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Expression of Matrix Metalloproteinase 9 in Oreochromis Niloticus Fish and Its Active Role in Initiation and Resolution of Ammonia

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A B S T R A C T

Dietary protein for reaching better growing Oreochromis niloticus including gradual protein levels, taking into account that optimum protein levels for grown fish will help to reduce the impact of ammonia on fish health. Herein, this study verified a theory that assumes ammonia induces changes in fish tissues responsible for ammonia excretion by a mechanism coupled to capillary endothelial cells. Fish feeding on a higher level of protein caused the expression of MMP markers. Alongside, ammonia raised the activity of interstitial matrix metalloproteinases MMP-9. The rise of cell permeability may be amended by using an inhibitor of MMP. The results support the idea that stimulation of MMPs in brain endothelial cells contribute to the variations in permeability and cerebral vasogenic component, renal and gill tissues associated with ammonia implication. Detailed considerate of the response to ammonia stress and tolerance in tilapia can provide useful information for fish breeding professionals to expand aquaculture.

Keywords: Protein, Ammonia, MMP-9, Fish Health.

1. Introduction

Nile tilapia is a famous fish that represents a high extent of the world's hydroponics yields because of its superb development rates and obvious adaptability to various natural conditions. It is perfect for hydroponics because of its hardness, fast development, stress and illness resilience, a short time frame, and a low prerequisite for advantageous feed (El-Sayed, 2002). Nile tilapia hydroponics will require figuring satisfactory food with ideal power during the developing out stage to meet the protein necessities. Ammonia is one of the most widely recognized and fundamental boundaries of water quality, which influences and impacts fish conduct and wellbeing. Protein is viewed as the primary constituent of the fish body, in this manner requiring suitable dietary supplements for ideal growth. In this way, since protein is the most excessive piece of the eating regimen, the eating regimen sum should be just enough for the development of fish where the overabundance protein in fish diets can be extreme and cause pointless expensive nourishments (Ullah-Khan et al., 2019). Accordingly, a key factor for effective hydroponics creation might be decreasing taking care of expenses. Data about Nile tilapia's dietary necessities is fairly appropriate (Nguyen et al., 2009) (Abdel-Tawwab et al., 2010). The dietary protein necessity for juvenile tilapia and more plentiful tilapia has been reported. Nonetheless, these examinations didn't show enhancements in the metabolism of ammonia fish when dietary protein is raised. The brain tissue is one of the most critical organs associated with the conflict of ammonia. Ammonia poisonousness can be anticipated by detoxifying ammonia to items, for example, glutamine in fish (Ip and Chew, 2010). Release of ammonia happens principally through fish gills, and prior ideas have proposed that ammonia may penetrate the expanding epithelium as NH_3.

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In any case, late studies discovered that ammonia is discharged through fish gills and can involve transporters (Weihrauch et al., 2009a), requiring transporters certifying the presence of an ammonia carrier in the internal layer of ammonotelic fish liver mitochondria. MMP-9 should be viewed as a functioning member in the natural insusceptible reaction in fish. Carp leucocyte incitement prompts the strong mobilization of MMP-9 with an unordinary biphasic profile (Chadzinska et al., 2008). Ammonia linked activation of MMPs has been shown repeatedly in the CNS and the peripheral tissues (Skowrońska et al., 2012).

ROS and RNS shaped by ammonia additionally energize the inherent action of MMPs in the cerebral microvessel EC. Though the system by which ammonia created RNS initiates MMPs in RBE-4 cells is presumably like that in different CNS cells and incorporates Which includes direct nitrosylation (McCarthy et al., 2008) or oxidation (Kessenbrock et al., 2010) activation of the pro-MMPs and increased expression of MMPs in response to inflammation (Li et al., 2010).

The current examination portrays the ideal degree of dietary protein to accomplish great development of diets took care of with Oreochromis niloticus containing evaluated protein levels, considering that ideal protein levels for developed fish will assist with diminishing the effect of ammonia on fish health. Henceforth, a point by point comprehension of the reaction to ammonia stress and resistance to ammonia in tilapia can give valuable data to fish reproducing specialists to improve aquaculture.

2. Materials and Method

2.1. Fish and experimental protocol

The tests were completed at the Aquaculture and Fish Nutrition Research Aquarium, faculty of veterinary medication, Damanhour University, Egypt. An eighty fish were acquired from a nearby commercial aquarium, Kafr Elsheikh, and adjusted to lab conditions for about fourteen days during which they were taken care of a commercial diet (crude protein 30%). A sum of 80 fish (20 ± 0.5 g) was irregularly conveyed into group 1 (35 % protein) and group 2 (25% protein), each group was separated into two duplicates in (100L capacity) fiberglass tanks (20 fish for each tank) containing circulated air through recycled freshwater.

Fish were admitted to eating regimens two times every day (08:00 and 17:00 hours) until clear satiation for about six weeks and raised at 29.7 ± 1.0oC with a 12:12 h light: dull photoperiod. Fish were weighed independently toward the start, and the finish and mass weighed week by week during the trial time frame. Water pH was kept up somewhere in the range of 7.1 and 7.6, dissolved oxygen somewhere in the range of 7.1 and 7.7 mg/L, and ammonium between 0.02 ± 0.001 mg/L Physico-substance boundaries, I-e., temperature, saltiness, pH, ammonia and disintegrated oxygen were observed every day. Fish support and trial techniques were affirmed by the Research Committee of the NIOF, Egypt, and were as per the Guide for Use and Care of Laboratory Animals (European Communities Council Directive 2010/63/EU).

2.2. Experimental diets

Two isonitrogenous (25% and 35% crude protein) exploratory eating regimens were detailed. The proximate formula of the dietary components appears in Table 1. The experiment feeds were then stored at – 20oC for feeding trials.

Diets: The present study used two types of artificial feed with different protein content: feed A (25%) and feed B (35%) with an E/P ratio ranging from 8 to 10 Kcal. Feed formulations are presented in Table 1.

2.3. Feeding protocol

A trial plan with 25% and 35% protein contents were tried to decide the ideal protein requirement of Nile Tilapia fish. Throughout this experiment, every single diet was provided to duplicate tanks in two equivalent meals for every day at 8:00 and 17:00 hours. Daily hand-fed of proportion 5% wet body weight every day for 42 days. The daily feed provided was noted, and uneaten feed was gathered two hours after the beginning of the feeding. The measure of food gave is being balanced after week after week testing to determine gain in weight per treatment, which endured 42 days. Each tank was entirely depleted and completely scoured upon the day of sampling.

2.4. Measurement and analysis

Calculation of growth parameters

Toward the finish of the analysis, all fish from each tank were separately weighed. Growth and feed ingestive behavior were checked in concerns to the last weight, weight gain (the percent of introductory body weight toward the finish of the analysis), specific growth rate (SGR)

(in final body weight – in initial body weight/time, expressed as %), feed conversion ratio (FCR) (feed fed / wet weight gain), the protein efficiency ratio (PER) (wet weight gain/protein intake).

Feed conversion ratio (FCR)=(feed intake (in grams))/(weight gain (in grams))

Specific growth rate (SGR)=100x (final body weight-initial body weight (in grams))/(experimental time (in days))

Protein efficiency ratio (PER)=(weight gain (in grams))/(protein intake (in grams))

Hematological parameters

At the end of the trial, blood tests for examination were collected in heparinized (Na-heparinised) capillary tubes from the haemal arch post serving of the caudal peduncle. Blood from each test group was put away in EDTA vacutainer plastic tubes for future tests. All the hematological investigation was completed within 2 hours after every extraction.

Hemoglobin determination:

The hemoglobin substance of blood was evaluated utilizing Drabkin's technique (Nkrumah et al., 2011). Quickly, 5 ml Drabkin's solution (Vitroscient Co., Egypt) was blended in with 20µl blood, and afterward, the blend was left to stand 15 minutes. Hemoglobin content was measured by estimating the absorbance at 540 nm wavelength, and afterward, it was contrasted with that of hemoglobin standard (Vitroscient Co., Egypt). Test specimens were centrifuged to expel scattered nuclear material before checking the absorbance.

Determination of hematocrit value:

Haematocrit value was measured utilizing a microhematocrit technique, where blood was taken in smaller scale hematocrit heparinized tubes and centrifuged in a miniaturized scale hematocrit rotator (VEB MLW Medizintechnik Leipzig, Germany) at a speed of 12,000 rpm for 5 min. The hematocrit value was recorded utilizing a microhematocrit reader and announced in percentage (Tsai et al., 2014).

Counting of red blood cell (RBC), white blood cell (WBC) and blood indices:

For RBC and WBC count, a blood sample was diluted (1:200) with Natt-Herrick's diluent (Williams et al., 1991). Then, the diluted sample was placed in a Neubauer improved hemocytometer (Precicolor, HBG. Germany), and then the blood cells were counted using a light microscope (Zuzi, Series 116, Auxilab S.L. Spain). Red blood cell indices including, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the method adopted by (Walsh, 1969).

Biochemical analysis

Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum were determined colorimetrically according to (Reitman and Frankel, 1957). Plasma ammonia levels (in micrograms per decilitre) were determined colorimetrically according to Spain react kit (Spain react Co., Egypt).

Tissue collection and processing

Ten fishes were used from each group for collections of tissue samples. The fish were sacrificed by cervical section. The first-gill arch, kidney, and brain were taken, rinsed in physiological saline, and fixed in 4% PFA in phosphate buffer saline for 24 hours at 40C. The tissues were dehydrated in ascending concentrations of ethyl alcohol series (50%, 70%, 80%, 90%, and absolute alcohol), cleared in xylene, and embedded in melted paraffin 600C. The paraffin blocks were sectioned at 3 µm on positive charge super frosted slides. The sections were examined by an Olympus CX31 microscope and imaged by an Olympus LC20 camera.

Immunohistochemistry

The sections have been deparaffinized with xylene and rehydrated with descending grades of ethyl alcohols (100%, 95%, 90%, 80%, and 70%) then washed with distilled water. Blocking of sections is applied to reduce the endogenous nonspecific background Ultra vision hydrogen peroxide block (Thermo scientific, Fremont, USA) for 10 minutes. Washing 4 times with buffer were then blocked for 5 minutes with Ultra vision Block (Thermo scientific, Fremont, USA). Anti-human MMP9 (Biogenix, EP127) diluted with 5% normal goat serum was incubated overnight at room temperature. Furthermore, after washing four times with buffer, the primary antibody enhancer was incubated for 10 min. at room temperature. HRP secondary antibody was applied for one hour with dilution 1:1000, and then the DAB chromogenic was incubated for one min in the dark. Sections were washed with distilled water, counterstained with hematoxylin coverslipped with aqueous mounting media, and sealed with nail varnish.

2.5. Statistical analysis

Result data were examined utilizing unpaired t-test with Welch's adjustment via GraphPad Prism 5 (San Diego, USA). All assertions of significances relied upon ($p \leq 0.05$). Data are expressed as mean ± S.E.M except if in any case is demonstrated.

3. Results

Growth performance

The growth performance of Nile tilapia fish was significantly affected by dietary protein level (Table 2). Bodyweight gain and SGR of the fish fed a 35% protein diet were significantly ($P<0.05$) higher than of those fed the 25% protein diets. Weight gain and SGR tended to plateau based on weight gain; the appropriate supplementation of dietary protein for the fish was estimated to be 35% of diet using broken-line regression analysis.

Feed conversion and condition indices

Based on a dry matter the feed intake was increased marginally by an upsurge in dietary protein level. Supplementation with 35% protein in fish diets displayed considerably higher ($P<0.05$) feed intake than low protein group which expresses ingestive behavior (Table 2). Feed conversion ratio (FCR) revealed similar results as well.

Protein utilization

Protein utilization was evaluated through protein-dependent parameters such as protein efficiency ratio (PER). Fish fed the 35% protein diets showed significantly higher ($P<0.05$) PER than the other group (Table 2).

Hematological parameters

The hematological parameters of Nile tilapia fed on diets containing different protein levels showed some significant differences, as shown in table (3). The group fed on a diet containing 35% protein had significantly higher hemoglobin content, red blood cell count, packed cell volume, and mean corpuscular hemoglobin concentration compared to the other group (Group 2). On the other hand, both groups didn't show any significant differences in their total leucocytic count mean corpuscular volume, and mean corpuscular hemoglobin.

Biochemical parameters

The ammonia excretion in the test fish is presented in Table 4. The results indicated that ammonia excretion increased in line with increases in dietary protein levels ($p<0.05$). The ammonia in plasma of fish group with 25% dietary protein was lower ($p<0.05$) than that of fish nourished on 35% protein. Besides, serum ALT was significantly affected by the high protein level ($P<0.05$; Tables 4). Meanwhile, AST, Creatinine, and urea weren't affected by the dietary protein level in Nile tilapia ($P<0.05$).

Immunohistochemistry

Brain

MMP-9 was mainly located around blood vessels (endothelial cell/ per endothelial layer) and slightly stained cell bodies of some neurons and scattered microglia (Fig.1 A&C). In contrast, in 35% group, principal MMP-9 positive cells were activated microglia and increased the intensity of MMP-9 in the neuron cell body expression (Fig.1 B&D).

Gills

The intensity of MMP9 expression decreased in 25% group and restricted to the epithelium of the primary and secondary lamellae (Fig. 1E). However, the strength of MMP9 immunostaining was found along the bronchial filaments of 35% group with increasing immunolabelling of the protein around the blood vessels of the secondary lamellae and the pillar cells (Fig. 1F). Some epithelial lifting is also noticed in the high protein group.

Kidney

In a 25% group, gelatinolytic activity was identified in individual cells throughout the entire glomerular tuft and was recognized at the periphery of the glomeruli. In contrast, it focally increased gelatinolytic along with Bowman's capsule. It was also found to have a robust MMP-9 sign in the tubules with an increase in the protein's intensity in the 35% group.

4. Discussion

Nutritive protein is the primary origin of amino acids in fish. The intestinal cells of the aquarium are adjusted to diets with high protein and lower saccharides. (Karlsson et al., 2006) approved alterations in plasma levels of amino acids and its pools in portal blood after ingestion in some fish and approved that amino acids can be catabolized in the intestine before influx to the portal circulation. Therefore, only a few percent of the digested nitrogen was released during the day following the feeding period. Brain tissue was effectually disallowed from revelation to ammonia intoxication post-feeding (Tng et al., 2008).

Fish with high dietary protein, its carbon is extracted from the carbon chain of amino acids after the release of the α-amino group. Approximately 50 % of the nitrogen intake from food is excreted within a day (Ip et al., 2004) (Lim et al., 2004).

Considerably ammonia byproduct of metabolism in fish comes from the α-amino group of amino acids that are catabolized. Ammonia can be formed directly in hepatic cytosol via specific enzymes (Youngson et al., 1982) or via the combined actions of cytosolic aminotransferases and mitochondrial GDH (French et al., 1981) where trans deamination is the chief mechanism for the metabolism of amino acids in the liver of fish.

Even though ammonia excretion is not a problem at acidic pH, it is a vast difficulty for fish exposed to high pH. This is because at high biological pH, the worst even with a high buffering ability (Weihrauch et al., 2009c), the NH3 diffusion ascent is decreased, leading to ammonia buildup within the fish. Death can arise once plasma levels of ammonia rise else hastily, and toxic levels of ammonia are extended (Wilkie, 1997).

In the presence of high concentrations of ambient ammonia, fish are simultaneously faced with the retention of endogenous ammonia and the absorption of exogenous ammonia and have particular adaptations to overcome ammonia toxicity (Ip et al., 2004); (Chew et al., 2006). Here, we demonstrated that protein levels increase the production of ammonia, the mechanisms of ammonia toxicity in the brains of certain ammonia-tolerant fish are different from those in mammalian brains (Wee et al., 2007) (Tng et al., 2009). In mammals, acute ammonia poisoning is induced by NMDA receptor activation (Kosenko et al., 1999) and, excessive activation of these receptors is neurotoxic, leading to oxidative stress, neuronal degeneration, and death (Miñana et al., 1996). The novel findings of this study are consistent with the hypothesis that ammonia induces excessive changes directly inside the endothelial cells under high protein conditions in the fish diet, leading to activation of MMPs and increased intercellular protein passage.

Numerous experiments on cultured cells have been treated with ammonia, a focus that mimics the in vivo reactions of brain tissues to intense hyperammonemia (Häussinger and Görg, 2010); (Skowrońska et al., 2012). Ammonia generated NO and ROS in RBE-4 cells, similar to the effects of ammonia in astrocytes (Norenberg et al., 2009) and neurons (Klejman et al., 2005). Here, MMP-9 was found basically around blood vessels alongside certain neurons' marginally stained cell bodies and scattered microglia in the sample. Upraising of protein in the diet, essential MMP-9 positive cells were activated microglia, and the strength of MMP-9 in the expression of neuron cell bodies expanded.

MMP activation has been demonstrated repeatedly in the CNS and at the peripheral tissue (Lehner et al., 2011). ROS and RNS generated by ammonia also stimulate the intrinsic activity of the cerebral microvessels MMPs in the EC. While the mechanism by which ammonia-generated oxidative stress activates MMPs, it is probably analogous to that in other CNS cells and involves direct nitrosylation activation of pro-MMPs (Gu et al., 2002) or oxidation (Meli et al., 2003) or increased expression of MMPs in response to inflammation (Huang et al., 2001).

In vivo studies of animal models have demonstrated that increased BBB permeability is associated with disruption of TJ proteins, elicited by activation of MMP-9 (Chen et al., 2009). The function of MMP was deduced in these studies from increased metalloproteinase titer in animal serum, and the fact that BBB leakage was prevented by treatment with an MMP inhibitor. Here we show that MMPs that participate actively in BBB disruption are mostly of endothelial origin. The MMP action targets, which are the existence of the TJ proteins involved, remain to be identified.

The gills are the essential site of ammonia excretion in fish (Weihrauch et al., 2009b) since they have a wide surface area, cardiovascular perfusion, high ventilation levels, short distances of diffusion, and interaction with a full mucosal medium (Evans et al., 2005). The current study showed that the rate of MMP9 expression in gills decreased in the low protein contents (25 percent) and was restricted to the primary and secondary lamellae epithelium. However, the MMP9 immunostaining strength was found along the bronchial filaments of a large dietary protein group that immunolabelling of the protein around the secondary lamellar endothelial cells and particularly the pillar cells with some observed epithelial lifting. While gill tissues exert an extremely high metabolic rate representing nearly 10 % of the total oxygen demand for osmoregulatory purposes, the overall metabolic expenditure for ammonia release appears to be lower (Evans et al., 2005). In most fish, most ammonia is excreted as NH3 through the branchial epithelium, down a desirable gradient of blood-to-water diffusion (Wilkie, 2002) (Evans et al., 2005).

Fish physiological status is an integral part of determining their state of health. Physiological changes can, however, be used as indicators of unhealthy environmental conditions or the existence of stressors such as harmful chemicals, excess organic compounds, and stressors in intensive fish crops (Řehulka et al., 2004). Dietary protein levels have greatly affected increases in RBCs and Hb. The increase in RBC count may have been due to its release from the spleen storage pool (Pulsford et al., 1994). Thus it appears that dietary protein level affects splenic function.

Results of this study revealed the effects of feeding different dietary protein levels on some hematological parameters in tilapia fish where red blood cells count, hemoglobin percentage, packed cell volume, and mean corpuscular hemoglobin concentration were significantly higher in fish fed on a high protein diet. These results are in agreement with (Elbanna et al., 2017) where rainbow trout and some species of cyprinids which fed on different dietary protein levels showed enhanced growth rate, increased erythrocyte count, hematocrit, and hemoglobin values. Also, previous studies (Abdel-Tawwab et al., 2010) (Miao et al., 2018) reported a positive relationship between RBC, Hb, Hct values, and dietary protein levels in Megalobrama amblycephala.

Besides, anemia in Siberian sturgeon was reported due to an insufficient amount of dietary protein to fulfill nutritional requirements for that species. Also, the reduced amount of hemoglobin in this study may result from the lower number of red blood cells in the group fed on the low dietary protein (Docan et al., 2011). Moreover, protein inadequacy causes disturbances in the absorption of vitamins and minerals with subsequent deterioration in body metabolism leading to a low concentration of both plasma proteins and hemoglobin, and finally, anemia occurs. The previous studies suggest that the relationship between blood parameters and dietary protein levels differ in various fish species (Kondera et al., 2017).

Our study revealed no significant differences in white blood cells count in both groups and this finding is in agreement with a previous study of (Kumar et al., 2005) who fed the same species of fish diets containing different protein levels. Also, (Baruah et al., 2009) reported no significant differences in white blood cell count of L. rohita fed on diets containing 25% and 35% protein levels. Also, (Qiang et al., 2013) showed that different dietary protein levels do not affect the white blood cell count of O. niloticus. These data proved that innate immune mechanisms in fish are insensitive to dietary protein levels (Kondera et al., 2017).

The raised levels of ALT show catabolism of proteins at significant levels of dietary protein. The overflow of amino acid from protein-rich diets cannot be contained right in fish and could be deaminated and changed into energetic compounds (Ballantyne, 2001) (Stone et al., 2003). Dietary protein significantly affected ALT in Nile tilapia. Gluconeogenesis is indicated by high ALT in the blood of increased dietary protein levels. The expression of main intermediate metabolism enzymes is modulated by the nutritional status of the fish (Metón et al., 2004). The levels of amino acid-metabolizing enzymes and nitrogen excretion are consistent indicators of dietary protein availability. The increase in ALT activities in Nile tilapia fed on the 30 percent CP diet that reflects the use of excess amino acid hydrocarbons to supply energy demands. Similar responses were observed in Oncorhynchus mykiss for ALT (Sánchez-Muros et al., 1998) and in R. queen for AST and ALT (Bibiano Melo et al., 2006). The increase in protein-metabolizing enzyme hepatic activity when fish are fed on a high percent CP diet may indicate the use of excess dietary amino acids for growth and gluconeogenesis substrates, particularly for AST and ALT activities.

In conclusion, the findings support the hypothesis that ammonia involves activation of MMP directly in the endothelial cells, which could be the cause for events resulting in the proliferation of ammonia and probably with poisoning in the tissue of fish gills, brain, and kidneys. The findings in the present study illustrate the MMP's central role in this pathogenic chain and it may apply a treatment pathway for the harmful impacts of ammonia on fish production.

5. Conflict of Interest

There is no conflict of interest

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Figure 1: Showing expression of MMP9 in different organs of Oreochromis niloticus fish. A&C fed on a diet (25% protein); the expression of MMP9 in the brain is concentrated mainly around the blood vessels and around the neuron, which is more intense in B&D fed on a diet with (35% protein). E (25% dietary protein); primary and secondary lamellae of gills showing light staining of MMP9. F (35% dietary protein) showed strongly expressed MMP9 in the primary and secondary lamellae, especially around blood vessels and pillar cells, epithelial lifting is also noticed (astrix). G (25% dietary protein); kidneys are showing MMP9 in the glomerular tuft (G) and tubules (T), which more intense in H (35% dietary protein).

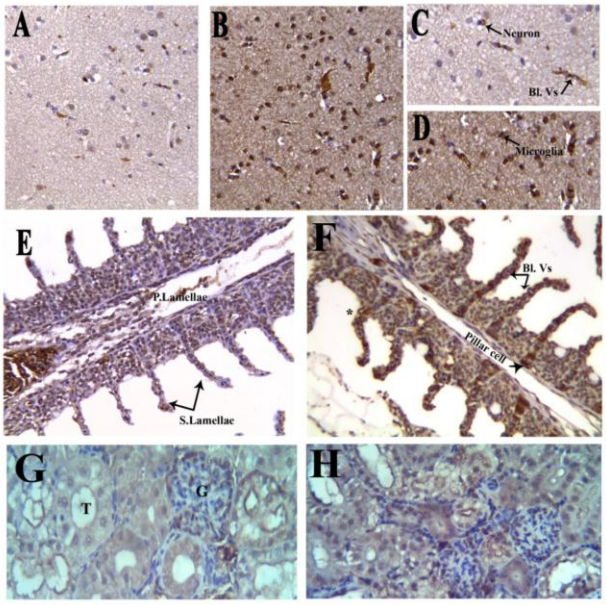

Table 1: Ingredients and chemical composition of the experimental diets (in percent; on a dry-matter basis)

Ingredients	**A (25% protein)**	**B (35% protein)**
Fish Meal	29.0	37.0
Soybean Meal	15.5	18.0
Wheat Flour	6.5	12.3
Bran Meal	15.0	10.0
Fish Oil	2.5	2.5
Corn Oil	2.5	2.5
Vitamin Mix [b]	3.0	3.0
Mineral Mix [b]	3.0	3.0
Choline Chloride	2.0	2.0
CMC	2.0	2.0
Filler	19.0	8.7
Chemical Analysis Results (%) [a]		
Protein	25.14	34.38
NFE [c]	34.34	30.53
Fat	11.55	11.59
Fiber	2.35	1.85
Total Energy (Kcal/G)	267.40	281.78
E/P (Kcal/G Protein)	10.64	8.84

Table 2: Growth rate, FCR, protein efficiency ratio of Oreochromis niloticus fed diets containing varying levels of dietary protein for six weeks (mean values of 2 replicates + SEM; n=20). Mean values sharing different superscripts are significantly different (P>0.05).

Parameter	**Group**	**Mean ± S.E**
Final weight (g)	G1 (35 %)	1576 ± 2.96[a]
	G2 (25 %)	1310 ± 5.78 [b]
Weight gain, % of initial weight	G1 (35 %)	74.42 ± 2.60 [a]
	G2 (25 %)	57.32 ± 1.76 [b]
Specific growth rate % (SGR)	G1 (35 %)	1.59 ± 0.04[a]
	G2 (25 %)	1.14 ± 0.01 [b]
Feed intake (g) per group	G1 (35 %)	1315 ± 3.22[a]
	G2 (25 %)	1077 ± 1.76 [b]
Feed conversion ratio (FCR)	G1 (35 %)	3.45 ± 0.03 [b]
	G2 (25 %)	3.64 ± 0.03 [a]
Protein efficiency ratio (PER)	G1 (35 %)	1.37 ± 0.03 [a]
	G2 (25 %)	0.96 ± 0.03 [b]

Table 3: Haematological parameters of Oreochromis niloticus blood-fed diets containing varying levels of dietary protein for six weeks (mean values of 2 replicates + SEM; n=20). Mean values sharing different superscripts are significantly different (P>0.05).

Parameter	**Group**	**Mean ± S.E**
Hemoglobin (g/dl)	G1 (35 %)	10.4±0.6[a]
	G2 (25 %)	7.5±0.6[b]
PCV (%)	G1 (35 %)	36.6±1.9[a]
	G2 (25 %)	30.2±1.7[b]
RBCs (million/µl)	G1 (35 %)	3.2±0.2[a]
	G2 (25 %)	2.5±0.2[b]
MCV (fl)	G1 (35 %)	118.7±3.4
	G2 (25 %)	124.5±3.6
MCH (pg)	G1 (35 %)	31.5±0.6
	G2 (25 %)	30.5±0.4
MCHC (%)	G1 (35 %)	28.3±0.2[a]
	G2 (25 %)	24.8±1.5[b]
WBCs (thousand/ µl)	G1 (35 %)	9.2±0.4
	G2 (25 %)	9.1±0.7

Table 4: Biochemical parameters and plasma ammonia of Oreochromis niloticus fed diets containing varying levels of dietary protein for six weeks (mean values of 2 replicates + SEM; n=20). Mean values sharing different superscripts are significantly different (P>0.05).

Parameter	**Group**	**Mean ± S.E**
Ammonia (ug/dl)	G1 (35 %)	537.5 ± 20.91[a]
	G2 (25 %)	337.0 ± 29.70[b]
AST (U/ml)	G1 (35 %)	11.63 ± 0.43
	G2 (25 %)	11.99 ± 0.22
ALT (U/ml)	G1 (35 %)	36 ± 2[a]
	G2 (25 %)	33 ± 3[b]
Creatinine (mg/dl)	G1 (35 %)	0.49± 0.01
	G2 (25 %)	0.46± 0.02
Urea (mg/dl)	G1 (35 %)	3.93± 0.48
	G2 (25 %)	4.05 ± 0.37