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The role of lectin in improving growth performance, feed utilization, immunity, and disease resistance of the Nile tilapia, *Oreochromis niloticus* fingerlings

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ABSTRACT

There is an interesting in using of medical and aromatic plants or spices as feed additives in fish diets instead of chemical products to avoid side effects and achieve organic aquaculture. Feeding experiment was conducted to evaluate the growth of the Nile tilapia fingerlings fed four isonitrogenous (30% crude protein) experimental diets containing different levels 0.0% (control), 1.0%, 1.5%, or 2% of *Moringa lectin* (MLM). The diet containing 1.5% MLM resulted in significantly greater ($P < 0.05$) specific growth rate (SGR), feed conversion ratio (FCR). Moreover, PER and APU values increased significantly at MLM-supplemented diets. While, there was no significant difference among all treatments of MLM in EU%. In regard to body composition, there was no significant difference in dry matter, lipids, and ash ($P > 0.05$) contents due to lectin supplementation. While, crude protein content increased significantly by increasing the MLM levels ($P<0.05$). Blood plasma profile showed an improvement in hemoglobin (HB), red blood cell (RBCS), hematocrite (Hct), WBCs, Lymphocytic, Monocytic, Granulocyte, glucose and gloubin, while, there was a decrease in total protein, total lipids, decrease in creatinine, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and cholesterol in fish fed 1.5% MLM. Moringa lectin supplementation enhance non-specific immune response of *O. niloticus* as lysozyme activity and respiratory burst activity showed significant increase in group fed 1.5 MLM then group fed 2 % followed by 1 % than control group. Moringa lectin was found to have an antibacterial activity antagonistic to *Aeromonas hydrophila* infection in fish. Improvement in Superoxide dismutase activity (SOD), Catalase activity (CAT), Glutathione S-transferase (GST) and Malondialdehyde (MDA) activities were observed at 1.5% MLM activities which was consistent with the immune response. Performance and FCR improvement translated into a 22.74% decrease in feed costs associated with the 2% MLM diet.

INTRODUCTION

Plants have played a significant role in maintaining human health and in improving the quality of life for thousands of years. In recent times, focus on plant research is increased throughout the world due to their extensive applications. *Moringa oleifera* is the most widely cultivated species of the genus Moringa, which is

the only genus in the family Moringaceae having many medicinal values which is largely grown in India (Fahey, 2005). The seeds contain high amounts of reserve storage proteins (MOCP), which has been used in the Philippine diet; leaves, flowers and green pods are edible as a human nutritious vegetable (Guevara *et al.*, 1999). In Malaysia, the young tender pods are cut into small pieces and added to curries (Abdulkarim *et al.,* 2005). A protein extract from *M. oleifera* seeds can remove humic acids from water reducing total and organic matter as well as aromatic content and color; may be due to the coagulant mechanism involves adsorption and neutralization of charges (Santos *et al.,* 2011).

The chemical constituents reported in the seeds are crude protein, crude fat, carbohydrates (Bhatta *et al.,* 1982), methionine, cysteine, benzylglucosinolate (Bennet *et al.,* 2003), moringyne, mono palmitic and di-oleic triglycerides. The mucilage from the pods is called drum stick polysaccharide, consists of Galactose, Dextrose, Xylose and potassium, sodium, magnesium, calcium salts of Glucoronic acid. Several properties have been identified in different parts of *M. oleifera*: the leaf extract is a potential source of antioxidants (Arabshahi *et al.,* 2007); hypocho lesterolemic effects have also been found in leaf extract (Ghasi *et al.,* 2000) and fruits (Mehta *et al.,* 2003); plant seeds contain hypotensive activity (Faizi *et al.,* 1995), strong antioxidant activity and chelating property against arsenic toxicity(Gupta *et al.,* 2005; Kumari *et al.,* 2006).

Plant seeds are sources of hemagglutinins – lectins or carbohydrate-binding proteins that exist in virus and all forms of life but most known are extracted from plants, especially from seeds (Jeyaprakash *et al.,* 2005), a storage organ, which is one of the main sources to obtain these molecules. In general, other plant tissues contain lower amount of lectins; also, not necessarily identical in structure or carbohydrate specificity to seed lectins (Correia *et al.,* 2008). Lectins can agglutinate cells and precipitate polysaccharides, glycoprotein or glycolipids (Lis and Sharon, 1998; Zhang *et al.,* 2009). These properties enable lectins to mediate different biological processes such as cell-cell interactions (Gabor *et al.,* 2004), induction of apoptosis (Perillo *et al.,* 1995), cytotoxic activity (Silva *et al.,* 2012), antibacterial and antiviral activity (Araújo *et al.,* 2012), antiproliferative activity for cancer cells (Bah *et al.,* 2011), mitogenic activity (Maciel *et al.,* 2004; Bah *et al.,* 2011) and antitumor activity (Andrade *et al.,* 2004). Lectins have been investigated in marine bio-resources by their various pharmacological applications to develop new drugs (Ogawa *et al.,* 2011).

Aquaculture is considered as the only possible solution to increase fish production in Egypt (Saleh, 2007). Nile tilapia, *O. niloticus (*L.*)* is one of the most important cultured. The attributes which make Nile tilapia very suitable for fish farming are its general hardiness and tolerance to a wide range of environmental conditions, ease of breeding, rapid growth rate, resistance to stress and disease, ability to efficiently convert a wide range of natural and artificial feed as well as organic and domestic wastes into high quality protein, ability to reproduce easily in captivity, and good taste *(*Zenhom, 2014*).*However there are limiting factors for Nile tilapia culture including bacterial diseases(Li *et al.,* 2006*)*

This study was conducted to evaluate the effects of *Moringa lectin* as natural feed additives on growth performance, feed utilization, survival rate, whole body composition, biochemical profile innate immunity and antioxidant enzymes assay of Nile tilapia "*Oreochromis niloticus"*.

MATERIALS AND METHODS

Extraction of Lectin

Mature malunggay seeds were obtained from Silang, Cavite. Lectin extraction from *Moringa oleifera* seeds were done at The National Research Center, NRC, Cairo, Egypt. The seeds were dehulled manually, ground on a mortar and pestle, and repeatedly defatted using n-hexane at a ratio of 1:5 (w/v) until a colorless liquid was obtained. The defatted powder was air-dried at room temperature and was homogenized for 15 min in a Mender using cold 0.2 M phosphate buffered saline pH 7.2 (PBS) containing 0.15 M NaC1 at a ratio of 1:5 (w/v). Protein extraction was continued by stirring the mixture for 12 hours under refrigeration. The extract was clarified by filtration using cheese cloth; this was followed by centrifugation at 900xg for 20 min at 4°C. The residue was discarded while the clarified PBS extract was used as the crude lectin source.

Isolation and Purification

The crude extract was precipitated using ammonium sulfate. The amount of $(NH_4)_2SO_4$ to be added was based on the monogram table by Maricel *et al.* (2004).

Ammonium sulfate was added to an aliquot of crude PBS extract in 3 lots over a 30-min period with constant stirring at 4°C. The mixture was allowed to equilibrate for 2 hours at 4°C. The precipitate was separated by centrifugation at 8000xg for 20 min at 4°C. The precipitate was kept at 0°C while the supernatant was further saturated with $(NH_4)_2SO_4$ to completely precipitate any remaining protein in solution. Likewise, the precipitate was collected by refrigerated centrifugation. The precipitate was dissolved in minimum amount of PBS (pH 7.2) and was subjected to gel filtration in a Sephadex G-25 for desalting. Eluates were pooled and lyophilized. The precipitate from the $(NH_4)_2SO_4$ fractionation was redissolved in PBS (pH.7.2) and was subjected to molecular sieving through the equilibrated Sephadex G-75 column eluted with PBS (pH 7.2). Then 5 mL of the sample was loaded to the column and a 3 mL fraction of the eluted sample was collected at a flow rate of 0.30 mL/min. The eluted fractions were monitored for spectrophotometric absorbance at 280 nm. Each fraction was tested for agglutinating activity. The eluted fractions showing agglutination activity were pooled and concentrated either by ethanol precipitation or lyophilization.

Experimental design and dietary treatments

Four experimental diets (30% crude protein and 8% lipid) were formulated containing 0.0 (control), 1.0, 1.5, and 2% lectin. The proximate chemical composition of lectin extraction and the main ingredients of the tested diets are shown in Table 1.

Dietary formulation and proximate composition of the experimental diets are shown in Table 2. The dry ingredients of each diet were thoroughly mixed, and 100 ml of water was added per kg diet. Afterwards, the mixture (ingredients and water) was blended using a kitchen blender to make a paste of each diet. Pelleting of each diet was carried out by passing the blended mixture through a laboratory pellet machine with a 1mm-diameter diet. The pellets were dried in a drying oven for 24h at 85°C and stored in plastic bags in a deep freezer at −2°C until use. The caloric value as digestible energy (DE) of each ingredient was estimated to be 5.65 kcal DE/g of protein, 9.45 kcal DE/g of lipid, and 4.12 kcal DE/g of carbohydrate (NRC, 1993).

Fish and Experimental Management

One hundred and eighty Nile tilapia (*Oreochromis niloticus*) fingerlings were obtained from the nursery ponds, CLAR, Abbassa, Abu-Hammad, Sharkia, Egypt. A feeding experiment was conducted in the Fish Nutrition Laboratory of CLAR, Egypt.

Table 1: Ingredients and chemical analysis of the experimental diets (on dry matter basis) containing different levels ofMoringa Lectin levels (%).

Ingredient	Control	*Moringa lectin* levels (%)		
	0.0	1.0	1.5	2
Fish meal (HF CP 70%)	7.5	7.5	7.5	7.5
Soybean meal (SBM CP 47 %)	45	45	45	45
Ground corn (CN)	20	20	20	20
Wheat bran (WB)	19	19	19	19
Cod fish oil	3.0	3.0	3.0	3.0
Corn oil	1.5	1.5	1.5	1.5
Vitamins[1] and Minerals premix[2]	1.5	1.5	1.5	1.5
Starch	2.5	1.5	1.0	0.5
Moringa lectins	0.0	1.0	1.5	2.0
Total	100	100	100	100
Proximate chemical analysis (%)				
Dry matter	94.2	94.5	94.7	94.8
Crude protein	30.0	30.1	30.3	30.4
Crude fat	7.0	7.1	7.2	7.4
Ash	8.2	8.3	8.3	8.4
Fiber	4.8	4.9	5.0	5.0
NFE[3]	50	49.6	49.2	48.8
GE(Kcal/100g)[4]	441.65	441.51	441.93	442.74
P/E ratio	67.92	68.17	68.56	68.66

1-Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; a-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

2- Mineral premix (g/kg of premix): CaHPO4.2H2O, 727.2; MgCO4.7H2O, 127.5; KCl 50.0; NaCl, 60.0; FeC6H5O7.3H2O, 25.0; ZnCO3, 5.5; MnCl2.4H2O, 2.5; Cu(OAc)2.H2O, 0.785; CoCl3.6H2O, 0.477; CaIO3.6H2O, 0.295; CrCl3.6H2O, 0.128; AlCl3.6H2O, 0.54; Na2SeO3, 0.03.

3 -Nitrogen-Free Extract (calculated by difference) = 100 – (protein + lipid + ash + fiber).

4- Gross energy (GE) was calculated from NRC, (1993) as 5.65, 9.45, and 4.12 kcal/g for protein, lipid, and carbohydrates, respectively.

After acclimation for two week on the control diet (without lectin extraction) the fish were divided into four triplicated groups of 15 fish per replace, with an average weight of 8.6± 0.1 g/fish. They were then randomly stocked in 12 glass aquaria (75x 40x 50 as w, l and h cm) with continuous aeration. The aquaria were daily cleaned before the first feeding and excreta were siphoned. Water quality parameters measured weekly included temperature (via a thermometer), PH (using Jenway Ltd., Model 350-pH-meter) and dissolved oxygen (using Jenway Ltd., Model 970-dissolved oxygen meter). Ambient water temperature, dissolved oxygen and pH through the experimental period were 27.0± 1.0°C, 5.6 ±1.0 mg/l and 8.0 ±0.2, respectively. The test diets were fed twice daily, at 09.00 and 13.00 h, for apparent satiation rate for 6 days a week for 12 weeks and fish were weighed biweekly. During the study period, the total amount of feeds consumed by the fish in each aquarium was determined and the feed consumed for each individual fish was calculated accordingly.

Evaluation of Growth Performance and Feed Utilization Efficiency

Growth performance and feed utilization including weight gain (WG, g), percent weight gain (%WG), specific growth rate (SGR, %/day), feed conversion ratio (FCR) protein efficiency ratio (PER), apparent protein utilization (APU %) and energy utilization (EU %) were determined as follows:

WG = FW – IW (g / fish)

WG% = 100 × [(final fish weight (g) - initial fish weight (g)) / initial fish weight]
SGR = 100 × [(ln final fish weight) - (ln initial fish weight)] / experimental days
FCR = feed fed (g) (dry weight)/weight gain (g)
PER = weight gain (g) / protein fed (g)
Apparent protein utilization (APU; %) = 100 [protein gain in fish (g) / protein intake in diet (g)];
Energy utilization (EU; %) = 100 [Energy gain in fish (g) / energy intake in diet (g)].

Proximate Analyses

Five fish were netted from each aquarium at the end of the feeding trial. They were then pooled together and homogenized for proximate composition (total of 15 fish per treatment). Moisture, total protein, lipid and ash contents were all determined by Standard Association of Official Analytical Chemist (NRC, 1993) methodology. Moisture content was estimated by drying samples in an oven at 85°C until constant weight was achieved. Nitrogen content was measured with a micro-Kjeldahl apparatus, and crude protein was estimated by multiplying total nitrogen content by 6.25. Total lipid content was determined by means of ether extraction for 16 h, and ash was determined by combusting samples in a muffle furnace at 550° C for 6 h. Crude fiber was estimated according to the method of Goering and van Soest, (1970). Gross energy was calculated according to the method of National Research Council, (1993).

Blood and serum sampling

At the end of the feeding trial, three fish from each aquarium were taken for physiological parameters analysis. The fish were anesthetized using buffered tricaine methane sulfonate (20 mg/l), and blood was collected from the caudal vein with a sterile syringe and divided equally among three clean and dry tubes. The first part was centrifuged at 3000 g for 15 min and the serum was stored at-20°C for further assays. The second part was mixed with sodium fluoride as an anticoagulant and centrifuged at 3000 g for 15 min for separation of plasma for glucose analysis. The last part was mixed with EDTA solution for measuring hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs) and hematocrite (Ht).other three fish/replicate were bled as above and both whole blood and serum were used for NBT activity and lysozyme activity respectively

Physiological analysis

Hb level was determined colorimetrically using a spectrophotometer according to the method of Stopkopf (1983). It was determined using the microhematocrit method Schalm (1975). RBCs were determined according to the method described by Natt and Herrick (1952). Total lipid content was determined colorimetrically according to the method of Joseph *et al.* (1972). Total protein content was determined colorimetrically according to the method of Henry (1964). Urea was determined following the method of Patton and Crouch (1977). Creatinine was determined calorimetrically as described by Henry (1964). Cholesterol was estimated as acolored complex according to Young (2001) method. Glucose was determined colorimetrically following the method of Trinder (1969). Colorimetric determination of serum albumin was carried out according to Wotton and Freeman (1982) using spectrophotometer. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined calorimetrically according to the method of Reitman and Frankel (1975).

Respiratory burst (NBT) Activity by Spectrophotometric Assays:

NBT activity was determined based on measuring Nitro-BlueTitrazolium activity (NBT) following the method described by Siwicki, (1989).

Lysozyme Determination:

Lysozyme level in serum of five fish in each aquarium was determined by turbidimetric assay according to the method described by Eills (1999). Briefly, test serum (0.1 ml) was added to 1.9 ml of a suspension of *Micrococcus lysodeikticus* (Sigma) (0.2 mg ml-1) in a 0.05 M sodium phosphate buffer (pH 6.2). The reaction was carried out at 25^{o}C and absorbance was measured at 530 nm after 0.5 and 4.5 min in a spectrophotometer. One unit of lysozyme activity was defined as the amount of sample causing a reduction in absorbance of 0.001 min-^{1}.

Antioxidant enzymes assay

After blood collection, gills and liver samples were collected from the euthanized fish for antioxidant enzymes assay. The gills and liver tissues were homogenized in 9 volumes of 20 mM phosphate buffer (pH 7.4) containing ethylene diamine tetra acetic acid (EDTA) and 0.1% Triton X-100. The homogenates were centrifuged at 600 ×g for 10 minutes and the supernatants were collected in clean Epindroff tube for antioxidant enzymes assay. Superoxide dismutase activity (SOD) was measured according to Kakkar *et al.* (1984), Catalase activity (CAT) was measured according to luck (1963) and Glutathione S-transferase (GST) and Malondialdehyde (MDA) activities (as a biomarker of lipid peroxidation) were measured according to Habig *et al.* (1974).

Challenges test

Finally at the end of experimental period (12 weeks), challenge test was carried out. All untreated and treated fishes were divided into two subgroups; the first group was injected intra peritoneal (IP) with 0.5 ml of pathogenic *Aeromonas hydrophila*. The second group was injected IP with 0.5 ml of saline solution and used as a negative control. Both subgroups were kept under observation for 14 days post challenge during which incidences of daily mortality were recorded. Challenge test were determined according to Brook *et al*. (1988) and Miles and Misra (1983).

Economic analysis

The cost of feed required to produce a unit of fish biomass was estimated using economic evaluation. The estimation was based on the local retail sale market price of all the dietary ingredients at the time of the study. These prices (in LE/kg) were as follows: herring fish meal, 22; soybean meal, 20; corn meal, 10; wheat bran, 4; starch, 4.0; fish oil, 13; corn oil, 18; vitamin premix, 10.0; mineral mixture, 4.0; and Lectins, 10.00.

Statistical analysis

Fish growth, feed utilization, survival rate, and proximate chemical composition data, Physiological analysis immunological parameters, antioxidant activity and were subjected to one-way ANOVA. Difference between means was tested at the 5% probability level using Duncans new multiple range test. All statistical analyses were done using SPSS program V.10 (SPSS, Richmond, USA) as described by Dytham (1999).

RESULTS AND DISCUSSION

Data in Table (2) shows that fish growth was enhanced significantly ($P<0.05$) with Moringa lectin meal (MLM) levels supplementation as compared to the control diet. Moreover, the highest final weight, weight gain, weight gain percentage and SGR were obtained at a diet containing 1.5%. In addition, the difference in fish performance between 1.5 and 2% MLM were non significant. This improvement may be related to the presence of readily available protein (lectins) in moringa meal that

recognize cells through carbohydrate binding sites, which is convenient for monogastric animals, and also to the higher levels of methionine and other essential amino acids when compared to a control diet. Also, the improvement of growth attributed to the higher level of vitamin A in moringa meal, as reported by Grubben and Denton (2004).

Table 2: Growth performances and feed utilization of Nile tilapia fingerlings fed different Moringa Lectin meal (MLM) for 12 weeks.

Items	Moringa lectin levels (%)			
	(0.0)	1.0	1.5	2.0
Initial weight (g)	8.63±0.01	8.64±0.02	8.68±0.1	8.63±0.1
Final weight (g)	27.23±0.41^{c}	32.69±0.33^{b}	36.13±0.14^{a}	35.40±0.32^{a}
Weight gain (g)	18.60±0.40^{c}	24.05±0.30^{b}	27.45±0.20^{a}	26.77±0.27^{a}
Weight gain %	215.52±4.54^{c}	278.35±2.57^{b}	316.24±4.57^{a}	310.19±1.62^{a}
SGR (% / day)	1.37±0.02^{c}	1.58±0.01^{b}	1.69±0.01^{a}	1.68±0. 01^{a}
Feed intake (g feed/fish)	31.74±0.17^{c}	35.61±0.15^{b}	37.89±0.69^{a}	35.05±0.63^{b}
FCR	1.71±0.04^{a}	1.48±0.02^{b}	1.38±0.03^{bc}	1.31±0.03^{c}
PER	2.05±0.07^{c}	2.39±0.06^{b}	2.53±0.06^{ab}	2.67±0.04^{a}
APU%	36.46±0.58^{c}	42.72±0.72^{b}	44.85±2.00^{ab}	46.63±0.64^{a}
EU%	21.36±0.58^{b}	24.71±0.43^{a}	26.19±1.29^{a}	26.77±0.53^{a}
Survival rate (%)	97.5	97.5	100	100

Means having the same letter in the same row is not significantly different at ($P < 0.05$).

Regarding the increase in protein digestibility with the addition of moringa meal, Fahey *et al.,* (2001) mentioned that moringa contains highly digestible protein. In addition to the presence of the major saturated fatty acids which present in the seeds as; palmitic, stearic, arachidic and benic acids. Oleic acid is the main unsaturated fatty acid Abdulkarim *et al.,* (2005), whose high concentration is desirable in terms of nutrition and stability during cooking diets. Moreover, as a natural source of benic acid, the *M. oleifera* seed oil has been used as a solidifying agent in margarines and other foodstuffs containing solid and semi-solid fat, therefore eliminating hydrogenation processes (FAO, 2012). These results agree with (Obasa *et al.*, 2013; Sidduraju and Becker, 2003). No significant differences were observed in survival among the treatments since its range was 97.5–100% ($P>0.05$; Table 2).

Feed intake increased significantly, while FCR decreased significantly ($P<0.05$) when fish fed MLM as compared to that fed on a control diet ($P<0.05$; Table 2). It was noticed that the highest and the lowest FCR were obtained at 0.0 (control) and 1.5% MLM (1.71±0.04 and 1.31±0.03, respectively). These are mainly due to low digestibility of energy and protein. This gradation may possibly related to the seed's bitter taste which contained alkaloids, saponins, cyanogenic glucosides and glucosinolates which enhanced appetite, so it used in animal diets. Moreover, PER and APU values increased significantly at MLM ($P>0.05$) and their highest values were obtained with 2% MLM (2.67±0.04 and 46.63±0.64 %, respectively; Table 2). While, there was no significant difference among all treatments of MLM in EU%. This related to the seeds contain an acidic protein with hemagglutinating activity, glucosinolates and phytates (Ferreira, 2004; Santos *et al.,* 2005).

The present study indicated no significant differences ($P>0.05$) in moisture, total lipid and ash contents in a fish body due to MLM supplementation and their ranges were (72.77–73.89%, 20.14-20.68 and 14.21- 15.10%, respectively) (Table 3). On the other hand, crude protein content increased significantly by increasing the MLM levels ($P<0.05$). However, there were insignificant differences ($P >0.05$) among treatments, it's ranged between 64.08 and 65.04 %. These results suggest that lectines

supplementation plays a role in enhancing feed intake with subsequent effects on fish body composition.

Table 3: Proximate chemical analysis (% on dry matter basis) of whole body of fingerlings Nile tilapia, *O. niloticus* (L) fed a diet containing different levels of Moringa Lectin meal (MLM) for 12 weeks.

Items	**Moringa lectin levels (%)**			
	(0.0)	**1.0**	**1.5**	**2.0**
Moisture	72.77±0.22	73.18±0.36	73.41±0.51	73.89±0.14
Crude protein	62.51±0.28[b]	64.08±0.50[a]	64.51±0.35[a]	65.04±0.39[a]
Total Lipids	20.31±0.72	20.57±0.38	20.68±0.35	20.14±0.08
Ash	15.10±0.08	14.61±0.22	14.56±0.23	14.21±0.42

Means having the same letter in the same row is not significantly different at ($P < 0.05$).

This agrees with Chiseva, (2006) that proved moringa can be considered as a potential feed component with high nutritive value for Nile tilapia. Contrarily, Abdel El-Naby, (2010) reported that there were no significant differences ($p<0.05$) in whole-body composition by supplementation of different levels of marjoram. Ahmad and Abdel-Tawwab, (2011) showed that there was no significant difference in dry matter, protein, lipids, and ash ($P > 0.05$) contents due to cinnamon supplementation.

Fish haematology is gaining importance in fish culture because of its importance in monitoring the health status of fish (Dienye and Qlumuji, 2014). Hrubec *et al.* (2001) reported that, haematological characteristics of most fish have been studied with the aim of establishing normal value range and deviation from it may indicate a disturbance in the physiological process, as regards hematological parameters analyzed (Hb, Ht, and RBCs) for Nile tilapia fed on MLM as natural extract of feed additives. Data in Table 4 showed that RBC and WBC counts increased with increasing MLM levels which may be a protective response of the fish to improve its immunity. However, there were only significantly ($P>0.05$) higher than control when dietary MLM levels increased to 1.5 and 2%. According to Akinwande *et al.* (2004) a measurable increase in WBC count of fish or any animal is a function of immunity or resistance to disease.

Similarly, percent lymphocyte of WBC was significantly higher in fish fed 1.5 and 2% MLM than that of fish fed 1.0 % MLM and non-supplemented diet. The inclusion of 1.5% of dietary MLM resulted in an increase in HB and Hct (9.27±0.03g/dI and 16.52±0.01 %, respectively). This increase observed in the fish fed plant additive diets might be an indication of high immunity on the fish (Soyinka and Boafo, 2015). Dietary MLM levels, however, significantly decreased the monocytes and granulocytes at 1.0, 2 % and control diet. This could be attributed to shift of water from the plasma to the muscle cells, thereby increasing the hemo concentration (El Mesallamy *et al.,* 2015). These probably suggest that a principle is in MLM that supports hemopoiesis since the value of RBC depends on those of Hb and Ht. The positive physiological effect of this plant extract may be related to the presence of lectin, trypsin inhibitor, tannin with a potent antioxidant activity.

The concentration of total protein in blood plasma is used as a basic index for the health status of brood fish (Rehulka, 1996) as the measurement of serum or plasma albumin is of considerable diagnostic value in laboratory animals as it relates to general nutritional status, and the integrity of the vascular system and liver function. Results indicated that total protein, globulin, creatinine, and glucose increased significantly ($P< 0.05$) as a result of increased growth (Table 4). These

results illustrated that high concentrations of total protein in fish serum were likely to be as a result of enhancement of the nonspecific immune response (El Mesallamy *et al.*, 2016). On the other hand, Cholesterol, AST, ALT, urea, serum albumin, and total lipid were decreased at 1.5% MLM. This decrease in AST and ALT suggests that the administration of MLM has a protective effect on the level of circulatory liver marker enzymes and hence liver damage.

Table 4: Some haematological and biochemical parameters in Nile tilapia, *O. niloticus* (L) fingerlings fed on different levels of Moringa lectin meal (MLM) as natural feed additives.

Items	Moringa lectin levels (%)			
	0.0	1.0	1.5	2.0
Hemoglobin (Hb g/dl)	4.54±0.01^{d}	7.17±0.06^{c}	9.27±0.03^{a}	8.70±0.06^{b}
Erythrocytes count (RBCsX10/cmm)	0.43±0.01^{c}	0.85±0.02^{b}	1.34±0.02^{a}	1.24±0.18^{a}
Haematocrite (Hct %)	5.88±0.01^{d}	7.57±0.04^{c}	16.52±0.01^{a}	10.83±0.02^{b}
WBCS (1x 10^{3}/mm^{3})	45.42±0.02^{c}	47.65±0.08^{b}	48.42±0.04^{a}	48.53±0.05^{a}
Lymphocytic	62.50±0.06^{b}	62.77±0.15^{b}	63.97±0.09^{a}	63.67±0.09^{a}
Monocytic	19.17±0.03^{b}	19.17±0.15^{b}	20.03±0.07^{a}	19.10±0.08^{b}
Granulocyte	15.47±0.12^{b}	15.13±0.09^{b}	15.97±0.12^{a}	15.30±0.15^{b}
Total protein (g/dl)	2.06±0.03^{b}	2.06±0.01^{b}	2.11±0.01^{b}	2.21±0.01^{a}
Albumin (g/dl)	0.80±0.02^{c}	1.71±0.02^{b}	1.74±0.02^{b}	1.91±0.01^{a}
Globulin (g/dl)	1.12±0.02^{d}	1.35±0.02^{c}	1.96±0.02^{a}	1.56±0.01^{b}
AST (GOT) U/L	48.33±1.20^{c}	52.33±1.45^{bc}	54.00±0.58^{b}	58.20±1.56^{a}
ALT (GPT) U/L	28.00±0.58^{a}	25.67±1.20^{ab}	24.00±1.15^{bc}	21.00±0.58^{c}
Total Lipids (mg/dl)	46.67±0.88^{a}	43.33±1.45^{ab}	42.33±0.88^{b}	40.33±0.88^{b}
Cholesterol (mg/dl)	61.67±1.45^{a}	61.33±0.67^{a}	52.33±1.76^{b}	61.67±0.88a
Urea (mg/dl)	1.95±0.03^{a}	1.92±0.03a	1.70±0.04^{b}	1.43±0.02^{c}
Creatinine (mg/dl)	0.24±0.01^{c}	0.29±0.01^{bc}	0.34±0.02^{ab}	0.36±0.03a
Glucose (mg/dl)	55.33±2.33^{b}	58.33±2.40^{ab}	63.00±1.73a	64.67±0.88^{a}

Means having the same letter in the same row are not significantly diff erent at ($P < 0.05$).

Several parameters such as lysozyme activity and respiratory burst activity are served as a good immunological indicator of fish health status (Chakrabarti *et al.*, 2014). At the end of the experimental period, lysozyme level and respiratory burst (NBT) activity increased in the serum of tilapia that were fed with diet containing MLM, whereas the highest value was recorded at 1.5% MLM diet (0.56±0.03 and 0.35±0.01, respectively) compared with fish group fed with control diet. These may be related to Lysozyme is an important component of the immune system of fish, as any form of pathogen challenge or environmental stress factor resulted in a subsequent change in lysozyme activity (Staykov *et al.*, 2005). These results suggest that the Moringa lectin supplementation could increase the nonspecific immune system of Nile tilapia resulting in fish resistance to *A. hydrophila* infection which has antimicrobial activities due to its phenolic compounds.

Table 5: Changes nitroblue tetrazolium (NBT), lysozyme, and post-challenge mortality of Nile tilapia fed dierent levels Moringa lectin meal (MLM) for 12 weeks.

Items	Moringa lectin levels (%)			
	(0.0)	1.0	1.5	2.0
Lysozyme (µg/ml serum)	0.30±0.03^{d}	0.48±0.03^{c}	0.56±0.03^{a}	0.51±0.06^{b}
Respiratory burst (NBT) activity (µg/mlserum)	0.15±0.03^{d}	0.31±0.02^{c}	0.35±0.01^{a}	0.32±0.05^{b}

Means having the same letter in the same row are not significantly dierent at $P < 0.05$.

MLM supplementation diets might enhance the antioxidant capability (CAT, SOD, GST and MDA) of *O. niloticus* in all concentration than control group which was consistent with the immune response as seen in Table (6). The highest value of CAT, SOD, GST were observed at 1.5% MLM as was supported by the fact that antioxidant enzymes are capable of scavenging reactive oxygen species and products

of lipid per oxidation, thereby protecting cells and tissues from oxidative damage (Li X and Liu, 2007; Abd El-Naby *et al.*, 2017).

Table 6: Changes in malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) of Nile tilapia fed diets supplemented by dierent levels of Moringa lectin meal (MLM) for 12 weeks.

Items	**Moringa lectin levels (%)**			
	(0.0)	**1.0**	**1.5**	**2.0**
CAT (IU/L)	2.38±0.22^{b}	3.02±0.08^{a}	3.24±0.09^{a}	3.19±0.04^{a}
SOD (IU/L)	55.09±0.66^{d}	76.18±0.83^{c}	101.51±0.90^{a}	84.69±0.32^{b}
GST (IU/L)	53.84±0.81^{c}	95.54±0.07^{b}	132.65±3.69^{a}	101.05±0.10^{b}
MDA (n mol/L)	13.07±0.46^{a}	6.69±0.11^{b}	6.58±0.10^{b}	6.71±0.03^{b}

Means having the same letter in the same row are not significantly diff erent at P <0 .05.

Also, data showed that the administration of MLM significantly reduced liver MDA content, indicating again that lectin could inhibit the process of lipid peroxide. This was supported by the fact that MDA level is a direct evidence of the toxic processes caused by free radicals (Livingstone, 2003). Antioxidant effects of *Moringa oleifera* were discussed by some researchers which Jabeen *et al.,* (2008) mentioned that the antimicrobial properties of the *Moringa oleifera* seed extracts may be due to lipophilic compounds, which attach to the cytoplasmic membrane. The authors also suggested that extracts of Moringa oleifera seeds may contain antibiotic metabolites, such as carboxylic acid, 2,4-diacetyl phloroglucinol, and cell wall-degrading enzymes and chitinases. The antioxidant effect of Moringa oleifera extract and fruit was explained by Luqman *et al.* (2012), who noticed that it was due to the presence of polyphenols, tannins, anthocyanin, glycosides, and thiocarbamates, which remove free radicals, activate antioxidant enzymes, and inhibit oxidases.

In this study the mortality rate of fish fed with lectin diets and challenged by *A. hydrophila* for 15 days was 10, 5 and 10 % for (1.0, 1.5 and 2% MLM, respectively) , whereas it was high in fish fed the control diet (75%). These results indicate that lectin had excellent results in improving fish immunity and overcome diseases so; it had high antibacterial effect against pathogenic *A. hydrophila* this is due to their antimicrobial activities. Various publications have documented the antimicrobial activities of plant extracts (Mathabe *et al.,* 2006; Ahmad and Aqil, 2007).

Table 7: Mortality rate (%) of Nile tilapia fingerlings fed a diet containing different levels of Moringa lectin and challenged by *Aeromonas hydrophila* for 15 days.

Items	**Moringa lectin levels (%)**			
	0.0	**1.0**	**1.5**	**2.0**
No. injected fish	**20**	**20**	**20**	**20**
No. of dead fish	**15**	**4**	**1**	**2**
Bacteria dose (5x10^{5} CFU)	**0.2 ml**	**0.2 ml**	**0.2 ml**	**0.2 ml**
Injection route	**IP***	**IP**	**IP**	**IP**
Mortality rate (%) after 10 days of injection**	**75**	**10**	**5**	**10**
RPS %		**86.7**	**93.3**	**86.7**

Means followed by the same letter are not significantly dierent at P <0 .05.
IP*= Interperitoneal injection

Thus plant extracts are promising natural antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. Plant materials contain mostly glycoproteins that are toxic in nature; they play a key role in the control of various normal and pathological processes in living organisms. So far more than hundred lectins have been purified and characterized but their antibacterial and toxicological studies against mortality of brine shrimp is scanty.

The lectins was reported to posses antibacterial activity against various gram positive and gram negative bacteria in particular against Bacillus subtilis, Bacillus megaterium, Streptococcus β -haemolyticus, Streptococcus aureus, Sarcina lutea, Shigella sonnei, E. coli, Klebsiella species, Shigella shiga, Shigella boydii, Shigella flexneriae, Shigella dysenteriae, Salmonella typhi and Pseudomonas aeuginosa (Ali *et al.*, 2003).

Economic evaluation of the experimental diets is shown in Table (8). There was a reduction in feed cost to produce 1 kg of fish weight gain of (18.99, 19.96 and 21.37 %, respectively) for the diet containing (2, 1.5 and 1%, respectively) MLM compared to the control diet (24.58%). Previous studies showed that the use of spices in small amounts reduced cost and profit in feeds of other fish species (Ahmad *et al.*, 2011).

Table 8: Economic evaluation of Nile tilapia fingerlings fed different of Moringa lectin levels (%) levels for 12 weeks.

Items	**Control**	**Moringa lectin levels (%)**		
	(0.0)	**1.0**	**1.5**	**2.0**
Cost/ kg feed (LE)	14.38	14.44	14.47	14.5
FCR (kg feed/ kg gain)	1.71	1.48	1.38	1.31
Feed cost/ kg gain (LE)	24.58	21.37	19.96	18.99
Reduction cost in kg gain (%)	100	13.05	18.79	22.74

CONCLUSION

In general results of the present study may lead us to conclude that incorporation of Moringa lectin at 1.5 – 2.0% level in Nile tilapia diets is worthy for obtaining better growth performance and higher immune response against pathogenic bacteria.

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