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RESEARCH ARTICLE

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Effect of partial or total replacement of fish meal with *Procambarus clarkii* by-product meal in *Oreochromis niloticus* diets

ABSTRACT:

The present study aimed to evaluate the effect of partial or total replacement of fish meal (FM) with *Procambarus clarkii* by-product meal (PBM) on *Oreochromis niloticus* growth performance, feed utilization and health. Five isonitrogenous (30% protein) experimental diets were formulated and applied to *O. niloticus* with initial body weight 10.94 g for four months in ceramic ponds. The control group (D1) contained 0% of PBM, while D2, D3, D4, and D5 contained 25, 50, 75, and 100% replacement of FM with PBM, respectively. The present study revealed that the protein content of PBM was 33.13% and its amino acids profile fulfilled the requirements of *O. niloticus*. All analysed heavy metals of PBM, except arsenic and iron, were in the permissible limits for *O. niloticus*. Moreover, the growth performance and feed utilization parameters of fish of all treated groups were significantly different than those of the control one, but the best results were recorded in fish fed diets D2 and D3, followed by fish fed diets D4 and D5. Moreover, the histological investigation revealed that the liver showed alterations only in fish fed diet D5, and the kidneys showed alterations in fish fed diets D4 and D5, however, the gills showed alterations in all treated groups.

KEY WORDS:

Oreochromis niloticus, *Procambarus clarkii*, Fish meal, growth performance, feed utilization, fish histopathology

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INTRODUCTION:

The Nile tilapia, *Oreochromis niloticus*, is considered as the most preferred cultured fish in the East Africa and the second most important cultured fish in the world after carps, representing 10.25% of total farmed fish production (El-Sayed, 2006; El-Sayed *et al*., 2016). Tilapia is widely cultured in about 100 countries in the tropical and subtropical regions. As a result, the production of farmed tilapia has increased from 383,654 metric ton in 1990 (2.28% of total global aquaculture production) to 5,670,981 metric ton in 2015, representing 11.63% of total global aquaculture production (FAO, 2017).

The fish meal (FM) is traditionally used as the main dietary protein source in fish feeds (Hu *et al*., 2013). The increasing of global demand and decreasing availability of fish have led to sharp increase in the price of FM, and hence, the cost of aquaculture production has increased as well (Ayoola, 2010). The price of FM has risen from US $ 600/metric ton in 2005 to US $ 1100/metric ton in 2017

and this trend is likely to continue (IMF, 2017). Thus, developing practical diets using cheap sustainable alternative non-FM protein sources for tilapia has the potential to reduce feed cost (Tacon and Metian, 2008; Hardy, 2010).

Moreover, several studies explored that plant proteins could be used as good candidates for total or partial replacement of FM, with the aim of testing the nutritional value of fish products and fish health (Caruso, 2015). However, it is well recognized that high dietary level of plant proteins for partial replacement of FM reduces feed efficiency and growth performances (Kaushik *et al*., 1995; Robaina *et al*., 1999). One of the key problems connected to the use of plants in aquaculture is the anti-nutritional factors such as protease inhibitors, saponins and phytic acid which not only damage the morphology and physiology of digestive tract but also suppress the fish growth (Usmani and Jafri, 2002; Nguyen *et al*., 2009; Akande *et al*., 2010).

The animal proteins are more palatable, available and cheaper than FM and are free of anti-nutritional factors thus making them perfect FM replacer for tilapia (El-Sayed, 1999). The main animal by-product meals that were tested as FM replacers for tilapia included poultry by-product meal (Yones and Metwalli, 2015; Palupi *et al*., 2019), blood meal (Aladetohun and Sogbesan, 2013), fish offal meal (Farahiyah *et al*., 2015), shrimp wastes (Kubiriza *et al*., 2016; Rathore and Yusufzai, 2018), feather meal (Rachmawati and Samidjan, 2019), and meat and bone meal (El-Sayed, 1998; Hasan *et al*., 2012).

The freshwater crayfish, *Procambarus clarkii* (Girard, 1852) was considered as the largest invertebrate in the freshwater systems (Nyström, 1999). Moreover, *P. clarkii* was highly tolerant to unfavourable conditions and as a consequence had an extraordinary production rate in farming, elevated adaptive capacity, and flexible feeding strategy (Alcorlo *et al*., 2004). Moreover, *P. clarkii*, is an exotic animal that was accidentally introduced into the River Nile in Egypt during early eighties of the last century; since that time, it spread rapidly in the freshwater ecosystems. The rapid growth of this species can be regarded as the main reason of dramatic environmental damage such as harm the fisheries productivity and damage the agricultural crops (Ibrahim *et al*., 1995).

The present study aimed to evaluate the effect of partial or total replacement of FM with *P. clarkii* by-product meal (PBM) on growth performance, feed utilization and the health of *O. niloticus.*

MATERIAL AND METHODS:

The experimental fish and culture technique:

The experimental work was conducted at the Fish Research Station Farm in the National Institute of Oceanography and Fisheries at El-Qanater El-Khayria, Al-Qalyubia, Egypt. *O. niloticus* fish were obtained from the Industrial fish hatchery at Saft Khaled**,** Beheira Governorate, Egypt. The average initial body weight of the fish ranged from 10.87 to 11.08 g. At May first, 2016, a total of 250 healthy fish were equally divided into ten groups and stocked into ten ceramic ponds (115 X 110 X 120 cm). The dietary treatments were tested in duplicate groups where each pond was considered as an experimental unit. The ponds were supplied with freshwater from the Darawa Irrigation Branch, Al-Qalyubia, were aerated continuously by using air blowers and were held under the natural light. During the 4-months experimental period, all fish were fed their respective diets at the level of 3% of the fish body weight for 6 day/week. The daily ration was divided into two equal amounts and given two times in a day at 8 am and 14 pm. Fish from each experimental treatment were weighed biweekly and the ration was adjusted accordingly.

The physico-chemical characteristics of water:

Water temperature was measured daily by using mercuric thermometers that were suspended in the ponds mid-daily and the average was recorded monthly. pH value was determined by pH meter (Orion pH meter, Abilene, Texas, USA) and the dissolved oxygen (DO) of water was measured by oxygen meter (LaMotte Tracer and Pocke Tester, USA). The phosphate and ammonia concentrations were measured biweekly according to APHA (1999).

The diets ingredients and the experimental diets:

PBM was prepared by the following method: 30 kg of crayfish, *P. clarkii*, were brought to the laboratory from Jiang's Fish Processor Company at El Obour city, Al-Qalyubia Governorate in ice boxes, and then the exoskeleton and the appendages were separated. This by-product was rinsed with tap water, then dried at 60°C for 48 hours and then it ground by mixer grinder (HL1616/D Philips, India limited, 500 W). A sample of PBM was taken to evaluate its chemical composition (protein, lipid, ash, fibre, amino acids profile, and heavy metals content). The protein, lipid, and ash contents of PBM were carried out according to AOAC (1995). Moreover, the fibre content, amino acids and heavy metals analysis was carried out in Regional Centre for Food and Feed, Agriculture Research Centre, Ministry of

Agriculture, Giza, according to AOAC (2012) by using fibre analyser, ANKOM^{2000}, high performance amino acid analyser (Biochrom 30) and using inductively coupled plasma ICP (optima 2000 DV – Perkin Elmer), respectively.

FM (60% protein, 6.8% lipid, 21.9% ash, and 0.8% fibres) was obtained from Bahr Al-Arab Fish Meal Factory, Modern Industrial Area, Umm Al-Quwain, The United Arab Emirates. The amino acids content of this FM was evaluated according to AOAC (2012). The fish oil was purchased from Egypt Feed Additives Company, 10th Of Ramadan Industrial Zone A2, Egypt. The other ingredients, which include soybean and yellow corn, were obtained from the local manufacturer. These ingredients were mixed with vitamins and minerals, which obtained from UNIVET Company, Tullyvin, Cavan, Ireland.

Five isonitrogenous diets (approximately 30% protein) were formulated to replace 0, 25, 50, 75, or 100% of the FM by the PBM. Each diet was prepared by thoroughly mixing the ingredients by the horizontal mixer and then the mixture was pressed into pellet mill (California Pellet Mill, San Francisco, California, USA). The diet pellets of size 2 mm in diameter and 6 mm in length were sun-dried for 2 days, and then were kept in a freezer at – 4°C. The chemical composition and the proximal analysis of the experimental diets were calculated according to NRC (1993) (Table 1).

Table 1. Chemical Composition and proximate analysis of the experimental diets

	Experimental diets				
Ingredients	Control (D1)	D2	D3	D4	D5
PBM	-	5	10	15	20
FM	20	15	10	5	-
Fish oil	2	2	2	2	2
Soybean	33	35	38	42	46
Yellow corn	43	41	38	34	30
Vitamins & Minerals	2	2	2	2	2
Sum	100	100	100	100	100
Proximate chemical composition (%)					
Dry matter (DM)	86.14	86.21	86.29	86.40	86.51
Crude protein (CP)	30.16	29.53	29.25	29.33	29.42
Ether extract (EE)	5.27	5.24	5.19	5.11	5.03
Ash	7.02	8.51	10.05	11.64	13.24
Crude fibre (CF)	3.56	4.48	5.46	6.48	7.50
Nitrogen free extract (NFE)[1]	53.99	52.24	53.78	47.44	44.81
Gross energy (GE) (kcal/100 g DM)[2]	441.73	430.72	435.02	408.64	397.60
Protein/energy ratio (mg CP/kcal)[3]	68.27	68.56	67.25	71.79	73.98

[1]NFE: Nitrogen free extract was calculated by using the following equation: NFE= 100 – (crude protein + ether extract + crude fibre + ash).

[2] GE: Gross energy was calculated as: 5.64, 9.44 and 4.11 kcal/100 g for protein, lipid and NFE, respectively (NRC, 1993).

[3] Protein/energy ratio (mg crude protein/kcal gross energy) = CP/GE X 1000.

Chemical composition of *O. niloticus* fed with different levels of PBM:

At the end of the experiment, six fish from each pond were randomly selected to serve as a final sample, the fish were autoclaved, ground into homogeneous powder, oven-dried and then stored at - 20ºC until further analysis for moisture, crude protein, lipid and ash.

All chemical composition of fish carcass was carried out according to AOAC (1995). The moisture of fish carcass using a thermostatically adjusted oven at 105ºC for 48 hours till it reached a constant weight. Moreover, protein content of fish carcass was determined by Kjeldahl's method using Kjeltech autoanalyzer (Model 1030, Tecator, Höganäs, Sweden). Fat content of fish carcass was determined by ether extraction method using a soxhelet apparatus (500 ml

capacity). Ash content of fish carcass was determined using a muffle furnace (550°C) for 12 hours.

The growth performance parameters:

Mean fish body weight in grams of each experimental treatment pond was determined by dividing the total fish weight by number of the fish in each pond. Weight gain (WG) was calculated according to Tacon (1990) as follow:

WG = final weight (g) – initial weight (g)

The specific growth rate (SGR) was calculated according to Brown (1957) as follow:

$$SGR = \frac{\ln W2 - \ln W1}{T} \times 100$$

Where In is the natural logarithm, W_1 is the initial fish weight (g), W_2 is the final fish weight (g) and T is the experimental period in days.

The feed utilization parameters:

The feed conversion ratio (FCR) was calculated according to Castell and Tiews (1980) by the following equation:

$$FCR = \frac{\text{Feed intake (g)}}{\text{weight gain (g)}}$$

The feed efficiency ratio (FER) was calculated according to Ballestrazzi *et al*. (1994) as shown in the following equation:

$$FER = \frac{\text{weight gain (g)}}{\text{weight of feed consumed (g)}}$$

The protein efficiency ratio (PER) was calculated by the following equation according to Halver and Hardy (2002):

$$PER = \frac{\text{Weight gain (g)}}{\text{protein intake (g)}}$$

The statistical analysis:

The statistical analyses were performed using SPSS (2013) (Statistical Package for Social Sciences). The experimental data were subjected to one-way analysis of variance (ANOVA) test. Duncan's multiple range test was used to compare differences between treatment means (Duncan, 1955). Differences between means were significant at a level of ($p < 0.05$).

The histological studies:

Five specimens of *O. niloticus* were randomly selected from each pond and were dissected. Small pieces of gills, liver and kidneys were fixed in aqueous Bouin's fluid for 24 hours. After fixation and washing, dehydration was carried out in ascending grades of ethyl alcohol. The samples were cleared in terpineol and embedded in molten paraplast. Sections of 5 µm thick were cut and stained with Harris' haematoxylin and counterstained with eosin Humason (1972). The sections were carefully examined by the light microscope and the photomicrographs were made using Sony Cyber-shot digital camera 5.1 MP and measurements were carried out using the eye piece micrometre, calibrated by the slide micrometre.

RESULTS AND DISCUSSION:

The physico-chemical characteristics of water:

All the physico-chemical characteristics of water throughout the experimental period were within the acceptable range for *O. niloticus*. The water temperature ranged from 27.2 to 30.5°C, pH ranged from 7.96 to 8.16 and the dissolved oxygen ranged from 4.9 to 5 mg L^{-1}. In addition, phosphate concentration (PO_4) ranged from 0.193 to 0.24 mg/L and ammonia concentration ranged from 0.049 to 0.158 mg/L. These results were in accordance with those of Makori *et al.* (2017) who reported that the optimum water temperature for tilapia ranged between 27 and 30°C and the optimum pH ranged from 6.5 to 9. In addition, Riche and Garling (2003) stated that the optimum dissolved oxygen value for tilapia growth was above 5 mg L^{-1}. Moreover, the tolerable range of phosphate (PO4) for fish culture is 0.005 to 0.2 mg L^{-1} (Boyd, 1998). In addition, Bhatnagar and Singh (2010) recommended that the level of ammonia < 0.2 mg L^{-1} was suitable for pond fishery.

The chemical composition of PBM:

It had been found that the protein content of PBM was 33.13%, lipid content was 7.24%, ash content was 49.74% and fibre content was 17.27%, while the FM had 60% protein, 6.8% lipid, 21.9% ash, and 0.8% fibres. Mjoun *et al*. (2010) stated that the protein requirements for optimum growth of the Nile tilapia were dependent on dietary protein quality, fish size and age, moreover, they reported that the required protein for juveniles tilapia (10.0 - 25.0 g) ranged from 30 to 35% and for on-growing tilapia (> 25.0 g) ranged from 28 to 30%. Thongrod (2007) found that the optimum dietary lipid concentration, ash content and crude fibres for tilapia were < 8%, 16%, and 8%, respectively.

Although the amino acids profile of FM appeared superior to PBM but PBM was still found to be suitable for the requirements of amino acids of *O. niloticus* as reported by NRC (2011). Besides, PBM was slightly deficient in histidine, leucine and methionine (Table 2).

Table 2. Amino acid profile of FM, PBM and the requirements of amino acids for *O. niloticus*

Essential amino acids	Amino acids of FM (%)	Amino acids of PBM (%)	*O. niloticus* essential amino acids requirements (%) [(1)]
Arginine	2.79	1.45	1.2
Histidine	1.43	0.75	1.0
Isoleucine	2.19	1.01	1.0
Leucine	3.66	1.60	1.9
Lysine	3.93	1.53	1.5
Methionine	1.46	0.44	0.8
Phenylalanine	2.07	1.25	1.1
Threonine	2.03	1.04	1.1
Valine	2.81	1.45	1.5
non-essential amino acids			*O. niloticus* non-essential amino acids requirements (%) [(1)]
Alanine	4.11	1.64	-
Aspartic acid	4.23	2.25	-
*Cystine	1.06	0.30	0.3
Glutamic acid	7.21	3.47	-
Glycine	3.07	1.62	-
Proline	2.06	1.28	-
Serine	1.56	1.01	-
*Tyrosine	1.56	0.90	0.5

[1]NRC (2011) and * the methionine requirement depends on dietary cystine concentration. If cystine is not supplied by the diet, the requirement of methionine is increased. Similarly, tyrosine can be synthesized from phenylalanine so if tyrosine is not supplied by the diet, the requirement of phenylalanine is increased.

The heavy metals analysis of PBM showed that cadmium, chromium, copper, lead, nickel and zinc were in the permissible limits for *O. niloticus*, but arsenic and iron were higher than the permissible limits for *O. niloticus* (FAO, 1983) (Table 3).

Table 3. Heavy metals of PBM and their permissible limits for *O. niloticus*

	Heavy metals of *P. clarkii* (mg/kg)	The permissible limits for *O. niloticus* (mg/kg)[(1)]
Arsenic (As)	9.3	6
Cadmium (Cd)	0.017	2
Chromium (Cr)	0.65	1
Copper (Cu)	16.6	30
Iron (Fe)	215.8	100
Lead (Pb)	0.25	2
Nickel (Ni)	0.60	6
Zinc (Zn)	72.3	100

[1]Source: FAO (1983).

Final chemical composition of *O. niloticus* carcass:

Concerning the effect of the diets on the final chemical composition of the fish carcass, there was significantly increased in body moisture and ash of fish of all treated groups in comparable with the control group. The final protein and lipid contents of the control diet, D2 and D3 showed the highest contents, while D4 and D5 showed the lowest contents. Moreover, there were significant decrease in lipid content of fish that fed D2, D3, D4, and D5 in comparable to fish fed the control diet (Table 4). The present study suggested that the substitution of 25% and 50% FM by PBM had the best effect on the fish protein and lipid contents and had the lowest moisture and ash contents in comparable with other treated groups. These results agreed with those of Leal *et al*. (2010) and Rathore and Yusufzai (2018). These results may be due to high level of ash and fibre contents of PBM, which caused low protein digestibility and may explain the low protein content in fish carcass (Rodde *et al*., 2008). Moreover, Garcia *et al*. (2010) stated that the addition of crab meal to the fish diets significantly reduced the total lipid content in whole fish, which could be due to the higher proportion of chitin in the crab meal, that binding to lipid in the digestive tract and reduce its absorption.

Table 4. Final chemical composition of carcass of *O. niloticus* that fed with different levels of PBM

The parameter	Final chemical composition				
	D1	D2	D3	D4	D5
Moisture	74.06 ± 3.23^{c}	75.73 ± 3.90^{b}	76.05 ± 1.25^{b}	79.68 ± 1.62^{a}	79.16 ± 1.62^{a}
Protein	63.15 ± 1.34^{a}	63.00 ± 0.88^{a}	62.15 ± 0.15^{b}	61.20 ± 0.31^{b}	61.15 ± 0.45^{b}
Lipid	18.28 ± 1.42^{a}	11.83 ± 1.70^{b}	11.61 ± 1.45^{b}	9.92 ± 0.90^{c}	7.88 ± 0.65^{d}
Ash	17.45 ± 0.24^{c}	19.74 ± 0.67^{b}	18.58 ± 1.67^{b}	24.27 ± 1.73^{a}	25.71 ± 0.07^{a}

Each value represents mean ± SEM (standard error of mean).

Values in the same rows with the same superscript letters are not significantly different (P > 0.05).

The growth performance parameters:

The weight gain and specific growth rate of fish of all treated groups were significantly lower than those of the control one. The highest weight gain and specific growth rate were recorded in D2 and D3, while the lowest values were recorded for fish fed D4 and D5 (Table 5). These observations are in accordance with those of El-Sayed (1998) who found that the replacement of FM by shrimp meal in the diets of *O. niloticus* lowered the fish growth. Moreover, Rathore and Yusufzai (2018) found that lower substitution level of FM by shrimp head meal for Nile tilapia gave the best growth compared to higher substitution level. Keremah (2013) explained that this decrease in growth parameters may be due to the lower feed consumption resulting from high ash contents in the crab meal**.** Moreover, Erickson *et al.* (2011) demonstrated that the diet-borne arsenic exposure had effects on fish growth.

Table 5. The growth performance parameters of *O. niloticus* fed with different levels of PBM

	D1	D2	D3	D4	D5
Initial weight (g)	10.89 ± 0.02^{a}	11.04 ± 0.00^{a}	10.94 ± 0.02^{a}	11.02 ± 0.06^{a}	10.92 ± 0.04^{a}
Final weight (g)	37.58 ± 2.69^{a}	32.52 ± 1.01^{b}	31.26 ± 0.44^{b}	26.19 ± 0.07^{c}	26.05 ± 0.74^{c}
WG (g)	26.69 ± 2.72^{a}	21.48 ± 1.01^{b}	20.34 ± 0.46^{b}	15.27 ± 0.11^{c}	15.03 ± 0.79^{c}
SGR (%)	1.24 ± 0.07^{a}	1.08 ± 0.03^{a}	1.05 ± 0.02^{a}	0.87 ± 0.01^{b}	0.86 ± 0.03^{b}

Each value represents mean ± SEM (standard error of mean).

Values in the same rows with the same superscript letters are not significantly different (P > 0.05).

The feed utilization parameters:

The feed conversion ratio, feed efficiency ratio and protein efficiency ratio of fish that fed D2 and D3 were insignificantly different in comparable with the control group, while, there were significant differences of fish that fed D4 and D5 in comparable with the control group (Table 6). The present study clearly demonstrated that PBM could partially replace (25% and 50%) FM for *O. niloticus* and gave good feed utilization parameters, while, 75% and 100% significantly reduced the feed utilization parameters compared with the control group. This could be explained by the low digestibility of chitin which found in crab meal and could prevent the action of digestive enzymes, thus affecting the utilization of the nutrients (Fall *et al*., 2012). In addition,the previous authours found that the lowest substitution levels of FM with shrimp head meal in the diet of hybrid tilapia gave the best FCR.

Table 6. Feed utilization parameters of *O. niloticus* fed with different levels of PBM

	D1	D2	D3	D4	D5
FCR (%)	3.14 ± 0.14^{a}	3.41 ± 0.26^{a}	3.42 ± 0.02^{a}	4.19 ± 0.14^{b}	4.34 ± 0.17^{b}
FER (%)	31.89 ± 1.49^{a}	29.54 ± 2.28^{a}	29.21 ± 0.17^{a}	23.91 ± 0.89^{b}	23.09 ± 0.89^{b}
PER (%)	1.06 ± 0.05^{a}	0.98 ± 0.08^{a}	0.97 ± 0.01^{a}	0.80 ± 0.03^{b}	0.77 ± 0.03^{b}

Each value represents mean ± SEM (standard error of mean).

Values in the same rows with the same superscript letters are not significantly different (P > 0.05).

Histological results of *O. niloticus* fed with different levels of PBM:

Histopathological studies are considered as a reliable and comprehensive bio-monitoring tool to assess the fish health in polluted aquatic ecosystems (Deore and Wagh, 2012).

The liver of the control group of *O. niloticus* was formed of a continuous compact field of the hepatocytes and scattered pancreatic tissue (Fig. 1a). The hepatocytes were polygonal in shape with large, rounded

and eccentric nuclei and their cytoplasm was filled with large lipid vacuoles. The central vein and the blood sinusoids are lined by flattened Kupffer cells that have a homogeneous attenuated cytoplasm and flattened darkly stained nuclei (Fig. 1b). Besides, the liver of fish that fed D2 showed normal architecture without any histopathological changes (Fig. 1c). However, the liver of fish that fed D3 and D4 showed normal structure, but there were sinusoidal blood congestions (Figs. 1d and 1e). The liver of fish that fed D5 showed severe lipid depletion, sinusoidal blood congestions and necrotic hepatocytes (Fig. 1f). Similarly, Omar *et al*. (2013) and Abdel-Khalek (2015) revealed that *O. niloticus* exposed to iron suffered from liver histopathological alterations such as blood congestion and hepatocytes degeneration. Moreover, Ahmed *et al*. (2013) revealed that there were histological changes of liver of arsenic exposed tilapia which were shrinkage of hepatocytes with pyknotic nuclei, blood congestion and increase in sinusoidal spaces.

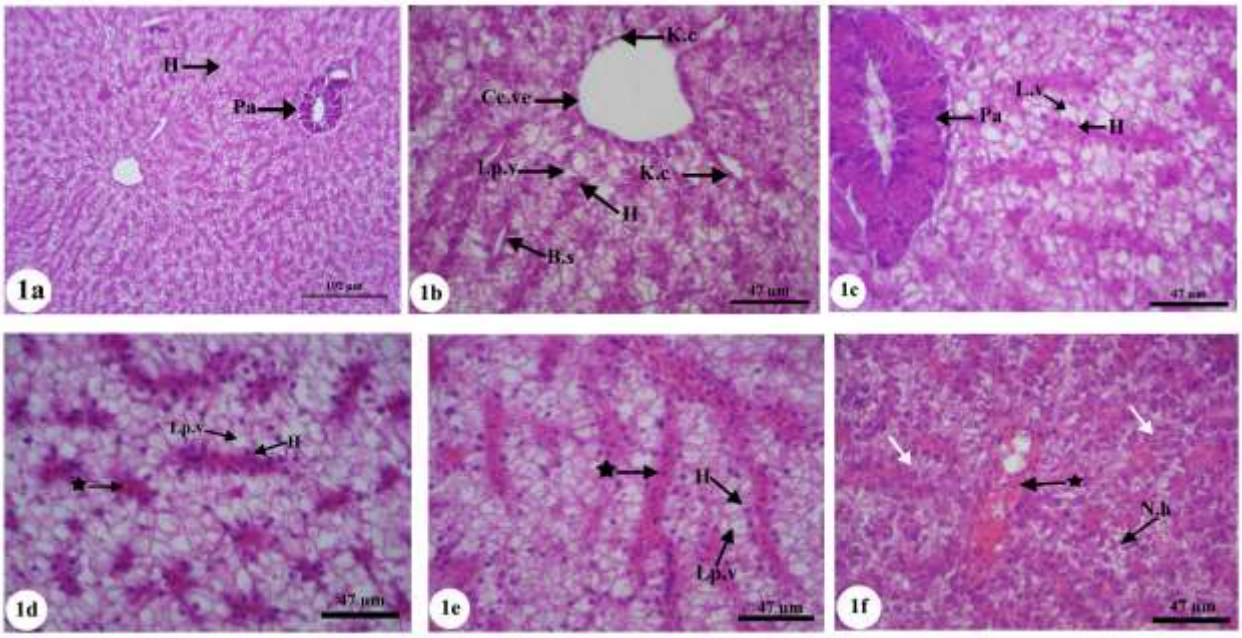

Fig. 1. photomicrographs of S. of the liver of *O. niloticus* **(a)** of the control group showing the hepatocytes (H) and pancreas (Pa), **(b)** of the control group showing the hepatocytes (H), central vein (Ce.ve), blood sinusoids (B.s), Kupffer cells (K.c) and lipid vacuoles (Lp.v), **(c)** of D2 group showing normal architecture of liver, hepatocytes (H), pancreas (Pa) and lipid vacuoles (Lp.v), **(d)** of D3 group showing hepatocytes (H), lipid vacuoles (Lp.v) and sinusoidal blood congestion (Asterisk), **(e)** of D4 group showing hepatocytes (H), lipid vacuoles (Lp.v) and sinusoidal blood congestion (Asterisk), **(f)** of D5 group showing lipid depletion of hepatocytes (White arrow), necrotic hepatocytes (N.h), and blood congestion (Asterisk). Hx & E stain.

The kidney of the control group of *O. niloticus* consisted of many nephrons, which in turn consisted of a Malpighian corpuscle, short neck tubule, proximal convoluted tubule and distal convoluted tubule. Each Malpighian corpuscle was built up of a highly vascularized glomerulus, which is enveloped by the Bowman's capsule. The neck tubule is lined with cuboidal epithelium that possesses homogeneous eosinophilic cytoplasm and large, rounded and centrally located nuclei. The proximal convoluted tubule is lined with columnar or pyramidal epithelial cells that were provided with prominent and deeply stained brush border. The distal convoluted tubule was lined with low pyramidal or short columnar epithelial cells that lacking the brush border (Fig. 2a). In addition, the kidney of fish that fed D2 and D3 was not affected and appeared normal as the control group without any histopathological alterations (Figs. 2b & 2c). However, the kidney of fish that fed D4 and D5 revealed tubular degeneration, glomerular atrophy and blood congestion (Figs. 2d & 2e). Likewise, Omar *et al*. (2013) and Alm-Eldeen *et al*. (2018) demonstrated that there were histopathological changes in the kidney of iron exposed *O. niloticus* which included damage of kidney tubules, tubular necrosis, separation of tubular epithelial cells and shrinkage of the glomeruli. In addition, Rani and Raju (2007) postulated that there were histological changes in the kidney of *Tilapia Mossambica* that exposed to arsenic toxicity included shrinkage of glomeruli and tubular degeneration.

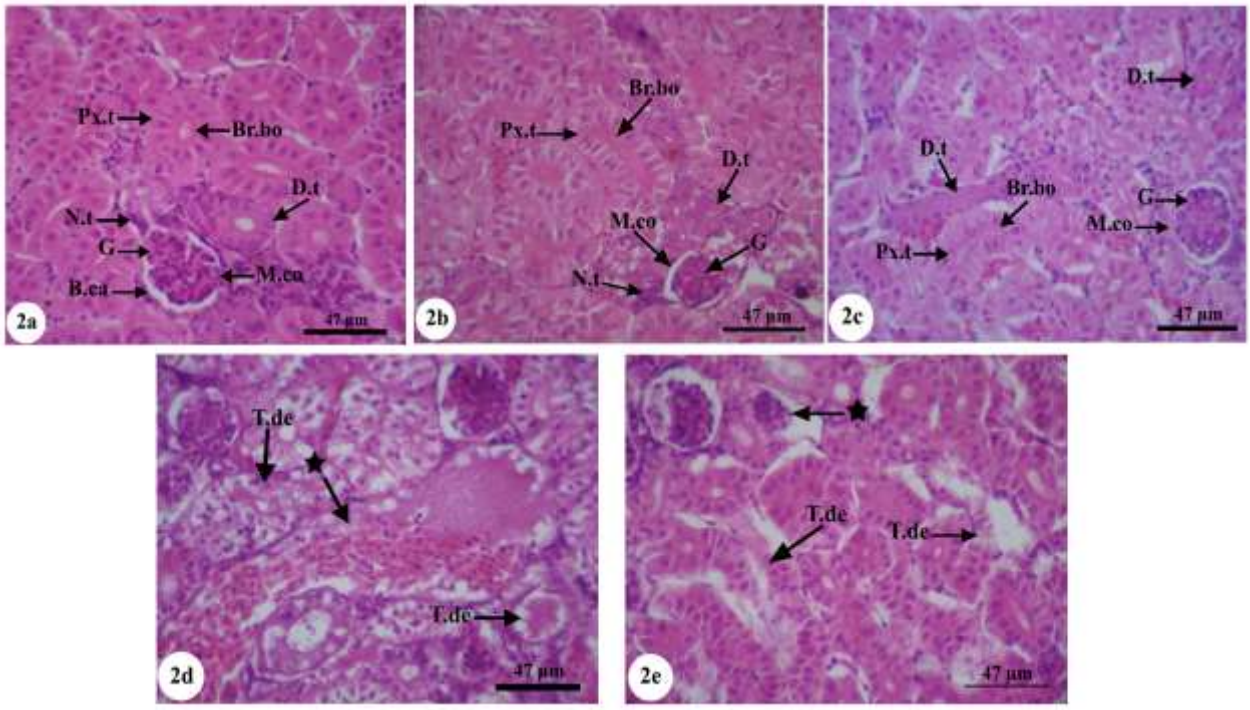

Fig. 2. photomicrographs of T. S. of the kidneys of *O. niloticus* **(a)** of the control group showing the Malpighian corpuscle (M.co), glomerulus (G), Bowman's capsule (B.ca), neck tubule (N.t), proximal convoluted tubule (Px.t), bruch border (Br.bo), and distal convoluted tubule (D.t), **(b)** of D2 group showing the normal histological structure, Malpighian corpuscle (M.co), glomerulus (G), neck tubule (N.t), proximal convoluted tubule (Px.t), Bruch border (Br.bo) and distal convoluted tubule (D.t), **(c)** of D3 group showing the normal histological structure, Malpighian corpuscle (M.co), glomerulus (G), proximal convoluted tubule (Px.t), bruch border (Br.bo) and distal convoluted tubule (D.t), **(d)** of D4 group showing tubular degeneration (T. De) and blood congestion (Asterisk), **(e)** of D5 group showing tubular degeneration (T. De) and glomerular atrophy (Asterisk). Hx & E stain.

The gills of the control group of *O. niloticus* were composed of numerous gill filaments that had numerous thin gill lamellae. The filament epithelium that lined the gill filaments consisted of a thin stratified epithelium which was formed mainly of pavement cells. The pavement cells are polygonal in shape and possess homogenous cytoplasm and centrally located nuclei. Moreover, the gill lamellae were lined by the lamellar epithelium that was composed of flattened respiratory cells. Each gill lamella was formed of a median vascular core, the pillar capillaries, which were separated by the pillar cells. The pillar cells were cuboidal in shape with rounded centrally located nuclei (Fig. 3a). However, the gills of all treated groups showed severe histopathological alterations including hyperplasia of filament epithelium, degeneration of pavement cells, lamellar fusion, epithelial lifting and lamellar aneurysms (Fig. 3b - 3f). The proliferative changes in gill filaments and lamellar fusions may lead to a great disturbance of gas exchange and ionic regulation (Mohamed, 2008). In addition, alterations like epithelial lifting, hyperplasia of the epithelial cells and fusion of lamellae were examples of defence mechanisms that lead to increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Flores-Lopes and Thomaz, 2011). The present gills histopathological changes were in accordance with Badr *et al*. (2014) and Abdel-Khalek (2015) who found that *O. niloticus* gills which exposed to iron showed abnormal lamellar arrangement, congestion, fusion of the secondary lamellae, epithelial lifting of secondary lamellae and hyperplasia. Besides, Ahmed *et al*. (2013) stated that the gills were the primary target tissue affected by arsenic and showed epithelial hyperplasia, epithelial lifting, lamellar fusion, aneurysm and necrosis.

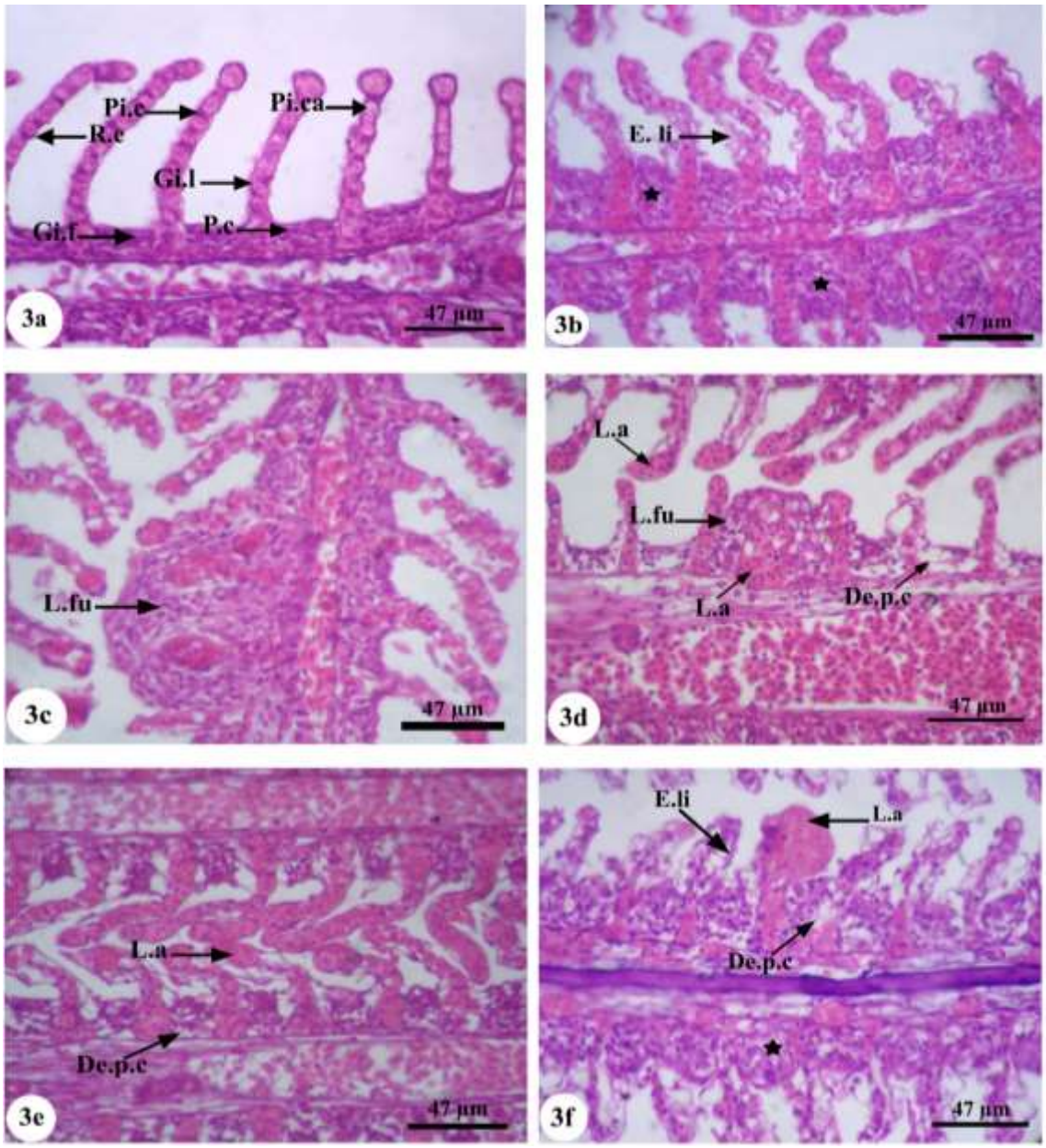

Fig. 3. photomicrographs of sagittal section of the gills of *O. niloticus* **(a)** of control group showing gill filament (Gi. f), gill lamellae (Gi. l), pavement cells (P. c), respiratory cells (R. c), pillar capillaries (Pi.Ca) and pillar cells (Pi. c), **(b)** of D2 group showing hyperplasia of gill filament epithelium (asterisk) and epithelial lifting (E. li), **(c)** of D2 group showing lamellar fusion (L.fu), **(d)** of D3 group showing degeneration of pavement cells (De.p.c), lamellar fusion (L. fu) and lamellar aneurysm (L.a), **(e)** of D4 group showing degeneration of pavement cell (De.P.C) and lamellar aneurysm (L.a), **(f)** of D5 group showing hyperplasia of filament epithelium (asterisk), degeneration of pavement cells (De.p.c) lamellar aneurysm (L.a) and epithelial lifting (E. li) Hx & E stain.

The present work concluded that PBM could replace FM at level of 25% and 50% for *O. niloticus* which showed the best growth performance, feed utilization and fish health. So, it is advisable to substitute FM by the cheap, available and safe PBM at levels of 25% and 50% in diets for *O. niloticus*.

REFERENCES:

Abdel-Khalek AA. 2015. Risk assessment, bioaccumulation of metals and histopathological alterations in Nile tilapia (*Oreochromis niloticus*) facing degraded aquatic conditions. Bull. Environ. Contam. Toxicol., 94(1): 77-83.

Ahmed MK, Al-Mamun MH, Parvin E, Akter MS, Khan MS. 2013. Arsenic induced toxicity and histopathological changes in gill and liver tissue of freshwater fish, tilapia (*Oreochromis mossambicus*). Exp. Toxicol. Pathol., 65(6): 903-909.

Akande KE, Doma UD, Agu HO, Adamu HM. 2010. Major anti-nutrients found in plant protein sources: their effect on nutrition. Pak. J. Nutr., 9(8): 827-832.

Aladetohun NF, Sogbesan OA. 2013. Utilization of blood meal as a protein ingredient from animal

waste product in the diet of *Oreochromis niloticus*. Int. J. Fish. Aquacult., 5(9): 234:237.

Alcorlo P, Geiger W, Otero M. 2004. Feeding preferences and food selection of the red swamp crayfish (*Procambarus clarkii*) in habitats differing in food item diversity. Crustaceana, 77(4): 435-453.

Alm-Eldeen AA, Donia T, Alzahaby S. 2018. Comparative study on the toxic effects of some heavy metals on the Nile Tilapia, *Oreochromis niloticus*, in the Middle Delta, Egypt. Environ. Sci. Poll. Res. Int., 25(15): 14636-14646.

AOAC. 1995. Official methods of analysis. Association of Official Analytical Chemists, 16th ed. Washington, USA, pp. 1298.

AOAC. 2012. Official methods of analysis, Association of Official Analytical Chemists, 19th ed. Washington, USA, pp. 3172.

APHA. 1999. Standard methods for the examination of water and wastewater. American Water Works Association, American Public Health Association, Water Environment Federation, Washington, USA.

Ayoola A. 2010. Replacement of fish meal with alternative protein sources in aquaculture diets. Thesis Degree of Master of Science Faculty of North Carolina State University, North Carolina, USA. Nutr. Food Sci., pp. 145.

Badr AM, Mahana NA, Eissa A. 2014. Assessment of heavy metal levels in water and their toxicity in some tissues of Nile tilapia (*Oreochromis niloticus*) in river Nile Basin at Greater Cairo, Egypt. Glob. Vet., 13(4): 432-443.

Ballestrazzi R, Lanari D, D'Agaro E, Mion A. 1994. The effect of dietary protein level and source on growth, body composition, total ammonia and reactive phosphate excretion of growing sea bass (*Dicentrarchus labrax*). Aquaculture, 127(2-3) 197–206.

Bhatnagar A, Singh G. 2010. Culture fisheries in village ponds: a multi-location study in Haryana, India. Agric. Biol. J. N. Am., 1(5): 961-968.

Boyd CE. 1998. Water quality for pond aquaculture. Research and Development Series No. 43. International Centre for Aquaculture and Aquatic Environments, Alabama Agricultural Experiment Station, Auburn University, Alabama.

Brown ME. 1957. Experimental studies on growth. In: "The physiology of fishes". Academic press, New York, pp. 361-400.

Caruso G. 2015. Use of plant products as candidate fish meal substitutes: an emerging issue in aquaculture productions. Fish. Aquacult. J., 6(3): 1-3.

Castell J, Tiews K. 1980. Report of the EIFAC, IUNS and ICES working group on standardization of methodology in fish nutrition research. Hamburg, Federal Republic of Germany.

Deore SV, Wagh SB. 2012. Heavy metal induced histopathological alterations in liver of *Channa gachua* (Ham). J. Exp. Sci., 3(3): 35-38.

Duncan NB. 1955. Multiple ranges and multiple F-tests. Biometrics, 11(1): 1-24.

El-Sayed AFM. 1998. Total replacement of fish meal animal protein sources in Nile tilapia (*Oreochromis niloticus*) Feeds. Aquacult. Res., 29(4): 275-280.

El-Sayed AFM. 1999. Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. Aquaculture, 179(1-4): 149-168.

El-Sayed AFM. 2006. Tilapia Culture. 1st Edn., CABI Publishing, Wallingford, UK., pp. 304.

El-Sayed AT, Martinez-Llorens S, Monino AV, JoverCerda M, Tomás-Vidal A. 2016. Effects of weekly feeding frequency and previous ration restriction on the compensatory growth and body composition of Nile tilapia fingerlings. Egypt. J. Aquat. Res., 42(3): 357-363.

Erickson RJ, Mount DR, Highland TL, Hockett JR, Jenson CT. 2011. The relative importance of waterborne and diet borne arsenic exposure on survival and growth of juvenile rainbow trout. Aquat. Toxicol., 104(1-2): 108–115.

Fall J, Tseng Y, Ndong D, Sheen S. 2012. The effects of replacement of soybean meal by shrimp shell meal on the growth of hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*) reared under brackish water. Int. J. Fish. Aquacult., 4(5): 85-91.

FAO. 1983. Compilation of legal limits for hazardous substances in fish and fishery products. Food and Agriculture Organization of United Nations. Rome, Italy.

FAO. 2017. Global Aquaculture Production1950-2015. Food and Agriculture Organization of United Nations. Rome, Italy.

Farahiyah IJ, Wong HK, Zainal AAR, Ahmed A. 2015. Fish offal meal as an alternative protein source of fish meal for tilapia, *Oreochromis* sp. Mar. J. Anim. Sci., 18(2): 81-86.

Flores-Lopes F, Thomaz AT. 2011. Histopathologic alterations observed in fish gills as a tool in environmental monitoring. Braz. J. Biol., 71(1): 179-188.

Garcia RJ, Kalinowski TH, Izquierdo SL, Robaina ER. 2010. Marine and freshwater crab meals in diets for red porgy (*pagrus pagrus*): effect on growth, fish composition and skin colour. Aquacult. Res., 41(12): 1759-1769.

Halver JE, Hardy RW. 2002. Fish Nutrition. 3rd edition. Academic Press; pp. 500.

Hardy RW. 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquacult. Res., 41(5): 770-776.

Hasan MT, Khalil SMI, Kashim M A, Hashem S, Mazumder SK. 2012. Substitution of fish meal by meat and bone meal for the preparation of tilapia fry feed. Int. J. Anim. Fish. Sci., 5(5): 464-469.

Hu L, Yun B, Xue M, Wang J, Wu XF, Zheng YH, 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, 372-375: 52-61.

Humason GL. 1972. Animal Tissue Techniques. 3rd edition. W. H. Freeman Company, San Francisco.

Ibrahim AM, Khalil MT, Mobarak FM. 1995. On the feeding behavior of the exotic crayfish *Procambarus clarkii* in Egypt and its prospects in the biocontrol of local vector snails. J. Union Arab Biol., 4(A): 321-340.

IMF. 2017. Primary Commodity Prices. International Monetary Fund, Research Department, commodities unit, pp. 1-23.

Kaushik SJ, Cravedi JP, Lalles JP, Sumpter J, Fauconneau B, Laroche M. 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 133(3-4): 257-274.

Keremah RI. 2013. The effects of replacement of fish-meal with crab-meal on growth and feed utilization of African giant catfish, *Heterobranchus longifilis*, fingerlings. Int. J. Fish. Aquacult., 5(4): 60-65.

Kubiriza GK, Akol AM, Arnason J, Sigurgeirsson O, Snorrason S, Tomasson T, Thorarensen H. 2016. Practical feeds for juvenile Nile tilapia (*Oreochromis niloticus*) prepared by replacing *Rastrineobola argentea* fish meal with freshwater shrimp (*Caridina nilotica*) and mung bean (*Vigna radiata*) meals. Aquacult. Nutr., 24(1): 94-101.

Leal ALG, De Castro PF, De Lima JPV, De Souza Correia E, De Souza Bezerra R. 2010. Use of shrimp protein hydrolysate in Nile tilapia (*Oreochromis niloticus* L.) feeds, Aquacult. Int., 18(4): 635-646.

Makori AJ, Abuom PO, Kapiyo R, Anyona DN, Dida GO. 2017. Effects of water physico-chemical parameters on tilapia (*Oreochromis niloticus*) growth in earthen ponds in Teso North Sub-County, Busia County. Fish. Aquat. Sci., 20(1): 30-39.

Mjoun K, Rosentrater K, Brown ML. 2010. Tilapia: Environmental biology and nutritional requirements. Fact Sheets. Paper 164.

Mohamed, FAS. 2008. Bioaccumulation of selected metals and histopathological alterations in tissues of *Oreochromis niloticus* and *Lates niloticus* from Lake Nasser, Egypt. Glob. Vet., 2(4): 205-218.

Nguyen TN, Davis DA, Saoud, IP. 2009. Evaluation of alternative protein sources to replace fish meal in practical diets for juvenile tilapia (*Oreochromis* spp.). J. World Aquacult. Soc., 40(1): 113-121.

NRC. 1993. Nutrient Requirements of Fish. National Research Council. The National Academies Press, Washington, D. C., USA, pp. 124.

NRC. 2011. Nutrient Requirements of Fish and shrimp. National Research Council. The National Academies Press, Washington, D. C., USA, pp. 392.

Nyström P. 1999. Ecological impact of introduced and native crayfish on freshwater communities: European perspectives. In: "Crayfish in Europe as Alien Species—How to Make the Best of a Bad Situation? (Gherardi F, Holdich DM. Eds)". Balkema, Rotterdam, pp. 63-85.

Omar WA, Zaghloul KH, Abdel-Khalek AA, Abo-Hegab S. 2013. Risk assessment and toxic effect of metal pollution in two cultured and wild fish species from highly degraded aquatic habitats. Arch. Environ. Contam. Toxicol., 65(4): 753-764.

Palupi ET, Setiawati M, Lumlertdacha S, Suprayudi MA. 2019. Growth performance, digestibility, and blood biochemical parameters of Nile tilapia (*Oreochromis niloticus*) reared in floating cages and fed poultry by-product meal. J. Appl. Aquacult., 31: 1-18.

Rachmawati D, Samidjan I. 2019. The effects of chicken feather silage substitution for fish meal in the diet on growth of saline tilapia fingerlings (*Oreochromis niloticus*). IOP Conf. Ser.: Earth Environ. Sci. 246 012015

Rani AS, Raju TN. 2007. Histological variations in freshwater teleost, *Tilapia mossambica* (Peters) on exposure to arsenic. Nat. Environ. Pollut. Technol., 6(4): 637-642.

Rathore SS, Yusufzai SI. 2018. Emerging animal protein source to replace fish meal in the diets of Nile tilapia (*Oreochromis niloticus*) Fry. Int. J. Curr. Microbiol. App. Sci., 7(5): 1227-1235.

Riche M, Garling D. 2003. Feeding tilapia in intensive recirculating systems. NCRAC extension fact sheets. 6: 1-6.

Robaina L, Corraze G, Aguirreb P, Blancb D, Melcionc JP, Kaushik S. 1999. Digestibility, postprandial ammonia excretion and selected plasma metabolites in European sea bass (*Dicentrarchus labrax*) fed pelleted or extruded diets with or without wheat gluten. Aquaculture, 179(1-4): 45-56.

Rodde R, Einbu A, Varum K. 2008. A seasonal study of the chemical composition and chitin quality of shrimp shells obtained from northern shrimp vandals borealis. Carbohydr. Polym., 71(3): 388-393.

SPSS. 2013. Computer Software, Ver. 17. SPSS Company, London, UK.

Tacon AGJ. 1990. Standard methods for the nutrition and feeding of farmed fish and shrimp. Redmond, Washington: Argent Laboratories Press, pp. 208.

Tacon AGJ, Metian M. 2008. Global overview on the use of fish meal and fish oil in industrially compounded aqua feeds: trends and future prospects. Aquaculture, 285(1-4): 146-158.

Thongrod S. 2007. Analysis of feeds and fertilizers for sustainable aquaculture development in Thailand. In: Study and Analysis of Feeds and Fertilizers for Sustainable Aquaculture Development. (Hasan MR, Hecht T, De Silva SS, Tacon AGJ. eds)". FAO Fisheries Technical Paper. No. 497. Rome, FAO, pp. 510.

Usmani N, Jafri AK. 2002. Influence of dietary phytic acid on the growth, conversion efficiency and carcass composition of *Cirrhinus mrigala* (Hamilton) fry. J. World Aquat. Soc., 33(2): 199-204.

Yones AMM, Metwalli AA. 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*). J. Aquacult. Res. Dev., 7(1): 6 pages.

تأثير الاستبدال الجزئي أو الكلي لمسحوق السمك بالمخلفات الثانوية لاستاكوزا المياه العذبة في علائق البلطي النيلي

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تهدف الدراسة الحالية الى تقييم تأثير الاستبدال الجزئي أو الكلى لبروتين مسحوق السمك بالمخلفات الثانوية لاستاكوزا المياه العذبة في علائق البلطي النيلي وتأثيرها على معدلات النمو والاستفادة الغذائية والحالة الصحية للأسماك. وقد تم تكوين خمس علائق غذائية تحتوي على 30% بروتين والتي تحتوي على نسب استبدال لمسحوق السمك بمخلفات استاكوزا المياه العذبة التالية: 0، 25، 50، 75 ،100% كما تم تغذية أسماك البلطي (10.94 جرام وزن مبدئي) عليها لمدة أربعة أشهر في أحواض سيراميك. و لقد أظهرت نتائج هذه الدراسة أن معدلات النمو والاستفادة الغذائية في جميع المجموعات مختلفة عن المجموعة الضابطة (ذات معدل استبدال 0%) ولقد سجلت المجموعات ذات نسب الاستبدال 25% و50 % أفضل نتائج ثم تليهما المجموعات ذات نسب الاستبدال 75% و 100 %. كما أوضحت الدراسة النسيجية للكبد والكلى والخياشيم وجود تغييرات نسيجية متفاوتة خاصة في المجموعات التي تمت تغذيتها على العلائق ذات معدل الاستبدال 75% و100 % والتي قد يرجع سببها الى وجود تركيزات من الزرنيخ والحديد أعلى من الحدود المسموح بها في مخلفات الاستاكوزا. وبناء على نتائج البحث الحالي يُوصى بإمكان استبدال مسحوق السمك بمسحوق مخلفات استاكوزا المياه العذبة رخيصة الثمن بمعدل 25% أو 50% حيث أعطت هذه العلائق أفضل معاملات نمو وأفضل استفادة غذائية وأحسن حالة صحية.