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Replacement of fishmeal with processed meal from knife fish *Chitala ornata* in diets of juvenile Nile tilapia *Oreochromis niloticus*

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ABSTRACT

A 60-day feeding trial was conducted to assess the effects of processed meal from knife fish *Chitala ornata* (KFM) as fishmeal replacement in diets of Nile tilapia *Oreochromis niloticus* juveniles. Five isonitrogenous (36.4% in dry matter) and isolipidic diets (8.6% in dry matter) with 0 (D1), 25 (D2), 50 (D3), 75 (D4) and 100% (D5) KFM inclusions were prepared. With a stocking density of 15 fish (0.59 ± 0.01 g) per tank, tilapia juveniles were distributed randomly in fifteen 30-L tanks. Results indicate a significant increase ($P < 0.05$) in the percent average weight gain, specific growth rate, and feed intake with increasing KFM inclusion up to the level of 75%. There were no adverse effects observed in both blood profile and carcass composition of the tilapia. Hepatosomatic index of fish significantly increased ($P < 0.05$) when KFM was included into the diet of tilapia, compared to those fed D1. Viscerosomatic indices were not significantly different ($P > 0.05$) among treatments. Results of hepatic histopathology showed absence of tumors, lesions and parenchymal inflammation in all treatments. However, mild cell membrane lysis and mild and mild to moderate apoptosis were evident in liver samples. Based on the results, KFM can partially and completely replace dietary protein from fishmeal. Moreover, D4 (75% KFM) is considered the optimal KFM replacement level for Nile tilapia juveniles.

1. Introduction

Exotic or non-indigenous species (NIS) are organisms that are displaced beyond their native ranges (Welcomme, 1988). NIS are introduced into new environments via various routes. Many fish species are introduced outside their natural aquatic habitat through international trade and aquaculture (Strayer, 2010). Some are imported intentionally and have accidentally escaped from captivity. Imported livestock and produce can also be unintentional routes for NIS (Lovell and Stone, 2005). Assessing the impacts on NIS is complex; it is difficult to quantify their effects across multiple taxa and regional scales (Nghiem et al., 2013). Some NIS fail to adapt to its new environment and eventually die; while some are able to survive causing destruction and replacement of other species (Lovell and Stone, 2005). Other NIS have the ability to cause harm on new ecosystems hence, termed as "invasive". Introduction of non-native fish species as a new functional group in natural aquatic systems has a serious effect on overall ecosystem functioning as well as on the behavior, number and distribution of native species (Simon and Townsend, 2003).

Biological invasion of NIS is difficult to undo or manage especially when the alien fish species already established itself and extensively proliferated. It is suggested that nutrient loading, which is common in fresh waters enhances the establishment of NIS as free resources become available to them (Strayer, 2010). And this is evidently magnified in waters used for aquaculture. Hence, an array of management strategies is being employed to reduce and suppress biological invasion. Strategies include using the invasive species as a potential biodiesel source, component of liquid fertilizers for soil applications, its bones as carbon filters, or for fishmeal production (Aranda et al., 2010; Gümüş et al., 2010; Punongbayan, 2012).

Various NIS have been utilized as fishmeal protein replacement. Utilization of invasive species namely, Asian carp *Hypophthalmichthys* spp. and menhaden fishmeal for largemouth Bass (*Micropterus salmoides*), a carnivorous fish (Bowzer et al., 2014). Results of the study suggest that performance is better among fish

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fed diets with higher levels of NIS fishmeal inclusion, regardless of origin. In Nile tilapia aquaculture, shrimp head meal, golden apple snail meal, blood meal, and Pangasius by-product meal (Gustavsson, 2016) have been utilized as fishmeal replacement. Pangasius by-product meal was reported to completely replace fishmeal without adverse effect on fish growth performance. Fermented golden snail meal with lactic acid bacteria and molasses was shown to be a potential protein source and may be used as complete fishmeal replacement in sex-reversed red tilapia (Chimsung and Tantikitti, 2014). Onslaught management of sand smelt invasion in Turkey was conducted by utilizing the invasive fish for fishmeal production (Gümüş et al., 2010). This strategy was also implemented in managing Hancok (*Pterigoplichthys multiradiatus*) invasion using the fish silage for feed formulation in Nile tilapia diets.

The future trend of utilizing non-indigenous and invasive species in aquafeeds is promising in the aspects of increasing productivity, nurturing environment sustainability, and reducing pressure on limited wild fisheries. Invasive species meals would also pose as an easy solution to eradicate an invasive species from the invaded ecosystem, translate to generation of income and market for the product, and a good source of long chain n-3 fatty acids and other essential nutrients (Rust et al., 2011).

Aquaculture production in Laguna de Bay contributed 2.33% (48,767 metric tons) to the 2,093,371 metric tons total aquaculture production of the Philippines in 2006 (BFAR Region 1V-A 2007). Moreover, aquaculture production of the bay comprised 1.11% of the 4,409,526 metric tons total fisheries production of the country (Israel, 2007). Unfortunately, the aquaculture industry of Laguna de Bay is currently facing knife fish infestation and has become the center of population control efforts.

Ornamental fish trade has contributed to the introduction of knife fish to the Philippines (Guerrero, 2014). Knife fish share similar features with arowana (*Scleropages* spp.) and easily became a popular ornamental fish (Punongbayan, 2012). Nonetheless, it has been recently branded as the latest invasive fish species to the country's local lakes and rivers (Sonido, 2012). It is believed that the fish found its way into Laguna de Bay in two ways: accidental escape from aquariums and ornamental fish farms primarily due to flooding, or deliberate release of hobbyists into waterways when the fish got too big for aquariums and they wanted to get rid of them (Despuez, 2012). Field surveys have recently revealed the presence of knife fish in Laguna. In Laguna de Bay, the knife fish caught are presumed to be the *Chitala ornata* species (Philippine News Agency, 2012).

Having an aggressive and carnivorous nature, knife fish preys on smaller fish species, especially cultured milkfish (*Chanos chanos*), bighead carp (*Aristichthys nobilis*), and tilapia (*Oreochromis niloticus*) in fish pens and cages (Mayuga, 2013). Knife fish fry is very small and can penetrate fish pens and cages. Once inside, the fish grows and consume cultured stocks inside the pens and cages (Despuez, 2012). Currently, knife fish is a regular catch among fishermen instead of cultured and indigenous species (Mayuga, 2013). Moreover, knife fish has a very low market demand and a market value of US$ 0.10–0.30 per kg only. Its low market demand is due to the consumer perception that the fish is exotic and not part of the regular fish staple (Despuez, 2012). The extremely high supply and low demand for knife fish translates huge investment loss on the livelihood of those dependent on the fishing industry. Proposed economic utilization strategies of knife fish include postharvest processing and fishmeal production. The reduction of knife fish into fishmeal is considered to intensify the aquaculture of cultured fish and shrimp species amidst the infestation. Furthermore, knife fishmeal processing is believed to facilitate the eradication of this invasive species.

Table 1
Proximate composition of processed meal from knife fish.

Nutrient component	(in% dry matter, DM)
Moisture	8.51 ± 0.04
Crude lipid	3.22 ± 0.14
Crude protein	62.36 ± 0.95
Crude fiber	0.18 ± 0.02
Ash	22.85 ± 0.41

Values are means of triplicate groups ± SEM.

Table 2
Test diets for partial and complete fishmeal replacement using KFM.

Ingredient (g per kg diet)	D1	D2	D3	D4	D5
Fishmeal	56.5	44	29.5	15	0
KFM	0	13	28	43	58.5
Cod liver oil	2	2.5	3	3.5	4
Soy lecithin	4	4	4	4	4
Vitamin mix[a]	3	3	3	3	3
Mineral mix[b]	1.5	1.5	1.5	1.5	1.5
Cornstarch	23	23	24	25	26
CMC	10	9	7	5	3
Total	100	100	100	100	100

[a] As reported by Monje et al. (1996).
[b] Monje et al. (1996).

The purpose of this study is to determine the efficiency of processed meal from knife fish *C. ornata* as fishmeal replacement in diets of juvenile Nile tilapia *O. niloticus*.

2. Materials and methods

2.1. Collection and authentication of knife fish

Knife fish samples were procured from the Fisheries and Aquatic Resources Management Council, Santa Cruz, Laguna, Philippines. Knife fish were caught from the major tributaries of Laguna de Bay, mainly Santa Cruz River from November to December 2014. Each fish was washed and kept at −25 °C prior to authentication. The fish samples were authenticated as *Chitala ornata* at the Bureau of Fisheries and Aquatic Resources-National Freshwater Fisheries Technology Center (BFAR-NFFTC), Central Luzon State University Compound, Science City of Munoz, Nueva Ecija, Philippines.

2.2. Processing of knife fish meal

Authenticated fish samples were cleaned and rinsed with water and cut into smaller portions (approximately 50–100 g). The portions were boiled for 30 min at 100 °C to separate water and oil. Then, the liquid was removed using a strainer. The press cake was sun-dried for 6–12 h or until brittle and flaky in texture. The product was ground and sifted (425 μm in size). The proximate composition (Table 1) of the knife fishmeal (KFM) was analyzed at Institute of Chemistry Analytical Services Laboratory, University of the Philippines-Los Baños (UPLB), Laguna, Philippines. Moisture and ash contents were measured using the Association of Official Analytical Chemists (AOAC, 1990) standard methods. Kjeldhal distillation method, Soxhlet extraction and Weende method were used for determination of crude protein, crude lipid and crude fiber contents, respectively.

2.3. Formulation and preparation of diets

Iso-nitrogenous and iso-lipidic test diets were formulated to meet the nutritional requirements of *O. niloticus* juveniles. The test diets (Table 2) were tailored to contain 36.5% protein and 8.2% lipid. KFM replaced 0% (D1), 25% (D2), 50% (D3), 75% (D4), and 100%

(D5) of the main protein source, fishmeal. D1 served as control diet and contained fishmeal as sole protein source. Cod liver oil and soy lecithin were used as lipid sources. Carboxymethyl cellulose (CMC) and cornstarch were used as carbohydrate sources and for improved pellet binding properties. All dry ingredients were mixed thoroughly using a large mixer for 10 min or until homogenized. Vitamin and mineral premix were added first in a small amount (i.e., 10% of the total batch) before blended into the rest of the mixture to ensure even distribution. Then, lipid sources were added and mixed for another 5 min. Water was then added slowly until the water content of the mash ranged from 30% to 40% to achieve desired feed consistency. The feed mixture was passed through a pellet machine to produce 1.2–1.5 mm pellet size. The extruded pellets were cut into similar lengths followed by dry heating at 60 °C for 2 h using a convection oven. After drying and cooling, prepared diets were stored in sealed containers kept at −25 °C until used.

2.4. Experimental design

Feeding trials were conducted at the Ateneo de Manila Biological Research House of the Ateneo de Manila University, Quezon City from November 2014 to April 2015. A total of five hundred (500) *O. niloticus* juveniles were obtained from BFAR-NFFTC in closed oxygenated bags. The juveniles were acclimatized and fed a commercial diet to apparent satiation for 2 weeks.

Nile tilapia juveniles with uniform body weight (0.59 ± 0.01 g) were distributed randomly in fifteen 30-L capacity tanks with a stocking density of 15 juveniles per tank. Each tank was filled with water delivered via recirculating aquaculture system and supplied with oxygen using air pumps continuously for 24 h. Temperature was monitored daily (26.1 °C ± 0.3). A photoperiod of 12 h light: 12 h dark was used throughout the experimental period. Experimental diets were randomly assigned among the fifteen tanks with 3 replicates per treatment. Fish was fed manually to apparent satiation twice daily at 08:00 h and 15:00 h for a period of 60 d. Uneaten feed was removed from the tanks 10 min after feeding. About 40% of the water was replaced daily. Complete water replacement was employed every 3 days.

2.5. Growth performance and feed utilization

After the 60-day feeding trial, parameters measured were percent average weight gain (% AWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF), feed intake (FI) and percent survival.

2.6. Fish carcass composition

Fish were starved for 24 h prior to final sampling. Fish was collected from each tank, weighed individually and anaesthetized by hypothermia treatment. Fish from each treatment was randomly selected for the removal of viscera and these were pooled for calculation of the viscerosomatic index (VSI). The liver was separated from the viscera, pooled and weighed to determine the hepatosomatic index (HSI). The VSI (Ighwela et al., 2014) and HSI (Agbo, 2008) were computed using the following:

$$\text{VSI} = (\text{viscera weight[g]}/\text{whole fish weight[g]}) \times 100$$

$$\text{HSI} = (\text{liver weight}/\text{body weight}) \times 100$$

The remaining fish were kept at −25 °C until analyzed for carcass composition. Carcass samples were sent to the Institute of Chemistry Analytical Services Laboratory-UPLB, College, Laguna, Philippines for proximate analysis. All proximate analyses were performed in triplicate according to Association of Official Analytical Chemists (AOAC, 1990) procedures.

2.7. Blood chemistry

Blood sample was also collected after anaesthetizing the fish by hypothermia treatment. From each replicate tank, blood was collected through the caudal vein of each fish using heparinized (Heparin, 5000 IU/mL) 25-gauge, 1-cc tuberculin syringes. The desired amount of extracted blood was contained in Eppendorf tubes rinsed with heparin. These were centrifuged to separate the serum at 3000 × *g* for 15 min using a microcentrifuge.

Blood chemical parameters such as total cholesterol (CHO), triglycerides (TGS), glucose (GLU), uric acid (URA), creatinine (CRE), serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic-pyruvic transaminase (SGPT) were determined for blood profile analysis using blood chemical analyzer kits (Stanbio Laboratory, Boerne, TX, USA) and a semi-automated chemistry analyzer (STATFAX Model 1904 plus, Awareness Technology Inc., Palm City, FL, USA) (Velasquez et al., 2016).

2.8. Hepatic histopathology

Immediately after weighing, liver samples were fixed in 10% formalin and sent to the Philippine Kidney Dialysis Foundation, Diliman, Quezon City for hepatic histological examination. The hepatic tissues were transversely cut and hematoxylin and eosin stain was applied. Samples were observed for abnormalities and lesions. Observations were recorded according to the reference used by Abdel-Moneim et al. (2012). Analysis was done in triplicate.

2.9. Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Analysis of variance was conducted using one-way ANOVA and *P* values were calculated to quantify levels of significance for each treatment type. Tukey post-hoc test was used to compare between means at $P \leq 0.05$. The IBM-SPSS v. 20 software was used for statistical analysis.

3. Results

3.1. Growth performance and feed utilization

The results (Table 3) indicate a significant increase ($P < 0.05$) in the% AWG, SGR, and feed intake with increasing KFM inclusion until 75% inclusion level. Fish fed 75% KFM diet exhibited numerically highest values. There are no significant differences ($P > 0.05$) in FCR, PER, CF, and% survival among treatments.

3.2. Blood chemistry

There were no adverse effects observed on the blood profile (Table 4) of KFM fed fish. Serum CHO, TGS, URA, CRE, SGPT and SGOT levels were not significantly different ($P > 0.05$) across all treatments. The GLU levels followed an increasing trend with increasing KFM inclusion levels.

3.3. Fish carcass composition

Significant differences ($P < 0.05$) were evident in the HSI of fish fed D1 when compared to those fed D2, D3, and D4. Numerically highest HSI was seen in fish fed D2 while numerically lowest was in fish fed D1. VSI levels were not significantly different ($P > 0.05$) among all treatments (Table 5).

Table 3
Growth performance of tilapia juveniles fed increasing KFM replacement levels.

Growth parameters	Diets				
	D1	D2	D3	D4	D5
% AWG	896.04 ± 37.78[a]	1195.37 ± 65.91[a]	1694.02 ± 57.27[b]	2021.14 ± 127.33[b]	1652.46 ± 84.53[b]
SGR	9.91 ± 0.18[a]	12.79 ± 0.49[a]	18.57 ± 0.90[b]	21.41 ± 1.78[b]	17.58 ± 0.90[b]
FCR	2.09 ± 0.06[a]	1.94 ± 0.10[a]	1.71 ± 0.16[a]	1.90 ± 0.05[a]	1.65 ± 0.07[a]
FI	0.21 ± 0.01[a]	0.25 ± 0.01[a,b]	0.32 ± 0.02[b]	0.40 ± 0.02[c]	0.29 ± 0.00[b]
PER	1.31 ± 0.04[a]	1.42 ± 0.07[a]	1.63 ± 0.16[a]	1.45 ± 0.04[a]	1.66 ± 0.07[a]
CF	1.75 ± 0.05[a]	1.64 ± 0.04[a]	1.67 ± 0.03[a]	1.69 ± 0.03[a]	1.62 ± 0.04[a]
% Survival	91 ± 4[a]	91 ± 4[a]	91 ± 2[a]	89 ± 4[a]	87[a]

Values are means of triplicate groups ± SEM. Means along a row with different letters are significantly different ($P < 0.05$).

Table 4
Blood chemistry of tilapia juveniles fed increasing KFM replacement levels.

Blood chemical parameters	Diets				
	D1	D2	D3	D4	D5
CHO (mg/dL)	124.33 ± 15.90[a]	115.73 ± 9.16[a]	133.27 ± 28.48[a]	139.53 ± 23.08[a]	127.57 ± 26.07[a]
TGS (mg/dL)	135.70 ± 8.42[a]	82.77 ± 4.29[a]	110.30 ± 17.52[a]	117.77 ± 25.83[a]	91.87 ± 7.09[a]
GLU (mg/dL)	100.53 ± 27.48[b]	93.00 ± 4.60[a]	105.00 ± 13.42[a,b]	110.90 ± 24.91[a,b]	127.60 ± 22.49[a,b]
URA (mg/dL)	5.90 ± 3.65[a]	3.83 ± 0.28[a]	5.50 ± 2.49[a]	3.27 ± 1.09[a]	3.63 ± 1.30[a]
CRE (mg/dL)	0.57 ± 0.13[a]	0.33 ± 0.09[a]	0.73 ± 0.30[a]	0.57 ± 0.09[a]	0.33 ± 0.15[a]
SGOT (U/L)	200.07 ± 20.44[a]	296.27 ± 23.75[a]	203.87 ± 104.53[a]	254.80 ± 39.19[a]	196.77 ± 73.20[a]
SGPT (U/L)	14.40[a]	12.80 ± 1.40[a]	11.70 ± 6.70[a]	11.90 ± 1.10[a]	11.87 ± 4.78[a]

Values are means of triplicate groups ± SEM. Means along a row with different letters are significantly different ($P < 0.05$).

Table 5
Fish body composition of tilapia juveniles fed increasing KFM replacement levels.

Parameters	Diets				
	D1	D2	D3	D4	D5
HSI	1.54 ± 0.54[a]	5.25 ± 0.51[b]	4.57 ± 0.20[b]	4.45 ± 0.56[b]	3.73 ± 0.60[a,b]
VSI	8.19 ± 0.50[a]	10.49 ± 0.73[a]	9.24 ± 1.07[a]	9.22 ± 0.39[a]	7.67 ± 0.38[a]

Values are means of triplicate groups ± SEM. Means along a row with different letters are significantly different ($P < 0.05$).

No significant differences ($P > 0.05$) were found in the ash content of fish fed increasing KFM inclusion levels. On the other hand, there were significant differences ($P < 0.05$) in the crude fat content of fish fed increasing KFM inclusion in diets except for fish fed D3 compared to fish fed D4 and D5. Highest crude fat content was evident in fish fed D3 while lowest was from fish fed D2.

Statistical analyses revealed significant difference ($P < 0.05$) between the moisture content of fish fed D2 and D4. Highest moisture content was obtained from fish fed D2 while the least was from fish fed D4. The protein content of fish fed D1 was significantly different with fish fed D2. Moreover, fish fed D2 had significantly different ($P < 0.05$) protein content compared with fish fed D4 and D5. Highest protein content was evident in fish fed D4 while fish fed D2 had the least protein content. Fiber content of fish fed D3 and D4 were significantly different ($P < 0.05$) with fish fed D5. The highest fiber content was from the control diet fed fish. Least fiber content was evident in fish fed D5 (Table 6).

Table 7
Hepatic histopathology for tilapia fed with increasing KFM replacement levels.

Parameters	Diets				
	D1	D2	D3	D4	D5
Presence of tumor/lesion	0	0	0	0	0
Parenchymal inflammation	0	0	0	0	0
Cell membrane lysis	1	1	1	1	1
Apoptosis	1	1	2	2	2

Legend: 0 = absent; 1 = mild; 2 = mild to moderate; 3 = moderate; 4 = severe.

3.4. Hepatic histopathology

Histological examination of hepatic tissues (Table 7) showed absence of tumors or lesions, and parenchymal inflammation. On the other hand, liver samples in all treatments showed mild cell membrane lysis, and mild, mild to moderate apoptosis (Fig. 1; Fig. 2).

4. Discussion

4.1. Growth performance and feed utilization

Increasing KFM replacement in diets resulted in better growth performance of tilapia juveniles. This can be attributed to improved protein composition and essential nutrients in the test diets (Chitmanat et al., 2009). The% AWG and SGR of KFM fed fish fol-

Table 6
Carcass proximate composition of tilapia juveniles fed increasing KFM replacement levels.

Composition (in% DM)	Diets				
	D1	D2	D3	D4	D5
Ash	6.00 ± 0.05[a]	5.58 ± 0.42[a]	5.14 ± 0.15[a]	5.41 ± 0.32[a]	4.80 ± 0.07[a]
Crude Fat	4.75 ± 0.03[b]	4.09 ± 0.05[a]	5.31 ± 0.06[c]	5.22 ± 0.07[c]	5.13 ± 0.06[c]
Moisture	72.26 ± 0.19[a,b]	73.57 ± 0.49[b,c]	72.89 ± 0.23[a,c]	71.83± 0.28[a]	73.05 ± 0.02[a,b]
Protein	15.59 ± 0.28[b,c]	13.90 ± 0.39[a]	14.71 ± 0.10[a,b]	15.96 ± 0.14[c]	15.31 ± 0.11[b,c]
Fiber	0.19 ± 0.03[b]	0.06 ± 0.01[a,c]	0.14 ± 0.03[b,c]	0.15 ± 0.02[b,c]	0.02 ± 0.00[a]

Values are means of triplicate groups ± SEM. Means along a row with different letters are significantly different ($P < 0.05$).

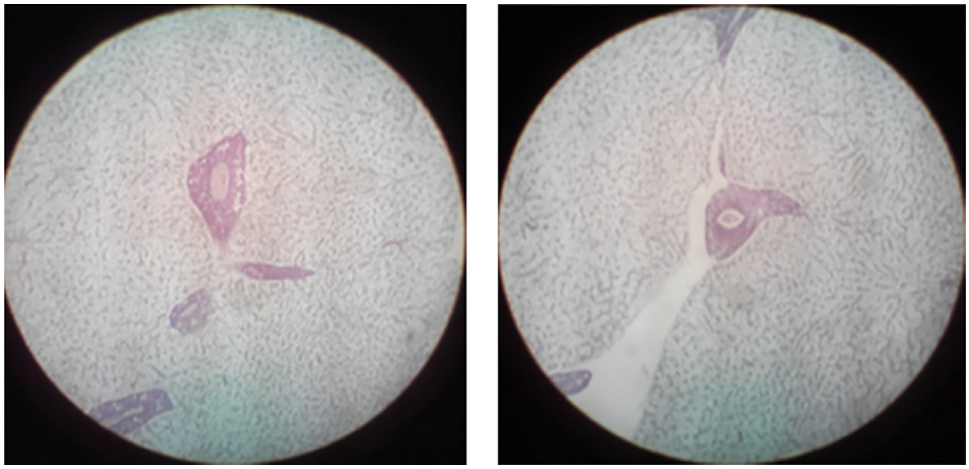

Fig. 1. Histopathology of hepatic tissues showing normal conditions without apoptosis (left) and mild cell membrane lysis (right).

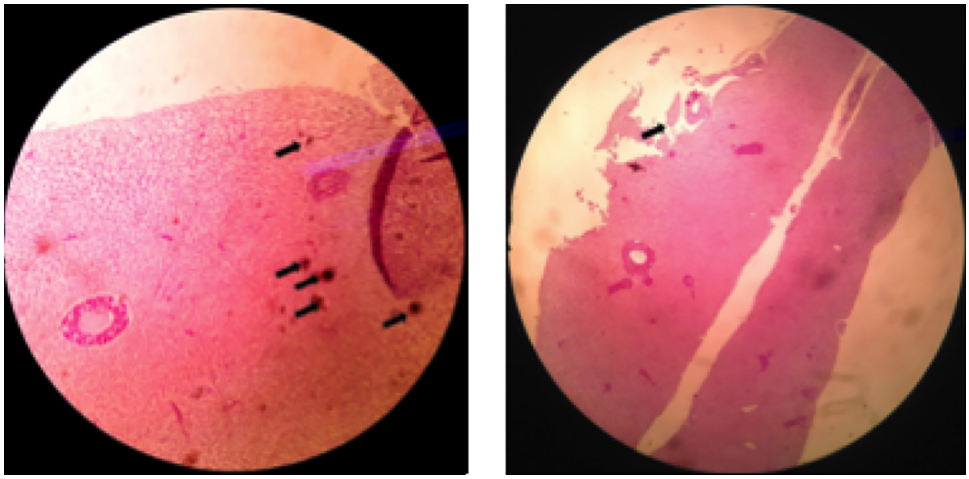

Fig. 2. Histopathology of hepatic tissues showing mild apoptosis (left) and mild cell membrane lysis (right).

lowed an increasing trend and this could signify that feed proteins were efficiently converted to fish tissues (Sogbesan and Ugwumba, 2008). Animal proteins as fishmeal replacement are reported to effectively improve fish growth performance (Lee et al., 2012). The results are comparable with earlier studies on positive response of farmed red tilapia juveniles when using blowfly maggot meals as fishmeal substitute in diets (Sing et al., 2014). Other protein sources such as processed meat meal and bone meal were also found to demonstrate no adverse effects on growth, survival, and feed conversion ratio of juvenile grouper (Millamena, 2002). Studies on common carp, cuneate drum and olive flounder suggested that combined protein sources were optimal than a single protein source for fish diets (Hossain and Jauncey, 1989; Kikuchi, 1999; Guo et al., 2007). Higher growth performance in KFM fed fish confirms the synergistic effect of two protein sources (i.e., fishmeal and KFM) as more superior than having one protein source (Sogbesan and Ugwumba, 2008).

Fish fed KFM inclusion diets showed higher feed intake values and was significantly different from the lowest FI value of the fish fed control diet. This shows that there was increased palatability and acceptability of the diets. Similarly, the use of termite meal as animal protein supplement in diets of *Heterobranchus longifilis* showed feed intake to be highest from fish fed 75% termite meal (Sogbesan and Ugwumba, 2008). Although present results show no significant differences in FCR and PER, a trend showing low FCR and high PER values in fish fed KFM diets may imply efficiency of feed use and consequent conversion to animal protein and reduced production of wastes (Boyd et al., 2007).

4.2. Blood chemistry

There were no significant differences among the values for total cholesterol and triglycerides across all treatments. This suggests the culture system provided optimal conditions for growth. Blood cholesterol levels are affected by various factors such as cholesterol metabolism and feed consumption (Tocher et al., 2008). The combination of animal protein in the test diet might have avoided hypocholesterolemic effects as reported in other studies involving plant-based fishmeal replacement (Chen et al., 2003; Borgeson et al., 2006; Soltan et al., 2008; Lim and Lee, 2009). Triglycerides along with cholesterol and glucose are the main energy reserves for fish and these are produced in excess during periods of stress.

Glucose levels increased with increasing KFM inclusion levels. Serum glucose in fish is affected by various factors such as stress, environment and diet formulation (Chen et al., 2003). High glucose values may indicate short-term intensive stress from external conditions and handling (Svobodova et al., 1991). At such stressful situations, effects of increased catecholamine action in the liver lead to higher cortisol and glucose in the serum followed by a slow decrease (Svobodova et al., 1991). Hyperglycemia can also be a sign of disrupted carbohydrate metabolism due to increased glycogen

breakdown in the liver and glucose synthesis from extra-hepatic tissue proteins and amino acids (Ozgur and Kargm, 2010).

The levels of uric acid and creatinine in the serum are useful indices for the overall health of fish kidney and gills (Campbell, 2004). In the present study, levels of uric acid and creatinine were not significantly different. Related research on sea bass and broiler chickens observed the reduction of serum uric acid and creatinine levels, indicating protein retention from balanced dietary amino acid (Tulli et al., 2007; Yilmaz et al., 2012; Ghazalah and Ali, 2008).

Serum enzymes such as SGOT and SGPT are known to be health markers of the animal's physiologic state (Ozgur and Kargm, 2010). It is known that SGOT and SGPT are sensitive indices even for minor cellular or tissue damage (De la Tore et al., 2000; Palanivelu et al., 2005). In the present study, there were no significant differences in SGOT and SGPT levels among all treatments. Studies on crucian carp fed dietary free gossypol at 260.57 mg/kg showed significantly higher SGOT and SGPT compared to those fed diets containing 104.23 mg/kg FG (Jiang et al., 2012). It is evident that although SGOT levels were high, SGPT serum levels of all KFM fed fish remain low. In elucidating true liver condition, all serum hepatic enzyme levels must express equivalent values. Thus, the contrasting results in serum SGOT and SGPT may not indicate direct presence and severity of liver damage.

4.3. Fish body and carcass composition

HSI value of the control group was significantly different with fish fed 25–75% KFM diets. This could be due to increased carbohydrate level in the diet, which is in agreement with similar studies on rainbow trout (Gumus and Ikiz, 2009). Increased glucose and SGOT concentration in the serum along with higher HSI values demonstrates the significant effect of KFM diets on the carbohydrate metabolism of the fish.

Studies on fish composition have shown a positive correlation between HIS and liver metabolism (Stephensen et al., 2000). Generally, increased lipidosis causes higher HSI (Liu et al., 2010). Nonetheless, effects of dietary inclusion of high fat and animal proteins on hepatic conditions of fish are contrasting and unclear. As in a study by Caballero et al. (1999), gilthead sea bream fed 22% lipid in diet showed no effects on fish quality and liver histology. Similarly, there was no alteration in liver histopathology observed when cod was fed a mixture of soy protein concentrate and wheat gluten at a maximum inclusion of 44% (Hansen et al., 2006). Moreover, there was no alteration in hepatocyte morphology and hepatic cell quantification when sharp snout sea bream was fed plant protein substitute (Nogales-Mérida et al., 2010). In another study, however, hepatic steatosis, high HSI and liver lipid values were evident in Japanese sea bass fed high levels of animal protein blend as substitute to prime steam dried fish (Hu et al., 2013). Moreover, hepatic lesions were evident in Japanese sea bass *Lateolabrax japonicas* fed diet with seven sources of protein (Han et al., 2011).

Dietary nutrient content is readily reflected in the fish body composition (Kikuchi et al., 1997; Lee and Kim, 2005; Li et al., 2009). Fish carcass composition was significantly affected by the dietary treatments. Nevertheless, fish proximate composition across all treatments was within the range for Nile tilapia (ash 0.5–1.5%; lipid 0.79–8.5%; moisture 72–80%; and protein 13–25%) (Hernández-Sánchez and Aguilera-Morales, 2012). In the present study, fish carcass exhibited high ash content, which is in direct relationship with the ash content of the diets. There is a correlation between dietary crude ash and carcass ash contents, as seen in fish fed maggot meal (Ogunji et al., 2008). High ash content in the tissues of salmonids and tilapia is influenced by the mineral composition of their diets (Pouomogne et al., 1997; Skonberg et al., 1997). Furthermore, increased ash content in diets negatively affects ingredient and diet digestibility, resulting in reduced protein digestibility and efficiency (Gully, 1980; Hajen et al., 1993; Robiana et al., 1997).

4.4. Hepatic histopathology

The liver is the main organ for detoxification and is susceptible to damage; alterations in its histology are useful markers, which indicate exposure to stressors from the environment (Bernet et al., 1999; Velmurugan et al., 2007). It also indicates the nutritional and physiological status of fish. Excessive digestible carbohydrate in the diet may produce negative effects in glycogen accumulation as well as liver morphology and function. Such effects may have a great effect in suppressing the immune system and thereby increase susceptibility to infectious diseases (Hemre et al., 2002). On the other hand, lipid sources such as fish oil, eicosapentanoic acid and docosahexanoic acid increase liver and plasma lipid peroxides (Fritsche and Johnston, 1988; Gonzalez et al., 1992). Hypertrophy of hepatic cells caused by an increase in dietary carbohydrate was also observed in *Labeo rohita* and *Catla catla* fry (Mohapatr et al., 2003). Higher glycogen deposits caused by excess in carbohydrate levels may impose metabolic burden for the fish. Moreover, excessive dietary lipid intake may also result in a fatty liver (Lu et al., 2013). Various hepatic alterations include vacuolization, irregular hepatocyte arrangement, inordinate and obscure hepatic cords, and stricture hepatic sinus, and apoptosis.

Alteration in hepatocyte cytoplasm is an indication of an early and unspecific hepatocellular homeostasis (Braunbeck, 1998). Numerous effects brought about by fish exposure to toxicants such as vacuolar degeneration, increase in hepatocyte lipid droplets, and hypertrophy have been reported (Biagianti-Risbourg and Bastide, 1995; Braunbeck, 1998; Dezfuli et al., 2006; Giari et al., 2007; Strmac and Braunbeck, 2002). Commercial feed may cause hepatocyte vacuolization., hepatic cell membrane degeneration, and lipid droplet accumulation in farmed fish (Bilen and Bilen, 2013; Coz-Rakovac et al., 2002, 2005). Nonetheless, histological changes are not considered pathological if extensive hepatic necrosis is not detected (Saraiva et al., 2015). There are undetermined threshold and factors in considering a fish farm healthy liver. The present study shows absence of liver alterations. While there was no presence of tumor or lesions and parenchymal inflammation in the hepatic tissues of the *O. niloticus* juveniles, mild apoptosis was still observed, which is a normal part of a cell's life cycle. Cell membane lysis cannot be attributed to the diet because of similar hepatic histopathological results across all treatments, and may be caused by prolonged formalin fixation (Peluso et al., 2014).

5. Conclusion

Processed meal from knife fish as fishmeal protein (partial and complete) replacement generally enhanced the growth performance and feed efficiency utilization, and had no adverse effects on body composition and blood chemistry of Nile tilapia juveniles. Results suggest 75% KFM diet as the optimal level of fishmeal replacement to achieve best growth performance in Nile tilapia juveniles.

Future studies on the amino acid and fatty acid profiles and digestibility of KFM will further assess its potential as a replacement for fishmeal. Influence of KFM on the immune response, intestinal histology and stress-related factors may also be the focus of succeeding studies.

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