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Original Article

Effect of fishmeal replacement with poultry by-product meal on serum parameters and histomorphology of liver and kidney in Nile tilapia ((*Oreochromis niloticus*), Linnaeus, 1758)

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Abstract

Today, *Oreochromis niloticus* has become one of the most popular fish in the world. Due to the increasing prices of fishmeal and its limited availability, various protein sources, including poultry by-product meals (PBM) can substitute fishmeal in the aquatic diet. This study was thus intended to investigate the effect of replacing fishmeal with different ratios of PBM on the liver, and kidney tissue structure changes, growth performance, and some serum parameters of *O. niloticus*. To that end, 120 *O. niloticus* were randomly distributed into four groups: the control, 25, 50, and 100% PBM meal instead of fishmeal which was fed for 44 days. At the end of the treatment period, growth parameters, blood serum, liver enzymes, and histomorphology of the liver and kidney of all fish were evaluated. The results showed that the liver enzymes increased significantly in the groups with higher replacement (100% PBM) compared to the control group; and in the histological examinations of the liver, the liver tissue lost its normal structure and function with the increase in the amount of replacement diets and there were fat vacuoles accumulated in the cytoplasm of hepatocytes. The level of urea plasma also showed a significant difference with the increase in the amount of substitute diets among the groups with upward substitution compared to the control group. These changes were evident in the structure of tubules and glomeruli. Data suggests that 100% PBM meal is not recommended for fishmeal substitution in *O. niloticus* but PBMs up to 50% can replace fishmeal for *O. niloticus* diet without adversely affecting the growth performance and biochemical parameters of the fish.

Keywords: Fishmeal, Growth performance, Histomorphometric changes, *Oreochromis niloticus*, Poultry by-product (PBM)

Introduction

Aquaculture and fisheries play important roles in providing food and income for hundreds of millions of people around the world (FAO, 2018). *Oreochromis niloticus* (Linnaeus, 1758) belonging to the Cichlidae family, in the order Perciformes, is one of the most species-rich families of fish, particularly in tropical freshwater fisheries

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(Metwalli, 2013; Rahmati et al, 2022). It is also the second most farmed fish worldwide and its production has multiplied over the past decade because of its suitability for aquaculture, marketability and stable market prices. Given its reasonable price and high meat quality, *O. niloticus* can play a key role in providing the animal protein needed by the rapidly growing global population, especially low-income people in many developing countries. Among the important species of *O. niloticus* in the world, the culture of *O. niloticus* is now under practice almost everywhere (FAO, 2018). However, fish feed and nutrition management in aquaculture has been always one of the main challenges of breeders. Fishmeal is a commercial nutrient-rich feed ingredient that is mostly made from fish and is usually used to feed farm animals in agricultural settings (Zhou et al, 2020). Given the increasing prices of fishmeal and its limited availability (Irm et al, 2020), various food items have been thus considered to find alternative protein sources in diets for farmed *O. niloticus*, including sources of animal protein such as fish by-products, pet by-products such as feather powder, blood powder, bone meal, and PBM, as well as vegetable protein such as soy powder, sesame, and canola, etc (Ogunji, 2004; Panserat et al, 2009). Poultry by-product meal (PBM) is a by-product of the poultry processing industry that is made from waste parts of the poultry body such as blood, legs, head, and internal organs (Yu, 2023). Replacing fishmeal with other alternative protein sources such as PBM in aquafeed has been regarded as a way toward more sustainable development of universal aquaculture, given the high content of protein, amino acid, and phosphorus in PBM diets (Irm et al, 2020). Qiu et al (2023) reported that a diet containing PBM could well provide adequate protein for Chinese soft-shelled turtles. Likewise, the results of Irm et al (2020) showed that PBM can partially substitute the fishmeal without leaving any negative impact on growth performance and feed utilization of juvenile black sea bream. The fish liver and kidney are important target limbs that are specifically affected by dietary as well as biological and environmental parameters (Zhou et al, 2020). Nutritional imbalances in dietary components can indeed result in changes in the structure, metabolism, and morphology of fish liver and kidney (Roberts, 2003). Nutritional compounds can also have significant effects on the composition of blood parameters, making it possible to evaluate the effects of different food treatments on the fish body by measuring serum parameters, and histomorphology of the liver and kidney in fish (Nogales-Mérida et al, 2011).

Many studies have been done on different types of alternative foods in aquaculture. However, no studies have been reported on histomorphometric liver and kidney following consumption of PBM. Therefore, the current research is designed to investigate the effects of replacing feeding with PBM instead of fish meal in tilapia fish and its effects on the histology of the liver, kidney, and some blood parameters in farmed *O. niloticus* as an indicator of fish health.

Materials and Methods

Diets and experimental design

The studied fish were randomly divided into 4 groups each with 30 fish (Department of Aquatic Health and Diseases, at Shahid Chamran University of Ahvaz) (i.e., 10 fish in 3 repetitions for each group). In doing so, three treatment groups received diets containing approximately 40% protein, while the fish in the control group were fed a daily commercial diet containing 40% protein (Purely fishmeal) (PBM0). More precisely, the fish in the first treatment group received 25% PBM instead of fishmeal, and the second treatment group received 50% PBM rather than fishmeal, while the third group was, in turn, fed 100% PBM instead of fishmeal. The composition of the experimental diets (based on the dry matter) was measured in one liter (weight) (Table. 1), and the fish diets were adjusted based on their standard nutritional needs so that all groups had the same diet in terms of all nutrients. Simultaneously with the addition of PBM to the groups' diets, two essential amino acids (namely, lysine and methionine) were also added to the diets for these groups so that they were similar in terms of amino acid levels (Zhou et al, 2020).

Table 1: Formulation and analyzed composition in different groups (dry-matter basis)

Ingredients	Dietary treatments			
	PBM0	PBM25	PBM50	PBM100
Ingredient Replacement of FM CP (%)	0.0	25	50	100
Fish meal (% dry matter)	52.5	39.4	24.2	0.0
Poultry by-product meal (PBM) (% dry matter)	0.0	13.7	25.9	52.8
Mineral premix (g. kg^{-1}) *	2	2	2	2
vitamin premix (g. kg^{-1}) **	2	2	2	2
Antifungal (g. kg^{-1})	0.1	0.1	0.1	0.1
Salt (g. kg^{-1})	5	5	5	5
Carbohydrates (%)	5.2	9.2	9.5	10.1
white flour (g. kg^{-1})	10	10	10	10
Chromium oxide (g. kg^{-1})	0.5	0.5	0.5	0.5
Analyzed composition				
Ash (%)	10.3	10.8	10.8	10.2
Moisture (%)	6.8	6.5	6.4	6.5
Dry matter (%)	91	90	91	91
Crude protein (%)	38.6	38.2	39.8	38.8
Crude lipid (%)	10.3	10.1	10.6	10.7

*Mineral premix was provided by Animal Feed Company Products (Mazandaran, Iran).
Mineral premix (g.kg^{-1}): choline chloride 1750 mg, copper 11 mg, iron 56 mg, zinc 92 mg, Magnesium 34 mg, cobalt 0.8 mg, iodine 3 mg, selenium 0.75.
** Vitamin premix was provided by Animal Feed Company Products (Mazandaran, Iran).
vitamin premix (g.kg^{-1}): D36000 IU, A9000 IU, K3 15 mg, E 600 mg, C 780 mg, Thiamine 45 mg, Riboflavin 75 mg, Inositol 350 mg, Cyanocobalamin 120 mg, Pantonic acid 135 mg, Niacin 450 mg, Folic acid 34 mg, Biotin 3 mg, Antioxidant 87 mg.

Dietary treatments belonging to different groups were indeed the same in terms of protein and energy. The average crude protein (41±5) % and energy (20±2) mJ were, in turn, measured based on the requirements of *O. niloticus* in the present weight range. To provide each treatment group with its respective diet, the researchers first weighted the required amounts of material for each diet using a digital scale that provided the weights nearest to one gram. Then, items that had smaller particles in the diet, including white flour, mineral supplements, vitamin supplements, antifungals, salt (used to enhance the taste of the diet) and chromium oxide (used to increase the digestibility of the diet) were well mixed and the resulting mixture was later mixed with items including coarser particles (i.e., fishmeal or PBM). Finally, water and a little soybean oil were added to the resulting mixture of 25-30% of the diet until a homogeneous dough was obtained. The resulting dough which was made into noodles was dried and finally made in the form of pellets considering the size of the fish mouth (less than 2 mm) (according to the guidelines of the Iranian Fisheries Organization). The prepared pellets were then kept at -20 °C till use.

Fish and experimental conditions

A hundred and twenty fish with an initial weight of about 40-60 g were prepared from the *O. niloticus* farm and transferred to 100-liter pre-disinfected aquariums in the Department of Aquatic Health and Diseases, at Shahid Chamran University of Ahvaz, Iran. At first, the fish were kept for

2 weeks and closely monitored to ensure their health and adaptation to their new environment in the laboratory. In the meantime, they were fed under natural photoperiod conditions of approximately 12 h light/12 h darkness. The physical and chemical factors of water were the same for all groups and were checked daily (Table 2). During storage and testing, aquarium water was siphoned off at a rate of 20% by volume every two days from the bottom of the aquarium floor to remove fish excrement. Fish were fed at 3% of weekly BW, twice a day (morning and afternoon) for 44 days with the prepared diets.

Table 2: List of physicochemical factors of water for all groups

Oxygen	Total pH	Total salinity	Ammonia	Nitrite	Nitrate	Hardness	Temperature
6-8 mg/L	8-8.4	1.2 ppt	0.1 mg/L	0.02 mg/L	5 mg/L	180 mg/L	24±2 ºc

Growth performance and feed utilization indices

At the beginning and end of the experiment, 5 fish (15 fish from each group) were taken from each aquarium and their weight and length were measured. To evaluate the growth rate of the fish in each treatment at the end of the final weight (FW) test period, the Total length (TL) of each of the studied fish was measured and recorded using a digital scale and ruler. Afterward, the fish growth indices including body weight gain index (BWI %), food conversion ratio (FCR), survival rate (SR), incidence cost (IC), and profit index (PI) of fish were examined. These indicators were calculated as BWI (%) =100 [final body weight(g)-initial body weight(g)]/initial body weight(g), FCR=dry feed intake(g.day-^{1}) /wet weight gain(g.day-^{1}), IC= cost per kilogram of feed consumed/per kilogram of fish produced PI=value per kilogram of fish product/per kilogram of feed consumed, and survival rate (%)=100 (final number of fish/ initial number of fish). These apparent digestibility coefficients (ADCs) of the experimental diets were calculated according to Qiu et al (2023).

Hematological and blood chemistry parameters

At the end of the experiment after a 24-hour fasting period (to minimize the possibility of error due to feed diet), the fish were anesthetized with clove oil solution (Giahine, Iran) at a dose of 75 mg/l. Then, blood samples taken from the caudal vein of the five fish in each replicate in all treatments were collected in Eppendorf tubes without anticoagulant and centrifuged at 3000 rpm for 15 minutes to separate their serum. The non-hemolysed serum was also collected and stored at -20°C until use. Levels of serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) were then determined and the samples were finally measured using an auto-analyzer (Hitachi, Japan) at a wavelength of 340 nm (laboratory kits of Pars Azmoun company, Iran). Serum albumin, urea (laboratory kits of Pars Azmoun company, Iran), Cholesterol, and triglyceride, were measured by the colorimetric enzyme procedure (Zist Shimi, Iran) (Basir and Abdi, 2015).

Liver and kidney histological evaluation

To prepare histological sections of the studied fish, liver and posterior kidney samples were taken from the fish immediately after blood sampling, and were placed in 10% neutral buffered formalin (Merck, German). Tissue samples that were dehydrated by a series of ethanol ascending solutions were then cleared in xylene and finally embedded in paraffin. Sections with 4-6 μm thickness were prepared from paraffin blocks with a rotary microtome machine (RM2245-LEICA, German), and then stained with hematoxylin and eosin (H&E) (Merck, German) using standard techniques (Moradkhani et al, 2020;

Nochalabadi et al, 2023). Histological changes were examined under a light microscope (Olympus BH-2, Tokyo, Japan) equipped with a Dino-lite lens with Dino capture2.0 software (Dino-lite, Taiwan). For grading histological changes, ten sections from each group and at least five fields in each section were analyzed microscopically at 400× magnification. In the histometric evaluation of liver tissue, the size of hepatocytes, the connective tissue between cells, and the presence of adipose tissue within liver cells in different groups were counted. According to Caballero et al., four degrees are considered for the vacuolation: Grade 0: No vacuolation within liver cells (zero vacuolation). Grade 1: Less than 1/3 cytoplasms of hepatocytes show vacuolation (low vacuolation). Grade 2: Between 1/3 and 2/3 cytoplasms of hepatocytes show vacuolation (moderate vacuolation). Grade 3: complete evacuation of the cytoplasm of liver cells (severe vacuolation) (Caballero et al, 2004). In the histometric evaluation of the kidney tissue, all changes in the diameter and epithelial cells of the urinary tubules, glomerular diameter, number of glomeruli, and the connective tissue between the urinary tubules in different groups were evaluated qualitatively according to Bernet et al., in three degrees: (-) without any visible histological changes in any microscopic field, (+) histological changes visible in each microscopic field were less than 20%, (++) The visible histological changes in each microscopic field were between 20-60%, (+++) Histological changes visible in each microscopic field were complete and more than 60% (Bernet et al, 1999). Slides were read in a 'blind' fashion to provide more accurate results**.**

Statistical analysis

The data were normalized using the Kolmogorov–Smirnov test. Then, to compare the means in different experimental groups with the control group, a one-way ANOVA test was followed by Tukey post hoc. Statistical analysis was performed using the Graph Pad Prism (V.5.03. San Diego, CA, USA). The results were presented as mean± SEM, and the level of significance was set at 0.05 for all the performed statistical tests.

Results

Growth performance and feed utilization indices

The effect of the experimental diets on various growth performances in the studied fish groups is shown in Table 3. All diets were well accepted by the fish and there were no significant differences in feed intake during the experiment period. Statistical analysis showed that increasing fishmeal replacement with PBM in *O. niloticus* diet caused significant changes in the bioassays of *O. niloticus* in different groups ($P<0.05$). As the replacement level of fishmeal with PBM increased, the level of BWI, IC, and survival rate decreased, while the amount of FCR and PI increased considerably, leading to significant changes among different groups ($P<0.05$). Also, these parameters were not significantly influenced by different fishmeal replacements (0%–25%) with PBM ($P>0.05$).

Table 3: Growth performance and feed utilization in different groups (mean± SEM, n = 5 of each tank in each repetition)

Growth performance	Dietary treatments			
	PBM0	PBM25	PBM50	PBM100
IBW[a]	58.4 ±8.10 [a]	58.2 ±7.52 [a]	58.1 ±9.50 [a]	58.1 ±7.26 [a]
BWI[b]	151.4 ±8.71 [a]	147.9 ±10.36 [a]	136.4 ±7.15 [ab]	124.9 ±4.21[b]
FCR[c]	0.87 ±0.05 [a]	0.96 ±0.03 [a]	1.09 ±0.15 [ab]	1.37 ±0.08 [b]
IC[d]	2.30[a]	2.13[b]	1.58[c]	1.02[d]
PI[e]	0.37[a]	0.48[a]	0.71[ab]	1.25[b]
Survival	100	100	100	98.60

[a]IBW (%): initial body weight.
[b]BWI (%): body weight gain index =100[final body weight(g)-initial body weight(g)]/initial body weight(g).
[c]FCR: feed conversion ratio= dry feed intake (g.day^{-1}) /wet weight gain(g.day^{-1})
[d]IC: incidence Cost= cost per kilogram of feed consumed/per kilogram of fish produced
[e]PI: profit index= value per kilogram of fish product/per kilogram of feed consumed
Survival (%): survival rate=100 (final number of fish/ initial number of fish)
a, b, c, d. Different superscripts within each row indicate significant differences among groups($P< 0.05$). Data are means ± SEM

Blood chemistry parameters

As the findings showed, the replacement level of fishmeal with PBM significantly ($P<0.05$) influenced plasma parameter contents (Figure 1). The plasma urea and albumin levels were significantly increased in the PBM100 group compared to the PBM0 group ($P<0.05$) and there was a significant difference between PBM50 and PBM100 groups ($P<0.05$). The plasma cholesterol and triglyceride levels were significantly decreased in the PBM100 group than in the PBM0 ($P<0.05$).

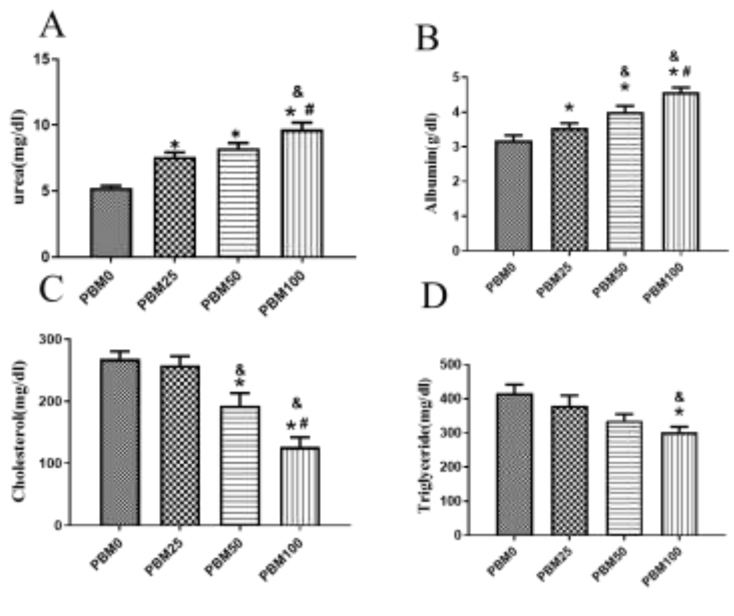

Figure 1: Blood chemistry parameters (Urea (A), Albumin (B), Cholesterol (A), Triglyceride (D)) in different groups determined up to 24h after the last meal (mean± SEM, n = 5 of each tank in each repetition).
*** Significant difference with PBM0 group (p< 0.05), #significant difference with PBM50 (p<0.05) and &significant difference with PBM25 group (p<0.05).**

The mean± SEM levels of liver enzymes in the fish groups are shown in Figure 2. Statistical analysis showed that increasing PBM levels in the diet of *O. niloticus* caused significant incremental changes in the level of liver enzymes in fish serum (P<0.05). There was also a significant difference between the PBM50 and PBM100 groups (P<0.05).

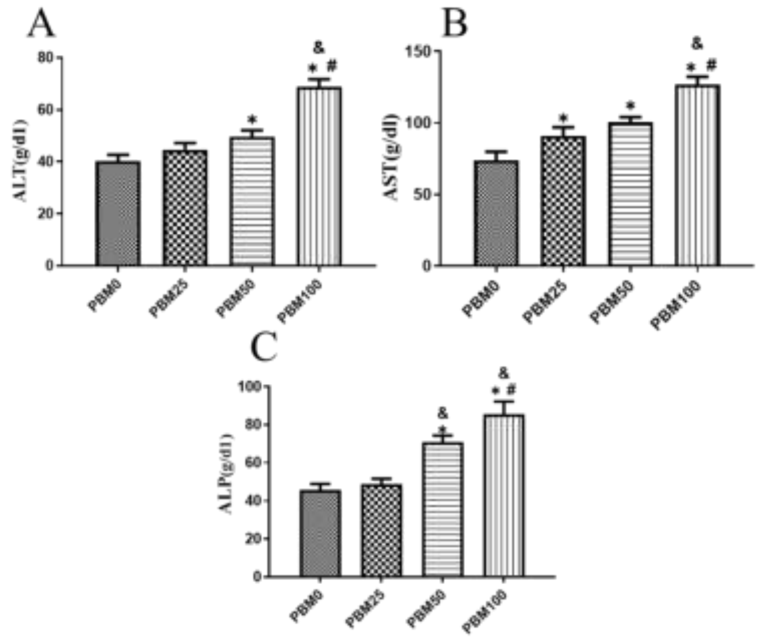

Figure 2: Liver enzymes (ALT (A), AST (B), ALP (C)) in different groups determined up to 24h after the last meal (mean± SEM, n = 5 of each tank in each repetition). * significant difference with PBM0 group (p<0.05), #significant difference with PBM50 (p<0.05) and &significant difference with PBM25 group (p<0.05).

Histological analysis

Macroscopically, *O. niloticus* liver was dark brown in the PBM0 group, while the liver with increasing PBM in the diet of PBM50 and PBM100 groups was turned brighter and whiter, which were, indicative of fatty liver. The liver histology of the fish fed with treatment diets is shown in Figure 3. In the control group (PBM0), normal hepatocytes are uniformly distributed and the integration of parenchymal tissues with a small number of lipid vacuoles is observed and the pancreatic acini are irregularly located in between them **(**3, A). Adding PBM rather than fishmeal to the fish diet in different proportions also caused fat to accumulate inside the hepatocytes, leading to fatty liver in the group receiving PBM 100% (3, D)**.** In effect, by increasing the amount of PBM in the fish diet, almost the entire cytoplasm of their liver cells became swollen with a lot of fat and changed to a vacuole-like appearance. This, in turn, compressed the sinusoids between them and reduced the interstitial tissue of the liver stroma. Likewise, in the PBM25 group, vacuolation is slightly detectable in hepatocyte cells (3, B). However, the hepatic vacuolization was found more in the group receiving PBM100 than PBM50.

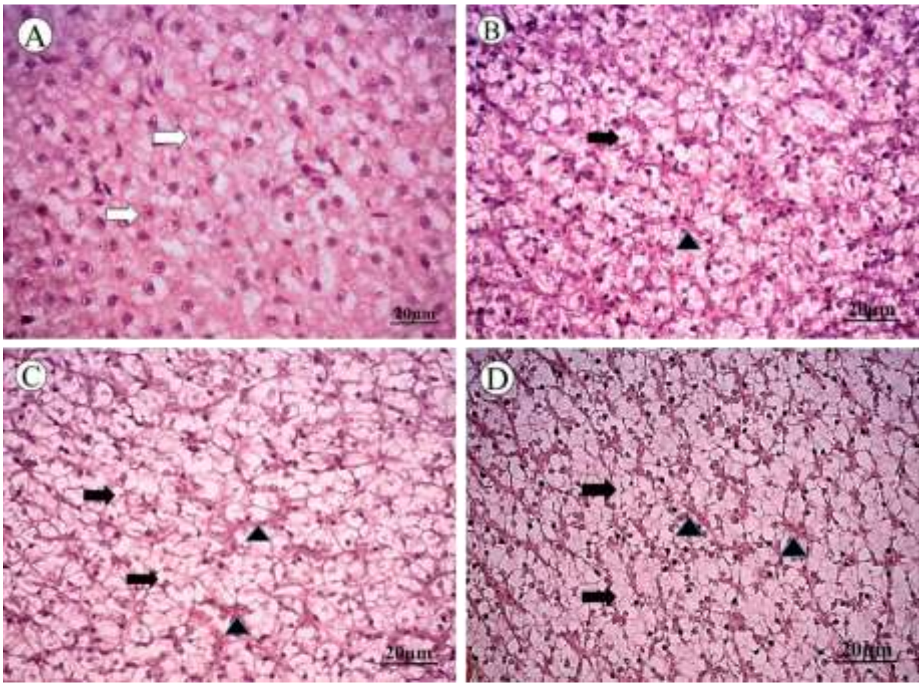

Figure 3: Sections of liver tissues in different groups (H&E, 400). (A) PBM0 group, normal liver morphology. (B) PBM25 group, hepatocytes, as well as sinusoids, are slightly degenerated. (C) PBM50 group, following the use of 50% replacing FM with PBM, liver tissue was a little more damaged. (D) PBM100 group, in response to high levels of replacing FM with PBM, severe damages to tissues including high degrees of vacuolation in the hepatocytes and compression of the sinusoids. White arrow indicates normal hepatocyte; a black arrow indicates swollen hepatocyte; arrowhead indicates compressed sinusoids

The swelling of hepatocytes and degree of vacuolation of liver tissue samples in different diets were initially measured by the magnification of 100 and then by the magnification of 400. The results are given in Table 4.

Table 4: The semiquantitative analysis of histological alteration assessment of hepatocyte size and degree of vacuolation in different groups (mean ± SEM, repetition of each tank in each n =5).

Liver changes	Dietary treatments			
	PBM0	PBM25	PBM50	PBM100
Hepatocyte size (µm)	8.83±0.42 ^{a}	9.64±0.52 ^{a}	11.65±0.19 ^{b}	14.76±1.29 ^{b}
Degree of vacuolation	0.40±0.24 ^{a}	1.0±0.20 ^{a}	2.20±0.20 ^{b}	2.80±0.20 ^{b}
a, b. Different superscripts within each row indicate significant differences amoung groups ($p<0.05$)				

The kidney of the *O. niloticus* is an elongated organ along the dorsal part of the body wall, below and along the spine, consisting of two parts, the anterior part of the kidney (head of the kidney), which includes mainly hematopoietic tissue, and the posterior part (posterior kidney), which often contains the tubes and glomeruli of the excretory system. The kidney histology of the fish in different groups is depicted in Figure 4. The gradual addition of different proportions of PBM rather than fishmeal to the fish diets causes the tubular cells to separate from the basement membrane, and increases the space between the basal part of these cells and the surrounding basement membrane, thus reducing the inner diameter of the tubule. Following that, the tubular cells are removed and fall into the space inside the tubule, causing epithelial cells to be seen inside the tubules. Following a gradual increase in the PBM ratio in the diets, the glomerulus inside of the Bowman's capsule condenses, and the diameter of the glomerule decreases, making the surrounding urinary space inside the Bowman's capsule larger. Moreover, hemorrhage, congestion, and the accumulation of mononuclear cells were observed between the tubules (4, D).

Changes in kidney tissue in different diets were initially measured by the magnification of 100, and then by the magnification of 400, the results of which are shown in Table 5.

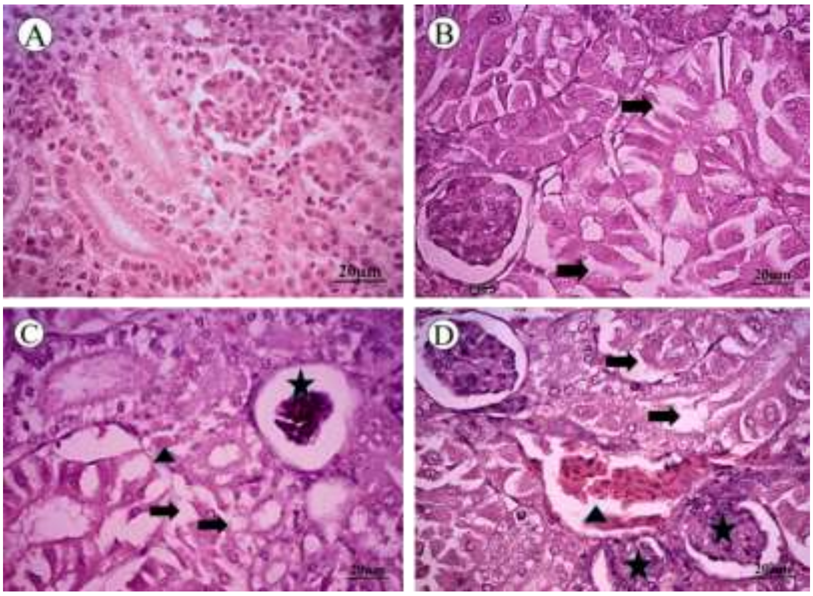

Figure 4: Sections of kidney tissues in different groups (H&E, 400). (A) PBM0 group, normal kidney morphology. (B) PBM25 group, kidney tissue slightly degenerates. (C) PBM50 group, following the use of 50% PBM, kidney tissue changes were partial. (D) PBM100 group, following replacement with %100, severe damages to tissues including glomerular compression, and degeneration of tubules. A star indicates glomerular compression; the black arrow indicates removal of tubule wall lining cells and the arrowhead indicates hemorrhage.

Table 5: Comparison of mean ± SEM of qualitative changes in kidney tissue in different groups (Repetition of each tank in each n =5)

Structures Kidney	Dietary treatments			
	PBM0	PBM25	PBM50	PBM100
Glomerular diameter (µm)	49.5 ±4.30 [a]	47.5 ±3.24 [a]	43.6 ±4.62 [ab]	34.7 ±2.70[b]
Bowman capsule diameter(µm)	58.7 ±6.15 [a]	56.9 ±5.20 [a]	57.8 ±4.20[a]	57.3±7.21 [a]
Internal diameter of the tubule(µm)	11.7 ±2.42 [a]	11.4 ±3.40 [a]	9.13 ±0.15 [b]	8.25 ±0.26 [b]

a, b. Different superscripts within each row indicate significant differences compared among groups ($p < 0.05$)

A comparison of qualitative changes in fish kidney tissues following the use of PBM in different proportions is presented in Table 6.

Table 6: Comparison of mean ± SEM of changes in kidney tissue in different groups (repetition of each tank in each. n =5)

Grading changes	Dietary treatments			
	PBM0	PBM25	PBM50	PBM100
Glomerular diameter (µm)	-	+	++	+++

Discussion

Aquatic animals are generally diverse in terms of food consumption, and *O. niloticus* as an omnivorous fish consumes a variety of food, making its proper nutrition one of the most important issues in aquaculture.

The results of the present study showed that by replacing PBM with fishmeal in *O. niloticus* diet between the groups of 100% PBM and the control group, the ratio of the final weight gain of the fish compared to their initial weight and specific growth rate index decreased, but the feed conversion ratio increased significantly. However, several studies have reported fish weight loss due to the replacement of PBM with fishmeal in the *O. niloticus* diet (Parés-Sierra et al, 2014; Irm et al, 2020; Qiu et al, 2023). In this research, after a 44-day feeding trial, the results showed that the growth performance of *O. niloticus* in the PBM25 group was similar to that in the PBM0 group.

Zhou et al, (2020) found that African catfish could tolerate up to 53.5% of PBM in their diet without significant changes in their performance and biometric parameters. In another study, Parés-Sierra et al, (2014) showed that PBM could be used up to 44% in diets for juvenile rainbow trout as a source of dietary energy. Nogales-Mérida et al, (2011) showed that substituting PBM up to 75% for fishmeal resulted in no change in biomarkers of European Cass.

Based on the findings of the present study, no significant variations in values of BWI%, FCR, PI as well as survival rate were observed in groups PBM0 and PBM25, indicating that up to 50% fishmeal could be replaced by PBM without negatively affecting the growth performance and feed utilization of *O. niloticus*. The findings of the present study are similar to those of Yones and Metwalli's in 2015 on juvenile Nile Tilapia (Yones and Metwalli, 2015). Many researchers attribute the fish weight loss and reduction in its growth index resulting from the replacement of PBM with fishmeal to a variety of reasons, including the presence of some compounds in PBM such as feathers as a horny tissue, connective tissue, as well as mixed skin and blood, or due to high temperature used during processing of PBM and its storage environmental conditions. In effect, these factors reduce digestibility and ultimately food consumption by fish (Parés-Sierra et al, 2014). In the present experiment, the lowest growth indices and the highest changes were observed in the treatment of PBM100% compared to the control group, indicating the inefficiency of PBM at higher levels in providing the energy required for daily fish consumption.

Plasma biochemical indicators are known as indicators of general fish health status (Liang et al, 2018). The results of the present study showed that serum albumin levels in the diet containing 50% and 100% PBM increased as compared to those in the control group. Albumin is a protein produced by the liver. Albumin helps to maintain the level of fluids in the blood so that they do not leak to other tissues, and the level of albumin is also a criterion for determining health and nutritional status. High serum albumin levels can be due to a diet containing too much protein or problems with the liver or kidneys (Lim et al, 2023). This finding is in line with the results of previous studies on *O. niloticus* (Metwalli, 2013), on Sturgeon and Shiny bass (Zhu et al, 2011), and on juvenile bighead carp (Liang et al, 2018). Plasma urea content in aquatic animals is the second most important nitrogen excretion product after ammonia whose changes are often used as an important parameter to evaluate the digestion of amino acids, proteins, and kidney function (Liang et al, 2018). In this study, the amount of plasma urea increased significantly in groups with 50 and 100% substitution of PBM compared to that in the control group. However, more studies are needed to confirm renal dysfunction, given that only a slight change in plasma urea level is not sufficient to conclude the presence of renal lesions (Rahmati et al, 2022). Therefore, the histomorphology of the fish kidney was considered in the present study to provide more reliable results. The results of this study showed that the amounts of total cholesterol and triglyceride in the groups with 50 and 100% PBM substitution significantly reduced compared to those in the control group, which is consistent with the results on fish parrots (Lim et al, 2023), on rainbow trout (Panserat et al, 2009), and on gilthead seabream (Sabbagh et al, 2019). Sabbagh et al, (2019) and Mata-Sotres et al, (2018) concluded that Fish are universally considered to have a low level of cholesterol and the Σn-3/Σn-6 ratio decreases significantly with the increase of vegetable oil or animal substitutes in the diet. This may be related to the low content of Σn-3 fatty acids in PBM and this imbalance of Σn-3 fatty acids and Σn-6 in the liver can lead to fat deposits in the liver. The liver secretes specific enzymes for its function. The results of this study revealed that the amount of liver enzymes of AST, ALP, and ALT increased significantly with the replacement of 50% and 100% PBM with fishmeal compared to those in the group control, which confirms the findings of Lin and Luo (2011). An increase in liver enzymes in the serum can be a sign of inflammation or destruction of cells in the liver. Inflamed or damaged liver cells release higher amounts of certain chemicals, including liver enzymes, into the bloodstream, which can lead to elevated liver enzymes in blood tests. One of the reasons for the increase of these enzymes is the accumulation of fat in the liver and fatty liver disease. As here, following histological investigations, fat vacuoles were seen more in the cytoplasm of hepatocytes in the groups with higher substitution (Bruslé and Anadon, 2017). To further investigate the findings, the histomorphology of the fish liver was also considered in this study. The alkaline phosphatase enzyme which is found in all tissues of the fish body (Soltan et al, 2011) has an important role in the transmission of material between cell membranes It is also highly active in the kidneys and intestines of aquatic animals (Zahran et al, 2024). Several studies have suggested that an increase or decrease in fish liver enzymes is likely attributable to decreased production, increased excretion, or a change in their half-life due to dietary changes, suggesting that these enzymes act as a link between carbohydrates and protein metabolism; moreover and when liver cells are damaged for any reason, the levels of ALT and AST enzymes will change in the fish body (Soltan et al, 2011; Nochalabadi et al, 2023). For instance, by reducing the level of liver enzymes, transaminations are inactivated and amino acid catabolism is reduced(Soltan et al, 2011). Both of these aminotransferases (namely, ALT and AST) can, indeed, function as an interface between carbohydrates and protein metabolism. As such, in other organisms under the pressure of adverse conditions leading to internal lesions in the kidney and liver tissues, mitochondria of damaged cells release their aminotransferase contents into the bloodstream, causing an increase in the levels of these enzymes in blood serum (Rahmati et al, 2022).

The liver is one of the most important metabolic organs which regulates nitrogen and other metabolites in the body (Peyghan et al, 2023). The findings of the present study showed that increasing PBM proportions in the fish diet led to the accumulation of dietary fat inside fish hepatocytes, and also increased vacuolation within hepatocytes which ultimately resulted in fatty liver in the fish. Histological alterations in the liver, which are likely to affect fish health, were thus found in the treatment groups rather than the control groups. Zhou et al, (2020) reported that the use of a diet with over 53.5% PBM meal protein and soybean meal protein increased the vacuolation of hepatocytes and the fat storage profile in fish muscle tissue, leading to the fatty liver as compared with the control group. As such, large vacuoles existed more in group PBM50 and PBM100 than PBM0 and PBM25 due to the direct relationship between unabsorbed and non-metabolizable fat at high levels of PBM use. The level and amount of alternative protein sources for different fish species have been already considered in several studies (Campos et al, 2019; Monteiro et al, 2018). There are different results here, In the study of Aydín et al, (2015), PBM inclusion in the diet of Nile tilapia (*Oreochromis niloticus*) up to 100% did not reveal any effect on the histological examination of the liver tissue and livers. Contrarily, Hu et al, (2013) demonstrated a negative effect of FM replacement by animal protein blend (a mixture of 40% PBM, 35% meat, and bone meal, 20% spray-dried blood meal, and 5% hydrolyzed feather meal) in Japanese seabass (*Lateolabrax japonicus*) livers that were characterized by enlarged hepatocytes and apparent hepatic steatosis with intense vacuoles in the hepatocytes resemble lipids. As in our results, the hepatocytes were abnormally shaped. The kidney in fish is mesonephric in shape and its posterior or excretory part consists of glomeruli of variable size and a long folded tubule leading from a glomerulus to an excretory duct (Gholami et al, 2018; Koohkan et al, 2024). The results of this study showed that adding different proportions of PBM rather than fishmeal to the fish diet increased the plasma urea level, thus affecting the tissue of urinary tubules and glomeruli. This way, changes in fish kidney tissue are detectable in the PBM50 and PBM100 groups compared to the PBM0 and PBM25 groups and are in line with results on other similar species (Yones and Metwalli, 2015).

According to the present study, the substitution of PBM instead of fishmeal up to 50% could not change the biological indicators of *O. niloticus*. Its liver enzymes increased significantly compared to the control group, and in the histological studies on the liver, the liver tissue had lost its normal structure and function with the increase in the amount of alternative diets. The amount of urea plasma also showed a significant difference between the different groups with the increase in the amount of alternative diets compared to the control group, and these changes are also evident in the histological examinations of the kidneys. Therefore, based on the obtained results, the use of PBM in the diet of *O. niloticus* can be replaced up to a maximum of 50% without any change in the growth and health of these fish. But again, to investigate the replacement rate more accurately, up to 50% replacement, alternative diets with shorter intervals and more tests should be tried.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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References

Aydin, B., GÜMÜŞ, E., & BALCI, B. (2015). Effect of dietary fish meal replacement by poultry by-product meal on muscle fatty acid composition and liver histology of fry of Nile tilapia, Oreochromis niloticus (Actinopterygii: Perciformes: Cichlidae). *Acta Ichthyologica et Piscatoria*, *45*(4), 343-351

Basir, Z., & Abdi, R. (2015). Red blood cells histology and study of some blood biochemical parameters in spotted catshark Chiloscyllium punctatum in Persian Gulf. *Journal of Animal Environment*, *7*(2), 119-124.

Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., & Wahli, T. (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. *Journal of fish diseases*, *22*(1), 25-34. DOI: 10.1046/j.1365-2761.1999.00134.x]

Bruslé, J., & i Anadon, G. G. (2017). The structure and function of fish liver. In *Fish morphology* (pp. 77-93). Routledge.

Caballero, M. J., Izquierdo, M. S., Kjørsvik, E., Fernandez, A. J., & Rosenlund, G. (2004). Histological alterations in the liver of sea bream, Sparus aurata L., caused by short-or long-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. *Journal of Fish Diseases*, *27*(9), 531-541. [DOI: 10.1111/j.1365-2761.2004.00572.x].

Campos, I., Matos, E., Maia, M. R., Marques, A., & Valente, L. M. (2019). Partial and total replacement of fish oil by poultry fat in diets for European seabass (Dicentrarchus labrax) juveniles: Effects on nutrient utilization, growth performance, tissue composition and lipid metabolism. *Aquaculture*, *502*, 107-120. [DOI: 10.1016/j.aquaculture.2018.12.004]

FAO. (2018). Food UN Organization A.The State of World Fisheries and Aquaculture 2018: Meeting the sustainable development goals: 1950-2016. [DOI: 3/i9540en/i9540en.pdf]

Gholami, A., Abdi, R., Shirali, S., & Basir, Z. (2018). Histophysiology of Head Kidney and Blood Lymphatic System in Acipenser persicus in Cold and Warm Seasons. *Journal of Oceanography*, *9*(33), 59-65. [DOI: 10.29252/joc.9.33.59]

Hu, L., Yun, B., Xue, M., Wang, J., Wu, X., Zheng, Y., & Han, F. (2013). Effects of fish meal quality and fish meal substitution by animal protein blend on growth performance, flesh quality and liver histology of Japanese seabass (Lateolabrax japonicus). *Aquaculture*, *372*, 52-61. [DOI: 10.1016/j.aquaculture.2012.10.025]

Irm, M., Taj, S., Jin, M., Luo, J., Andriamialinirina, H. J. T., & Zhou, Q. (2020). Effects of replacement of fish meal by poultry by-product meal on growth performance and gene expression involved in protein metabolism for juvenile black sea bream (Acanthoparus schlegelii). *Aquaculture*, *528*, 735544. [DOI: 10.1016/j.aquaculture.2020.735544]

Koohkan, O., Morovvati, H., & Taheri Mirghaed, A. (2024). Histomorphological Study and biochemical changes in kidney of gray mullet (Mugil cephalus) exposed to iron oxide nanoparticles and Spirulina platensis. *Iranian Veterinary Journal*, *19*(4), 120-131. [DOI: 10.22055/ivj.2022.328211.2442]

Liang, H., Mi, H., Ji, K., Ge, X., Re, M., & Xie, J. (2018). Effects of dietary calcium levels on growth performance, blood biochemistry and whole body composition in juvenile bighead carp (Aristichthys nobilis). *Turkish Journal of Fisheries and Aquatic Sciences*, *18*(4), 623-631. [DOI: 10.4194/1303-2712-v18_4_14]

Lim, C., Lee, C. S., & Webster, C. D. (Eds.). (2023). Alternative protein sources in aquaculture diets. CRC Press. 74.

Lin, S., & Luo, L. (2011). Effects of different levels of soybean meal inclusion in replacement for fish meal on growth, digestive enzymes and transaminase activities in practical diets for juvenile tilapia, Oreochromis niloticus× O. aureus. *Animal Feed Science and Technology*, *168*(1-2), 80-87. [DOI: 10.1016/j.anifeedsci.2011.03.012]

Mata-Sotres, J. A., Tinajero-Chavez, A., Barreto-Curiel, F., Pares-Sierra, G., Del Rio-Zaragoza, O. B., Viana, M. T., & Rombenso, A. N. (2018). DHA (22: 6n-3) supplementation is valuable in Totoaba macdonaldi fish oil-free feeds containing poultry by-product meal and beef tallow. *Aquaculture*, *497*, 440-451. [DOI: 10.21608/ejabf.2013.2159].

Metwalli, A. (2013). Effects of partial and total substitution of fish meal with corn gluten meal on growth performance, nutrients utilization and some blood constituents of the Nile tilapia Oreochromis niloticus. *Egyptian Journal of Aquatic Biology and Fisheries*, *17*(1), 91-100. [DOI: 10.1016/j.aquaculture.2018.08.015].

Monteiro, M., Matos, E., Ramos, R., Campos, I., & Valente, L. M. (2018). A blend of land animal fats can replace up to 75% fish oil without affecting growth and nutrient utilization of European seabass. *Aquaculture*, *487*, 22-31. [DOI: 10.1016/j.aquaculture.2017.12.043]

Moradkhani A., Abdi R., Salari-Ali Abadi M.A., Nabavi S.M.B., Basir Z. (2020). Quantification and description of gut-associated lymphoid tissue in, shabbout, Arabibarbus grypus (Actinopterygii: Cypriniformes: Cyprinidae), in warm and cold season. *Acta Ichthyology Piscatoria, 50* (4), 423-432. [DOI: 10.3750/AIEP/02910].

Nochalabadi, A., Morovvati, H., & Abdi, R. (2023). Histomorphometry of Liver and some Blood Factors of Nile Tilapia, Oreochromis niloticus Exposed to Different Concentrations of Ammonia. *Pollution*, *9*(3), 1225-1235. [DOI: 0.22059/poll.2023.352271.1716].

Nochalabadi, A., Morovvati, H., & Abdi, R. (2023). The Effect of different concentrations of ammonia on histomorphometry of kidney and some blood factors of Nile tilapia, Oreochromis niloticus. *Iranian Veterinary Journal*, *19*(3), 64-71. [DOI: 10.22055/ivj. 2023.375952.2529]

Nogales-Mérida, S., Tomás-Vidal, A., Cerdá, M. J., & Martínez-Llorens, S. (2011). Growth performance, histological alterations and fatty acid profile in muscle and liver of sharp snout sea bream (Diplodus puntazzo) with partial replacement of fish oil by pork fat. *Aquaculture International*, *19*, 917-929. [DOI: 10.1007/s10499-010-9410-z]

Ogunji, J.O. (2004). Alternative protein sources in diets for farmed tilapia. Paper presented at the Nutrition Abstracts and Reviews. Series B, Livestock Feeds and Feeding.

Panserat, S., Hortopan, G. A., Plagnes-Juan, E., Kolditz, C., Lansard, M., Skiba-Cassy, S. & Corraze, G. (2009). Differential gene expression after total replacement of dietary fish meal and fish oil by plant products in rainbow trout (Oncorhynchus mykiss) liver. *Aquaculture, 294*(1-2), 123-131. [DOI: 10.1016/j.aquaculture.2009.05.013].

Parés-Sierra, G., Durazo, E., Ponce, M. A., Badillo, D., Correa-Reyes, G., & Viana, M. T. (2014). Partial to total replacement of fishmeal by poultry by-product meal in diets for juvenile rainbow trout (Oncorhynchus mykiss) and their effect on fatty acids from muscle tissue and the time required to retrieve the effect. *Aquaculture Research, 45*(9), 1459-1469. [DOI: 10.1111/are.12092].

Peyghan, R., Momeni, H., Bashiri, M., & Basir, Z. (2023). Histomorphological study of liver, spleen and pancreas in four cichlid species. *Iranian Veterinary Journal*, *19*(2), 32-38. [DOI: 10.22055/IVJ.2021.299539.2386].

Qiu, Z., Xu, Q., Xie, D., Zhao, J., Yamamoto, F. Y., Xu, H., & Zhao, J. (2023). Effects of the replacement of dietary fish meal with poultry by-product meal on growth and intestinal health of Chinese soft-shelled turtle (Pelodiscus sinensis). *Animals*, *13*(5), 865. [DOI: 10.3390/ani13050865]

Rahmati, M., Morovvati, H., & Abdi, R. (2022). Histomorphometric analysis of gills in Nile tilapia (Oreochromis niloticus) exposed to different concentrations of ammonia. *Iranian Veterinary Journal*, *18*(1), 63-70. [DOI: 10.22055/ivj.2022.323544.2432]

Rahmati, M., Morovvati, H., & Abdi, R. (2022). Histomorphometric analysis of gills in Nile tilapia (Oreochromis niloticus) exposed to different concentrations of ammonia. *Iranian Veterinary Journal*, *18*(1), 63-70. [DOI: 10.22055/IVJ.2022.323544.2432]

Roberts, R. J. (2003). Nutritional pathology. In *Fish nutrition* (pp. 453-504). Academic press.

Sabbagh, M., Schiavone, R., Brizzi, G., Sicuro, B., Zilli, L., & Vilella, S. (2019). Poultry by-product meal as an alternative to fish meal in the juvenile gilthead seabream (Sparus aurata) diet. *Aquaculture, 511*, 734220. [DOI: 10.1016/j.aquaculture.2019.734220]

Soltan, M., Fath El-Bab, A., & Saudy, A. (2011). Effect of replacing dietary fish meal by cottonseed meal on growth performance and feed utilization of the Nile tilapia, (Oreochromis niloticus). *Egyptian Journal of Aquatic Biology and Fisheries*, *15*(2), 17-33.[DOI: 10.21608/ejabf.2011.2089]

Yones A., Metwalli A. (2015). Effects of fish meal substitution with poultry by-product meal on growth performance, nutrients utilization and blood contents of juvenile Nile Tilapia (Oreochromis niloticus). *Journal of Aquaculture Research and Development, 7*(1), 389-395. [DOI: 0.4172/2155-9546.1000389]

Yu, Y. (2023). Replacement of fish meal with poultry by-product meal and hydrolyzed feather meal in feeds for finfish. In *Alternative protein sources in aquaculture diets* (pp. 51-93). CRC Press.1st Edition.,

Zahran, E., Elbahnaswy, S., Ahmed, F., Risha, E., Mansour, A. T., Alqahtani, A. S., ... & Sebaei, M. G. E. (2024). Dietary microalgal-fabricated selenium nanoparticles improve Nile tilapia biochemical indices, immune-related gene expression, and intestinal immunity. *BMC Veterinary Research*, *20*(1), 107. [DOI: 10.1186/s12917-024-03966-4

Zhou, Z., Yao, W., Ye, B., Wu, X., Li, X., & Dong, Y. (2020). Effects of replacing fishmeal protein with poultry by-product meal protein and soybean meal protein on growth, feed intake, feed utilization, gut and liver histology of hybrid grouper (Epinephelus fuscoguttatus♀× Epinephelus lanceolatus♂) juveniles. *Aquaculture, 516,* 734503. [DOI: 10.1016/j.aquaculture.2019.734503]

Zhu, H., Gong, G., Wang, J., Wu, X., Xue, M., Niu, C., Yu, Y. (2011). Replacement of fish meal with blend of rendered animal protein in diets for Siberian sturgeon (Acipenser baerii Brandt), results in performance equal to fish meal fed fish. *Aquaculture Nutrition, 17*(2), 389-395. [DOI: 10.1111/j.1365-2095.2010.00773.x]

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اثر جایگزینی پودر ماهی با پودر ضایعات طیور بر پارامترهای سرمی و هیستومورفولوژی کبد و کلیه (*Oreochromis niloticus*, Linnaeus ۱۷۵۸) در ماهی تیلاپیا نیل

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چکیده

امروزه *Oreochromis niloticus* به یکی از محبوب‌ترین ماهی‌ها در جهان تبدیل شده است. با توجه به افزایش قیمت پودر ماهی و محدودیت دسترسی به آن، منابع پروتئینی مختلف از جمله پودر ضایعات طیور می‌توانند جایگزین پودر ماهی در جیره آبزیان شوند. بنابراین این مطالعه به منظور بررسی اثر جایگزینی پودر ماهی با نسبت‌های مختلف پودر ضایعات طیور بر روی تغییرات ساختار بافت کبد و کلیه، عملکرد رشد و برخی پارامترهای سرمی *O. niloticus* انجام شد. برای این منظور، ۱۲۰ ماهی *O. niloticus* به طور تصادفی در چهار گروه کنترل، ۲۵، ۵۰ و ۱۰۰ درصد تقسیم و به مدت ۴۴ روز از پودر ضایعات طیور به جای پودر ماهی تغذیه شدند. در پایان دوره درمان، پارامترهای رشد، سرم خون، آنزیم‌های کبدی و هیستومورفولوژی کبد و کلیه ماهیان مورد بررسی قرار گرفت. نتایج نشان داد که آنزیم‌های کبدی در گروه‌های با جایگزینی بالاتر در مقایسه با تیمار شاهد به طور معنی‌داری بالا رفت که در بررسی‌های بافت‌شناسی بر روی کبد نیز بافت کبد با افزایش مقدار جیره‌های جایگزین ساختار طبیعی و عملکرد خود را از دست داده بودند و در سیتوپلاسم هپاتوسیت‌ها واکوئل‌های چربی تجمع یافته بود. میزان اوره پلاسما هم با افزایش مقدار جیره‌های جایگزین در بین گروه‌های با جایگزینی صعودی، اختلاف معنی‌داری نسبت به تیمار شاهد داشته که در بررسی‌های بافت‌شناسی بر روی کلیه نیز این تغییرات در ساختار توبول‌ها و گلومرول‌ها مشهود بود. به طور کلی، داده‌ها نشان می‌دهد که ۱۰۰ درصد پودر ضایعات طیور برای جایگزینی پودر ماهی در *O. niloticus* توصیه نمی‌شود، اما پودر ضایعات طیور تا سقف ۵۰ درصد می‌توانند جایگزین پودر ماهی برای جیره *O. niloticus* بدون تأثیر نامطلوب بر عملکرد رشد و پارامترهای بیوشیمیایی ماهی شوند. اما باز هم برای بررسی مقدار دقیق‌تر باید جایگزینی‌هایی با مقدار کم‌تر از ۵۰ و به فواصل کم‌تر انجام شود.

کلمات کلیدی: پودر ماهی، عملکرد رشد، تغییرات هیستومورفومتری، *Oreochromis niloticus*، پودر ضایعات طیور

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