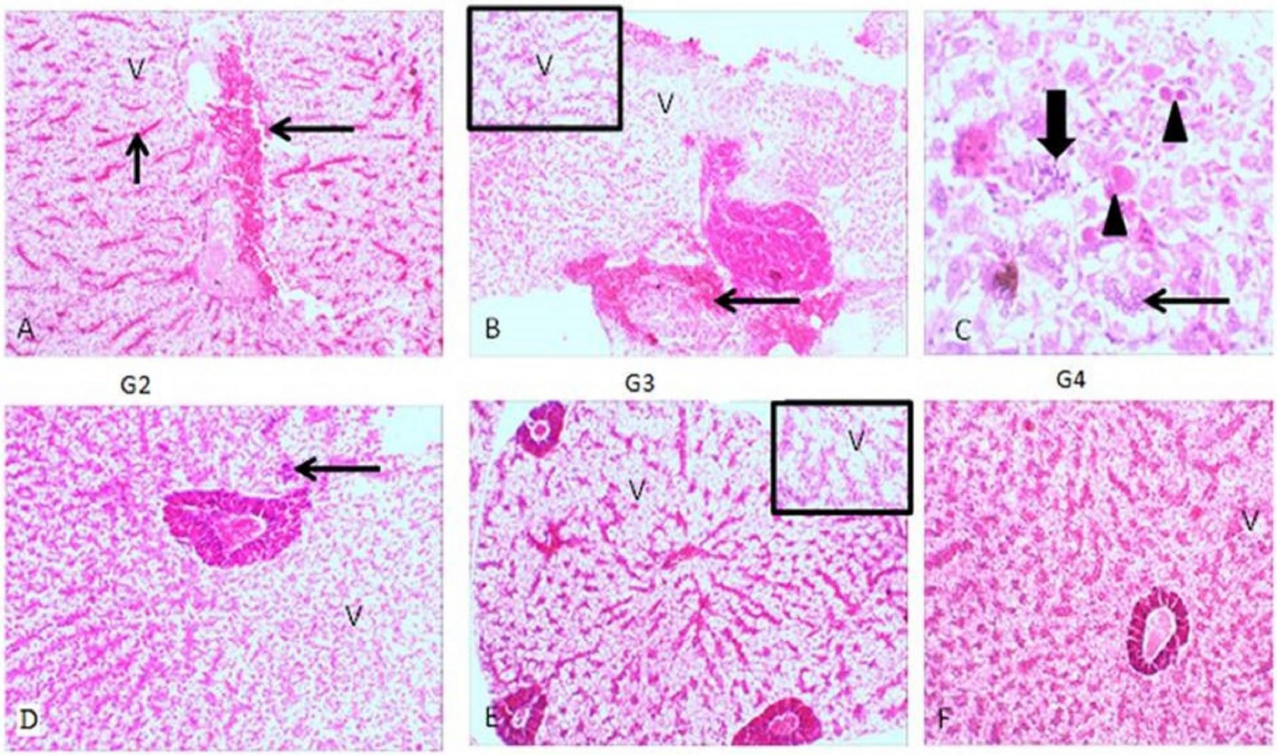

Fig. 1. Representative photomicrograph of Nile tilapia liver from different groups.

fish in farms.

Despite the improvement in growth performance, several changes in biochemical parameters, antioxidant status markers, and immune responses can be found either negatively or positively (Chaklader *et al*., 2020). In this investigation, the substitution protein of FM with protein of PBM (75 and 100%) resulted in a detectable rise in ALT activity with a non-significant change in AST and ALT activities in comparison with the control group that was also concomitant with the histopathological examination, which was also detected in a current research work by Chakladder *et al*. (2020) in juvenile barramundi using 100% replacement, (Chaklader *et al*., 2021) in juvenile barramundi using 70% PBM supplemented with defatted Hermetia illucens larval meal. In an experiment carried out by Panicz *et al*. (2017) on female tenches, there were non-significant changes in length, fillet weight, weight, liver, and gonadosomatic index with a significant appearance of lipidosis in the flesh of fish with 100% PBM substitution. In yellow catfish, the replacement of 30% of PBM showed a substantial elevation in ALT activity with normal AST activity (Luo *et al*., 2017). The excessive appearance of lipidosis with a high concentration of PBM might clarify the significant elevation of the levels of MDA in fish supplemented with 100% PBM which was found also to be elevated in yellow catfish during 40% and 60% replacement of PBM (Luo *et al*., 2017). The investigation of lipid peroxidation is important for the determination of the quality of meat (Sicuro *et al*., 2010), where high levels of lipid peroxidation indicate a great amount of polyunsaturated fatty acids in tissues of tilapia (Menoyo *et al*., 2002). In the present experiment, both groups of fish replacing 50% & 75% from protein of FM by protein of PBM induce a non-significant change in MDA levels which indicates an improvement in meat quality.

Dietary intake has a direct impact on the liver's function and morphology. Replacement of 100% protein of FM with protein of PBM resulted in minimal to mild hepatic vacuolation. Similarly, Sabbagh *et al*. (2019) observed that *S. aurata* fish's livers did not change when replaced FM with PBM at 100% of the original amount. Therefore, this total using the protein of PBM can be utilized effectively in the feeding of Nile tilapia fish.

CONCLUSION

According to the study's findings, Nile tilapia fish can accept the substitution of PBM for a FM up to 100% as a source of protein in their diets. PBM had a positive impact on the growth, body composition, and histological structure of the liver of Nile tilapia fish. The best level for maintaining the flesh quality & some blood parameters of Nile tilapia fish is to replace 75% of the FM protein with PBM protein.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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