DOI: 10.2478/cjf-2018-0021

CODEN RIBAEG ISSN 1330-061X (print), 1848-0586 (online)

sciendo

EFFECTS OF DIETARY *Moringa oleifera* LEAF MEAL AS A REPLACEMENT FOR SOYBEAN MEAL ON GROWTH, BODY COMPOSITION AND HEALTH STATUS IN *Cyprinus carpio* JUVENILES

Ibrahim Adeshina[1,3*], Ramatu Abdulkareem Sani[2], Yusuf Adetunji Adewale[2,4], Lateef Oloyede Tiamiyu[1], Samuel Bem Umma[5]

[1]Department of Aquaculture and Fisheries, University of Ilorin, Nigeria
[2]Department of Animal Science, University of Maiduguri, Nigeria
[3]School of Life and Environmental Science, Faculty of Science, Engineering and Built Environment, Deakin University, Australia
[4]Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria
[5]Department of Fisheries and Aquaculture, Federal University, Wukari, Nigeria

*Corresponding Author, Email: adesina.i@unilorin.edu.ng

ARTICLE INFO

Received: 23 August 2017
Received in revised form: 14 August 2018
Accepted: 9 October 2018
Online first: 15 November 2018

Keywords:
Nutrition
Growth performance
Moringa oleifera
Soybean
Cyprinus carpio

ABSTRACT

The growth performance, nutrient utilization and health status of *Cyprinus carpio* fed various levels of *Moringa oleifera* leaf meal as a replacement for soybean meal was investigated. Six isonitrogenous diets were formulated with *Moringa oleifera* leaf meal at 0%, 10%, 20%, 30%, 40% or 50% crude protein replacement. The diets were fed to the fish at 5% body weight to 360 *Cyprinus carpio* juveniles (8.12±0.21 g) allotted to 18 happas (1 m^3) in a completely randomized design for 12 weeks. The results revealed that crude protein replacement levels of 30% had significantly better final weight, weight gain, specific growth rate, protein efficiency ratio and feed conversion ratio, while survival rates were not significantly different. Also, haematological, biochemical and immune responses of the fish fed *Moringa oleifera* leaf meal fortified diets were significantly improved. The results further suggest that higher inclusion replacement is possible but opined that, for growth and economic consideration, *Moringa oleifera* leaf meal could be used to replace 30% crude protein of soybean in the diet of *Cyprinus carpio* juveniles.

How to Cite

Adeshina, I., Sani, R.A., Adewale, Y.A., Tiamiyu, L.O., Umma, S.B. (2018): Effects of dietary *Moringa oleifera* leaf as a replacement of soybean meal on growth, body composition and health status in *Cyprinus carpio* juveniles. Croatian Journal of Fisheries, 76, 174-182. DOI: 10.2478/cjf-2018-0021

INTRODUCTION

There is a progressive increase in the demand for fish which could be attributed to the increase in the consumption pattern, rise in population and awareness on nutritional quality of the fish (Adeshina et al., 2016). Currently, consumption of farmed fish is higher than of the captured fish (FAO, 2016). The significant progress achieved by aquaculture has been

hindered/hampered with the fish feed which is accountable for about 50-60% of total input (Solomon et al., 2016). However, fish feed industry, otherwise known as aquafeed industry, is challenged with ingredient use in fish feed. Most of the ingredients such as fish, soybean, groundnut, cotton seed, etc. (Tiamiyu et al., 2014; Tiamiyu et al., 2015; Abdel-Tawwab et al., 2018; Musa et al., 2018) are in competition with other users such as direct human consumption, poultry and terrestrial animal feed leading to price hikes of fish feed (Tiamiyu et al., 2016), hence the need for less competitive and low-price alternative such as *Moringa oleifera*. Plant materials as a protein source in the diet of different fish species has been investigated (Bello et al., 2013; Tiamiyu et al., 2015). However, *Moringa oleifera* is one of the economic plants commonly grown in tropical Africa. *Moringa oleifera* with about 30% crude protein and important minerals (WHO, 1986; FAO, 1990) has been tested in the diets of some fishes (Tiamiyu *et al.,* 2016), however, there is paucity of information on its utilisation in the diet of *Cyprinus carpio,* hence the need for this study. Although crude protein of soybean (45%) is higher than the crude protein of *Moringa oleifera* leaf (25 to 30%), the high percentage of the crude protein postulates its utilisation in the diet of fish. *Cyprinus carpio* is important fish species and has become one of the most traded and consumed fish species in the world (FAO, 2016). This study examines the effect of *M. oleifera* leaf meal as replacement for soybean in the diet of *Cyprinus carpio.*

MATERIAL AND METHODS

This study was conducted in the indoor laboratory of School of Life and Environmental Science, Faculty of Science, Engineering and Built Environment, Deakin University, Australia. Three hundred and sixty (360) *Cyprinus carpio* juveniles (8.12±0.21 g) were purchased from a reliable farm and acclimatised for 14 days. The fish were allotted to 18 happas ($1m^3$) in a completely randomised design with each happa containing 20 fish. The happas were attached to a sinker and floater at each edge to properly spread the happa as designed. The fish were fed at 5% body weight twice daily and the proportions were adjusted fortnightly while the experiment lasted for 12 weeks. Water parameters were monitored twice a day (08:00 and 18:00 h). Water temperature was measured by mercury-in-glass thermometer. The dissolved oxygen (DO) was measured using a digital DO meter (Model AVI-660, Labtech International Ltd, Heathfield, UK). The pH degree was assessed by a digital pH-meter (Model Photoic 20, Labtech International Ltd, Heathfield, UK). The water temperature, DO and pH ranges throughout the experimental period were 24.1 - 25.7°C, 5.6 - 6.6 mg/L, and 7.24-7.81, respectively. These ranges are appropriate for fish farming (Boyd and Tucker, 2012).

Moringa oleifera leaves were obtained in a botanical garden and air-dried at the room temperature. The meal was prepared using the method of Hardy (2000). The leaves were steam-heated in an autoclave for 15 minutes at 60°C to neutralise the anti-nutrient factors and grinded to fine powder in an electro-operated hammer mill. *Moringa oleifera* meal was incorporated to form six experimental diets (40% crude protein) including control (Table 1) to replace soybean meal. The diets were prepared with the addition of *Moringa oleifera* meal at 0%, 10%, 20%, 30%, 40% or 50% replacement. The feed ingredients including *Moringa oleifera* meal were ground in a hammer, pelleted to 2.0 mm pellet size and air-dried. The experimental diets were stored in a refrigerator until used and reproduced every fortnight. The moisture contents were determined by pre-weighing the samples and air-drying in a hot air-oven. The final weight was subtracted from the initial weight (AOAC, 2005). Crude protein was determined using micro-Kjeldahl distillation method of AOAC (2005). The percentage protein was calculated by multiplying the nitrogen content of the sample by a factor of 6.25. This was determined by burning the samples in a muffle furnace at 550°C for three hours, the samples were allowed to cool, weighed and expressed as percentage as content (AOAC, 2005). The samples were extracted in a soxhlet extractor using petroleum ether (40-60°C) for three hours. The solvents were evaporated and the ether extract was determined as the residue obtained (AOAC, 2005). This was achieved by subjecting the residual sample from the ether extraction to a successive treatment with boiling acid (0.25N sulphuric acid) and alkali of defined concentration (0.313N sodium hydroxide) under controlled conditions (AOAC, 2005). The ingredients contained fish meal (CP 72%), soybean (CP 45%), *Moringa oleifera* leaf meal (CP 25%) and maize (CP 9.2%).

Growth parameters were monitored bi-weekly. Initial weight, weight measured every two weeks and final weight were recorded using weighing scale. Growth and nutrients utilization parameters were calculated using the following formulae. Fish in each tank were group-weighed and average mean was recorded. Three (3) fish were used from each experimental unit for proximate analysis. Similarly, three (3) fish were used from each experimental unit for haematological and innate immune response parameters.

a. $$\text{Weight gain (\%)} = \frac{W_1 - W_0}{W_0} \times 100$$

b. $$\text{Specfic growth rate} = \frac{\text{Log}W_1 - \text{Log}W_0}{\text{Length of culture period}} \times 100$$

c. $$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Dry weight of feed fed (g)}}{\text{Fish weight gain (g)}}$$

d. $$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Wet body weight gain (g)}}{\text{Crude protein fed}}$$

e. $$\text{Survival rate (\%)} = \frac{\text{Initial number of fish stocked - Mortality}}{\text{Initial number of fish stocked}} \times 100$$

Where: W_1 = Final mean weight; W_0 = Initial mean weight.

Table 1. Gross and proximate compositions of experimental diets

	Percentage crude protein replacement levels					
Ingredients	Control (0% CP)	T1 (10% CP)	T2 (20% CP)	T3 (30% CP)	T4 (40% CP)	T5 (50% CP)
Fish meal	31.32	31.32	31.32	31.32	31.32	31.32
Soybean	32.45	26.77	21.00	15.33	9.61	3.89
MOM	0.00	5.68	11.45	17.12	22.84	28.56
Maize	31.73	31.73	31.73	31.73	31.73	31.73
Starch	1.00	1.00	1.00	1.00	1.00	1.00
Vegetable oil*	1.50	1.50	1.50	1.50	1.50	1.50
Premixes**	2.00	2.00	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition (%) dry weight						
Crude protein	40.08±0.21	40.60±0.23	40.51±0.67	40.43±0.36	40.33±0.81	39.69±0.11
Moisture	7.17±0.22	8.03±0.62	8.13±0.15	8.36±0.41	8.60±0.20	8.85±0.72
Ash	6.30±0.31	7.92±0.26	9.10±0.35	8.08±0.13	8.81±0.32	8.07±0.38
Fibre	9.21±0.37	11.01±0.39	10.93±039	11.00±0.42	11.45±2.00	11.68±0.47
***NFE	37.24±0.06	32.44±0.21	31.33±0.53	32.13±0.35	30.81±0.45	31.71±0.13

* Premixes = HI-MIX®AQUA (Fish) each one kilogram (1 kg) contains; vitamin A, 4,000,000 International Unit (IU); vitamin D3, 8,00,000 IU; vitamin E, 40, 000 IU; vitamin K3, 1,600 mg; vitamin B1, 4,000 mg; vitamin B2, 3,000 mg; vitamin B6, 3,800 mg; vitamin B12, 3 mcg; Nicotinic acid 18000 mg; Pantothenic acid, 8000 mg; Folic acid, 800 mg; Biotin, 100 mcg; Choline chloride 120,000 mg; Iron, 8000 mg; Copper, 800 mg; Manganese, 6000 mg; Zinc, 20,000 mg; Iodine, 400 mg; Selenium, 40 mg; Vitamin C C (coated), 60,000 mg; Inositol, 10,000 mg; Colbat, 150 mg; Lysine, 10,000 mg; Methionine, 10,000 mg; Antioxidant, 25,000 mg; ** mamador vegetable oil; ***NFE (Nitrogen Free Extract); MOM = *Moringa oleifera* meal; Values are presented in mean ± standard deviation in the same column.

Blood samples were obtained from the caudal vein of the anaesthetized fish and gently transferred into lithium heparinized tubes at room temperature. The extracted blood was divided in two groups of Eppendorf tubes containing anticoagulant (sodium heparinate 20 U/L) (Merrifield et al., 2011) for hematology and empty bottle at 4°C to allow clotting, and centrifuged at 5000 × g for 10 min at the room temperature to obtain serum. Hematocrit (Ht) values were estimated after sampling by placing fresh blood in glass capillary tubes, furthermore centrifuged for 10 min in a microhematocrit centrifuge, after that assessing the packed cell volume. Hemoglobin (Hb) levels were assessed colorimetrically by estimating the formation of cyanomethemoglobin according to Vankampen and Ziglstra (Vankampen and Ziglstra, 1961). Red blood cells (RBCs) and white blood cell (WBCs) counts were determined following the methods of Brown (Brown, 1988). The differential count (lymphocytes, heterocytes, monocytes, eosinophils and basophil) was displayed by staining blood films with Wright Giemsa stain after fixing in methanol. Glucose was determined colorimetrically according to Trinder (1969). Cholesterol was determined by the method of Allain et al. (1974). Total protein and albumin were assessed colorimetrically according to Henry (1964). Globulin was obtained by the subtraction of albumin from total protein and albumin - globulin ratio was calculated. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed colorimetrically according to Reitman and Frankel (Tietz et al., 1983). Alkaline phosphatase (ALP) was measured by the colorimetric method of Tietz et al., 1983). Urea and creatinine were assessed using methods of Coulombe and Favreau (1963), and Lausen (1972), respectively.

The parameters of antioxidant and immune response in fish sera were measured using the diagnostic reagent kits (Randox® Laboratories, Crumlin, County Antrim, UK). The activity of superoxide dismutase (SOD) was assessed spectrophotochemically at 550 nm (McCord and Fridovich, 1969). Catalase (CAT) activity was assessed by measuring the decrease of H2O2 concentration at 240 nm according to Aebi (1984). Lysozyme activity of fish sera was determined by using lysoplate technique (Grinde, 1989). In brief, 0.60 mg/mL Micrococcus luteus was cast in 1% agarose gel (Difco, USA) with 50 mM phosphate buffer (pH 6.2). Wells (6 mm) created nutrient agar plates and were filled with 25 µL of serum samples, and incubated for 20 h at 25°C. Lysozyme activity was calculated from a standard curve prepared

with lysozyme from chicken egg white. The respiratory burst activity was measured using diagnostic reagent kits (Randox® Laboratories, Crumlin, County Antrim, UK) as described by Secombes (1990). In brief, 100 ml of the blood suspension were added to each well of a 96-well microtitre plate (Nalge-Nunc, Hereford, UK). The plate was incubated at 25°C for two hours to allow cell attachment. Unattached cells were washed off three times using fresh L-15 medium, which was subsequently supplemented with NBT (1 mg/ml) and phorbol 12-myristate 13-acetate (PMA, Sigma Aldrich; 1 mg/ml) dissolved in dimethyl sulphoxide (DMSO, Sigma), and 100 ml added to each well of the microtitre plate and incubated for 1 h at room temperature. After incubation, the supernatant was removed from the plate and NBT reduction fixed with 100% methanol for 10 min. The plate was then washed with 70% methanol and left to air-dry. A mixture of 120 ml of 2 M potassium hydroxide and 140 ml DMSO was added to dissolve the resulting formazan blue crystals. The NBT reduction was estimated using the microplate reader (Optica, Mikura Ltd, UK) at 630 nm, and respiratory burst activity was expressed as a NBT reduction. Data obtained were analysed using descriptive statistics and one-way analysis of variance at $\alpha_{0.05}$. Polynomial contrast using quadratic regression was used to determine the optimum level of *Moringa oleifera* to replace soybean crude protein for crude protein for weight gain. The differences in the means were separated using Duncan Multiple Range Test with the aid of Statistical Package for Social Science (SPSS) IBM version 20.0.

RESULTS

Growth performance and nutrients utilization of *Cyprinus carpio* juveniles fed various inclusion levels of *Moringa oleifera* as replacement for soybean in terms of crude protein were presented in Table 2. The mean final weight (29.04±1.25 g) and mean weight gain (20.92±1.02 g) of the fish fed T3 diets containing 50% crude protein replacement of soybean were significantly higher (16.33±0.06 g), while the least (8.20±0.13 g) were observed in the group treated diet T1. There were significant differences in the mean final weight and mean weight gain ($P<0.05$) of fish treated with experimental diets. Also, specific growth rate, feed conversion ratio and protein efficiency ratio and survival rate of fish fed experimental diets differed significantly ($P<0.05$), as shown in Table 2. Highest specific growth rate (0.66±0.02 g/day) and protein efficiency ratio (0.52±0.01) were obtained in fish fed diet T3 and least 0.36±0.00 g/day and 0.21±0.01, respectively, were obtained in fish fed diet T1. In the same way, fish fed control diet had the highest feed conversion ratio (1.59±0.21) and lowest (1.61±0.17) was recorded in fish fed diet T3. However, survival rate was highest in fish fed T2 diet (100.00±0.00 %) and lowest in group treated with diet T4 (91.67±1.78%). There was significant difference in the survival rate of the fish fed experimental diets as indicated in Table 2.

The proximate composition of the fish carcass fed the diets fortified with *Moringa oleifera* revealed an increase in most of the proximate parameters except moisture content which is relatively stable (Table 3). The highest crude protein (12.84±0.37%) and ash (2.35±0.21%) were obtained in fish fed diet T4, while least crude protein (11.25±0.21%) and ash (2.04±0.15%) were obtained in fish fed basal diet. Moisture content ranged from 70.30±0.03% to 72.76±0.19% in fish fed experimental diets. Significantly higher ether extract (4.15±0.13%) was recorded in diet T5, while least (1.62±0.43%) was obtained in fish fed T1 diet. Furthermore, nitrogen free extract was the highest (13.65±0.74%) in fish fed basal diet, while lowest (6.81±0.06%) was obtained in group T5. Fish fed diet T5 had the highest (1.13±0.28%) fibre content, while least (0.80±0.01) was recorded in fish fed diet T1. There were significant differences in the crude protein, moisture, ash, ether extract and nitrogen free extract contents of fish fed experimental diets ($P<0.05$), while fibre values obtained were not significantly different ($P>0.05$), as shown in Table 3.

Table 2. Growth performance and nutrient utilization of *Cyprinus carpio* juveniles fed *Moringa oleifera* based diets

Treatment	MIW (g)	MFW (g)	MWG (%)	SGR (g/day)	FCR	PER	SR (%)
Control	8.12±0.02^a	18.43±0.23^c	126.97±2.16^c	0.42±0.01^c	1.59±0.21^c	0.26±0.01^b	98.33±2.50^a
T1	8.13±0.05^a	16.33±0.06^c	100.86±2.20^d	0.36±0.00^c	2.22±0.07^b	0.21±0.01^b	95.00±2.33^a
T2	8.10±0.10^a	21.74±0.19^b	168.40±3.17^b	0.51±0.03^b	1.89±0.13^c	0.34±0.02^b	100.00±0.00^a
T3	8.12±0.11^a	29.04±1.25^a	257.64±5.19^a	0.66±0.02^a	1.61±0.17^a	0.52±0.01^a	98.33±2.50^a
T4	8.12±0.10^a	27.75±0.57^a	241.75±3.07^a	0.64±0.01^a	2.45±0.04^a	0.49±0.03^a	91.67±1.78^b
T5	8.13±0.03^a	27.62±0.38^a	239.73±2.86^a	0.63±0.01^a	2.54±0.16^a	0.49±0.01^a	93.33±2.50^a

MIW = Mean Initial Weight; MFW = Mean Final Weight; MWG = Mean Weight Gain; SGR = Specific Growth Rate; FCR = Feed Conversion Ratio; PER = Protein Efficiency Ratio; SR = Survival Rate; Means ± standard deviations with different superscripts within the same column are significantly different ($P<0.05$).

Table 3. Proximate composition of *Cyprinus carpio* juveniles fed *Moringa oleifera* based diets (%)

	Experimental diets					
Parameters	Control	T1	T2	T3	T4	T5
Crude Protein	11.25±0.21^a	12.03±0.02^a	12.51±0.72^a	12.82±1.03^a	12.84±0.37^a	12.83±0.04^a
Moisture	70.30±0.03^a	70.73±1.29^a	70.96±0.22^a	71.55±2.07^a	70.03±0.35^a	72.76±0.19^a
Ash	2.04±0.15^b	2.16±0.02^a	2.34±0.13^a	2.33±0.01^a	2.35±0.21^a	2.32±0.22^a
Ether extract	1.72±0.11^b	1.62±0.43^b	1.81±0.03^a	2.51±0.21^a	2.76±0.16^a	4.15±0.13^a
NFE	13.65±0.74^a	12.43±1.06^b	11.57±0.16^b	10.02±0.03^b	11.22±0.30^b	6.81±0.06^c
Fibre	1.04±0.01^a	1.03±0.01^a	0.81±0.11^a	0.77±0.01^a	0.80±0.01^a	1.13±0.28^a

NFE = Nitrogen Free Extract; Means ± standard deviations with different superscripts within the same column are significantly different (P<0.05).

Relationship between inclusion levels of *Moringa oleifera* leaf meal and weigh gain *Cyprinus carpio* were established in Fig 1. A progressive increase in the weight gain was observed with increase in the inclusion level of *Moringa oleifera* leaf meal. Estimation of the optimum inclusion level from the expression revealed 67% as optimum inclusion level (Fig. 1). The hematological profile of *Cyprinus carpio* fed various *Moringa oleifera* leaf levels is shown in Table 4. Significant increases in PCV, Hb, RBC and WBC levels were observed in fish fed diets fortified with *Moringa oleifera* levels with insignificant difference in fish fed 11.45 to 28.56% (P < 0.05; Table 3). Similarly, platelet lymphocytes and eosinophils counts increased significantly in fish fed fortified diets when compared with the group fed control diet. However, significant reductions in heterocytes, monocytes and basophil counts were recorded in fish fed dietary MOM.
Biochemical profiles of *Cyprinus carpio* fed diets supplemented with MOM for 12 weeks were presented in

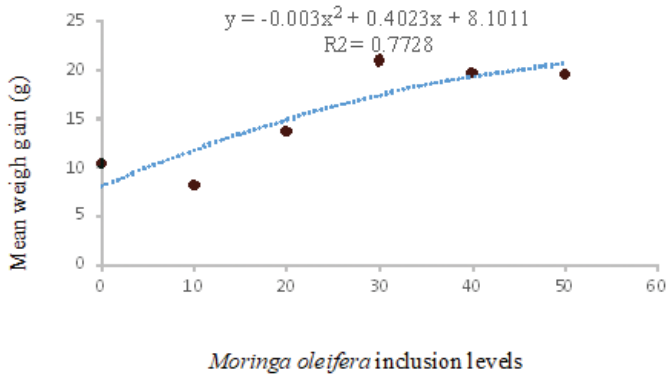

Fig 1. Relationship between various inclusion levels of Moringa oleifera fortified diets and weight gain of *Cyprinus carpio*

Table 4. Hematological variables of *Cyprinus carpio* fed diets containing different levels of *Moringa oleifera* leaf extract (CBLE) for 12 weeks

Parameters	Treatments					
	Control	T1	T2	T3	T4	T5
PCV(%)	18.00±1.26^b	19.00±0.31^b	20.00±1.05^a	24.00±1.27^a	24.00±1.18^a	26.00±1.54^a
Hb (g/dL)	4.60±0.95^c	4.80±0.12^c	6.00±0.27^b	7.40±0.32^b	8.30±0.41^a	8.50±0.28^a
RBC (	1.07±0.37^b	1.07±0.04^b	1.15±0.03^b	1.43±0.01^b	2.21±0.20^a	2.22±0.05^a
WBC (	10.60±1.19^a	12.58±1.03^{ab}	13.40±0.93^a	13.52±1.14^a	13.64±0.68^a	13.78±1.00^a
Platelets (	59.00±4.22^c	88.00±3.17^c	139.00±8.92^b	161.00±8.17^a	163.00±7.54^a	186.00±6.15^a
Lymphocytes (%)	55.00±2.73^c	63.00±4.04^b	64.00±7.19^b	68.00±5.03^a	72.00±4.42^a	74.00±5.75^a
Heterocytes (%)	41.00±1.04^a	31.00±2.21^b	30.00±2.64^b	29.00±2.17^b	25.00±1.23^c	23.00±2.67^c
Monocytes (%)	1.00±0.03^a	1.00±0.12^a	2.00±0.05^a	1.00±0.10^a	1.00±0.04^a	2.00±0.06a
Eosinophils (%)	3.00±0.03^a	4.00±0.05^a	3.00±0.01^a	2.00±0.00^a	2.00±0.01^a	1.00±0.03^b
Basophils (%)	0.00±0.00^a	1.00±0.01^a	1.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a

Means ± standard deviations with different superscripts within the same row are significantly different (p<0.05)

Table 5. Glucose and cholesterol levels decreased significantly with the highest value recorded in fish fed control diet and lowest in fish fed T5 diet. Total protein, albumin and globulin were significantly increased in fish fed diets supplemented with MOM when compared with fish fed control diet ($p < 0.05$). However, albumin-globulin ratio was not significantly different in fish ($p > 0.05$) fed experimental diets.

In addition, enzymes of the fish were presented in Table 6. The AST, ALT, ALP, urea and creatinine levels were significantly increased in the fish fed experimental diets when compared with the group fed control diet with respect to increase in the inclusion levels of MOM. Highest AST (183.00±9.59 IU/L), ALT (25.00±2.83 IU/L), ALP (250.00±7.03 IU/L), Urea (10.40±1.55 mg/dL) and creatinine (0.70±0.01 mg/dL) were obtained in fish fed T5, while fish fed control diets had the lowest values (Table 6).

Antioxidant variables of the fish fed diets fortified with *Moringa oleifera* leaf meal were increased (Table 7). There was a greater increase in SOD, CAT, RBA and lysozyme activities of fish fed experimental diets than in the fish fed control diet. Highest SOD (1.30±0.03 U/mL), CAT (2.28±0.13 U/mL), RBA (217.16±5.53 µmole) and lysozyme activities (15.52±1.23 U/mg) were recorded in fish fed diet T5, while the lowest were obtained in fish fed control diets.

Table 5. Biochemical profiles of *Cyprinus carpio* juveniles fed *Moringa oleifera* based diets (%)

Treatments	Glucose (mg/dL)	Cholesterol (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A:G ratio
Control	211.00±24.56^{a}	173.00±7.21^{a}	3.10±0.26^{b}	0.50±0.02^{a}	2.80±0.15^{b}	0.18±0.01^{a}
T1	193.00±12.34^{a}	141.00±3.10^{b}	3.40±0.18^{b}	0.50±0.02^{a}	2.50±0.13^{b}	0.20±0.03^{a}
T2	180.00±9.28^{b}	120.00±5.17^{b}	3.60±0.52^{b}	0.70±0.04^{a}	3.10±0.16^{b}	0.23±0.01^{a}
T3	162.00±11.10^{b}	110.00±8.39^{b}	4.80±0.78^{a}	0.80±0.01^{a}	4.30±0.18^{ab}	0.19±0.02^{a}
T4	154.00±14.32^{b}	103.00±4.52^{b}	5.20±0.13^{a}	0.90±0.03^{a}	4.50±0.21^{a}	0.20±0.02^{a}
T5	142.00±15.20^{b}	100.00±4.26^{b}	6.10±0.16^{a}	1.20±0.01^{a}	5.00±0.28^{a}	0.24±0.01^{a}

Means ± standard deviations with different superscripts within the same column are significantly different ($p<0.05$)

Table 6. Aspartate aminotransferase (AST), Alamine aminotransferase (ALT), Alkaline phosphate (ALP), urea and creatinine of *Cyprinus carpio* fed diets supplemented with *Moringa oleifera* leaf meal

Treatments	AST(IU/L)	ALT (IU/L)	ALP (IU/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	173.00±12.30^{b}	16.00±1.28^{b}	112.00±7.53^{c}	9.60±0.46^{a}	0.40±0.01^{c}
T1	172.00±9.17^{b}	17.00±1.17^{b}	113.00±8.39^{c}	9.70±0.72^{a}	0.50±0.06^{b}
T2	177.00±10.65^{a}	21.00±2.13^{ab}	184.00±6.43^{c}	9.95±1.02^{a}	0.60±0.00^{b}
T3	178.00±11.20^{a}	22.00±2.05^{ab}	200.00±9.52^{b}	10.00±2.13^{a}	0.61±0.01^{b}
T4	181.00±8.45^{a}	24.00±2.18^{a}	236.00±8.35^{b}	10.00±1.79^{a}	0.63±0.02^{a}
T5	183.00±9.59^{a}	25.00±2.83^{a}	250.00±7.03^{a}	10.40±1.55^{a}	0.70±0.01^{a}

Means ± standard deviations with different superscripts within the same column are significantly different ($p<0.05$)

Table 7. Antioxidant parameters of *Cyprinus carpio* diets fortified with *Moringa oleifera* leaf meal

Treatments	SOD (U/mL)	CAT (U/mL)	RBA (µmole)	Lysozyme Activity (U/mg)
Control	0.45±0.11^{b}	1.21±0.14^{b}	118.20±7.53^{c}	9.07±0.52^{b}
T1	0.61±0.01^{ab}	1.54±0.12^{a}	145.62±6.35^{b}	9.25±0.81^{b}
T2	0.80±0.11^{ab}	1.77±0.13^{a}	181.04±6.51^{b}	9.84±0.19^{b}
T3	1.07±0.03^{ab}	2.01±0.06^{a}	206.15±8.36^{a}	10.22±1.02^{b}
T4	1.25±0.10^{a}	2.21±0.02^{a}	208.89±9.48^{a}	13.73±0.89^{a}
T5	1.30±0.03^{a}	2.28±0.13^{a}	217.16±5.53^{a}	15.52±1.23^{a}

(Superoxide dismutase, SOD; Catalase, CAT; Respiratory Burst Activity, RBA and Lysozyme Activity); Means ± standard deviations with different superscripts within the same column are significantly different ($p<0.05$)

DISCUSSION

The geometric and progressive increase in the demand for fish emphasised the need for aquaculturist to produce high quality fish that would reach an adult size within the shortest possible time and reduce the production cost, especially the cost of feeding. Cost of feeding was estimated to be 40%-60% of total production cost (Fagbenro, 2005). To obtain an optimum growth within expected culturing period, nutrients requirement of the fish must be met and supplied appropriately in addition to good quality fish seed among other factors (Davies and Gouevia, 2010; Tiamiyu et al., 2014). Protein is one of the major nutrients in the diet of fish and should be provided in diet to ensure adequate growth.
In the present study, *Cyprinus carpio* juveniles were fed diets containing 40% crude protein. The growth performance showed that mean final weight and mean weight gain of the fish fed fortified diets were better and higher than fish fed basal diet. This result is similar to the results of Bello et al. (2013), Shazali et al. (2013) and Tiamiyu et al. (2014) who reported on fish fed diets of walnut leaves, onion bulb residues and watermelon seed meal, respectively. The values obtained in this study were higher than values reported by Tiamiyu et al. (2016) on *Orechromis niloticus* fed *Moringa oleifera* leaf meal. However, this variation may be attributed to the replacement nature. In the study by Tiamiyu et al. (2016), soybean was replaced with *Moringa oleifera* leaf meal weight for weight while the present study replaced crude protein for crude protein. The study further revealed that feed conversion ratio, specific growth rate and protein efficiency ratio of the fish fed experimental diets were improved compared to the fish fed control diet. The least feed conversion ratio observed in fish fed diet T3 which is not significantly different from group fed control diets corroborate the weight gain recorded. Also, specific growth rate and protein efficiency ratio followed a similar trend with weight gain. The better feed utilization recorded in treated groups falls within recommended values of SGR - less than 2 (FAO, 2004). This conforms the findings of Craig and Helfrich (2002), Bello et al. (2013) who reported that protein-sparing effect of balance diets would enhance growth performance and nutrient utilisation of the fish. The higher percentage of survival rate recorded in this study indicated that diets were not harmful to the fish, which is similar to the observation of Bello et al. (2013) and Tiamiyu et al. (2016) who reported survival rates of more than 90%, more than *Clarias gariepinus* and *Cyprinus carpio* fed diets fortified with walnut leaves extract and *Moringa oleifera* leaf meal, respectively. Proximate compositions of the fish fed fortified diets were higher than the fish fed basal diets, which could be attributed to the better utilisation of nutrients in the feed by the fish. The results share similar observation with the findings of Majappa et al. (2011).

Previous studies have shown that haematological and biochemical parameters are good indicators of health status of the fish (Sula and Aliko 2017; Abdel-Tawwab et al., 2018), especially erythrocytes. The higher RBC, WBC and Hb observed in the fish fed *Moringa oleifera* meal revealed that the maturity of the RBC and level of Hb might promote a higher presence of oxygen. Similarly, most of the haematological parameters were improved in fish fed fortified diets when compared to the fish fed control diet. Biochemical parameters revealed that apart from glucose and cholesterol, others such as total protein, albumin and globulin were increased significantly. The blood enzymes measured, such as AST, ALT, ALP, urea and creatinine, were significantly higher in the fish fed *Moringa oleifera* meal based diets than the control. The higher values recorded suggest better functionality of the internal organs such as liver, kidney, etc. The results of innate immune response parameters (SOD, CAT, RBA and lysozyme activity) were greatly higher in fish fed *Moringa oleifera* meal fortified diets. The increases haematological, biochemical and innate immune response parameters of *Cyprinus carpio* fed *Moringa oleifera* meal as replacement for soymeal reveal immunodulatory performance of *Moringa oleifera* meal in the diet. The result of the present study is in agreement with the findings of Sula and Aliko (2017) and Abdel-Tawwab et al. (2018).

CONCLUSION

Investigation of the effects of *Moringa oleifera* leaf meal as replacement (protein for protein) in the diets of *Cyprinus carpio* juveniles revealed that significant growth improvement, reduction in the cost of soybean and enhanced immunity of the fish could be achieved. This would go a long way in raising juveniles with fast growth to meet the astronomic demand for the fish. In this study, fish fed diet containing 30% crude protein replacement had the highest weight gain. However, estimation of optimum values shows crude protein for crude protein replacement of *Moringa oleifera* leaf meal for soybean meal up to 67% is possible. Further studies should be performed with different processing methods of *Moringa oleifera* leaf for the possibility of total replacement in the diet of *Cyprinus carpio.*

SAŽETAK

UČINAK BRAŠNA LIŠĆA MORINGE *Moringa oleifera* KAO HRANIDBENE ZAMJENICE SOJINOM BRAŠNU NA RAST, SASTAV MESA I ZDRAVSTVENI STATUS MLAĐI ŠARANA *Cyprinus carpio*

U radu je istraživana učinkovitost rasta, iskoristivost hranjivih tvari i zdravstveno stanje šarana *Cyprinus carpio* hranjenih različitim razinama brašna listova moringe *Moringa oleifera* kao hranidbene zamjenice za sojino brašno. Izrađeno je šest izo-dušičnih hranidbenih smjesa s postotnom zamjenom sirovih proteina od 0%, 10%, 20%, 30%, 40% i 50% brašnom listova moringe. Mlađ šarana (n=360; W=8.12±0.21 g) su razdijeljeni u 18 hapasa (1m^3) te su hranjeni hranidbenim smjesama u omjeru od 5% ukupne mase tijela ribe. Rezultati su ukazali kako riba hranjena smjesom s razinom zamjene sirovog proteina od 30% ima značajno bolju završnu masu, prirast, specifičnu stopu rasta, omjer proteinske učinkovitosti i omjer pretvorbe hrane, dok su stope preživljavanja nisu bile značajno različite. Također, značajno su poboljšane hematološke, biokemijske i imunološke reakcije riba hranjene smjesama s zamjenom *Moringa oleifera*. Rezultati upućuju i na to da je viša razina zamjene moringom moguća, ali je i mogući utjecaj na rast ribe i ekonomsku isplativost. Brašno lišća moringe *Moringa oleifera* može biti korišten za zamjenu 30% sirovog proteina sojinog brašna u hranidbi mlađi šarana *Cyprinus carpio*.

Ključne riječi: hranidba, performansa rasta, *Moringa oleifera*, soja, *Cyprinus carpio*

REFERENCES

Abdel-Tawwab, M., Adeshina, I., Jenyo-Oni, A., Ajani, E.K. Emikpe, B.O. (2018): Growth, physiological, antioxidants, and immune response of African catfish, *Clarias gariepinus* (B.), to dietary clove basil, *Ocimum gratissimum*, leaf extract and its susceptibility to *Listeria monocytogenes* infection, Fish and Shellfish Immunology, 78, 346–354.

Adeshina, I., Jenyo-Oni, A., Ajani, E. K., Adewale, Y.A. (2016): Natural occurrence of *Diplostomum spp.* In farm-raised African catfish (*Clarias gariepinus*) from Oyo State, Nigeria, International Journal of Veterinary Science and Medicine 4, 41-45.

Aebi, H. (1984): Catalase in vitro Methods, Enzymology, 105, 121–126.

Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W., Fu, P.C. (1974): Enzymatic determination of total serum cholesterol, Clinical Chemistry, 20, 470-475.

AOAC, (2005): Official methods of analysis AOAC international methods 934.01, 988.05, 920.39 and 942.05. Arlington, VA, USA: AOAC International.

Bello, O. S., Olaifa, F. E., Emikpe, B. O., Ogunbanwo, S. T. (2013): Potentials of walnut (*Tetracarpidium conophorum* Mull. Arg) leaf and onion (*Allium cepa* Linn) bulb extracts as antimicrobial agents for fish. African Journal of Microbiology Research, 7, 19, 2027-2033.

Boyd, C.E., Tucker, C.S. (2012): Pond aquaculture water quality management, Springer Science & Business Media.

Brown, B. A. (1988): Routine haematology procedures, in: B.A. Brown (Ed.), Hematology, principles and procedures, Leo and Fabiger, Philadelphia, Pennsylvania, USA, 7-122.

Coulombe, J. J., Favreau, L. (1963): A new simple semi micro method for colorimetric determination of urea, Clinical Chemistry, 9, 102-108.

Craig, S., Helfrich, I. A. (2002): Understanding fish nutrition, feeds and feeding. Department of Fisheries and Wild Life Science, Virginia Tech., 420, 256.

Davies, S. J., Gouevia, A. (2000): Response of common carp fry fed diets containing a pea sed mean (*Prisum sativum*) subjected to different thermal processing methods. Aquaculture, 205, 14, 117-123.

Fagbenro, O. A. (2005): Aquaculture in Nigeria: history, status and prospects. Small-Scale Aquaculture in sub-Saharan Africa: Revisiting the Aquaculture Target Group Paradigm, Report of the FAO-World Fish Centre Workshop, CIFA Occasional Paper. J. Moehl, M. Halwart and R. Brummett. Eds. Limbe, Cameroon, Rome. 54.

FAO (1990): Protein quality evaluation (Report of Joint FAO/WHO constitution) held in Bethside, M.D., USA, DE, 10-35.

FAO (2016): The State of World Fisheries and Aquaculture: Contributing to Food Security and Nutrition For All, FAO, Rome Italy 24.

Grinde, B. (1989): Lysozyme from rainbow trout, *Salmo gairdneri* Richardson, as an antibacterial agent against fish pathogens, Journal of Fish Diseases, 12, 95-104.

Hardy, J. (2000): Anti- nutritional of moringa and mineral composition, source for tilapia (*Oreochromis niloticus* L.). Aquaculture, 217, 599-611.

Henry, R. J. (1964): Colorimetric Determination of Total Protein, Harper Row Publ., New York, USA, 181.

Lausen, K. (1972): Creatinine assay in the presence of protein with LKB 8600 reaction rate analyser, Clinical Chemistry Acta, 38, 475-476.

Manjappa, K., Keshavanath, P., Gangadhara, B. (2011): Influence of Sardine oil supplemented fish meal free diets on common carp (*Cyprinus carpio)* growth, carcass composition and digestive enzyme activity. Journal of Fisheries and Aquaculture Science, 6, 1-10.

McCord, J. M., Fridovich, I. (1969): Superoxide dismutase an enzymic function for erythrocuprein (hemocuprein), Journal of Biology and Chemistry, 244, 6049-6055.

Merrifield, D. L., Harper, G. M., Mustafa, S., Carnevali, O., Picchietti, S., Davies, S. J. (2011): Effect of dietary alginic acid on juvenile tilapia (*Oreochromis niloticus*) intestinal microbial balance, intestinal histology and growth performance, Cell Tissue Research, 344, 135-146.

Musa, S. O., Tiamiyu, L. O., Solomon, S. G., Ayuba, V. O., Okomoda, V. T. (2018): Nutritional value of hydrothermally processed *Jatropha curcas* kernel and its effect on growth and hematological parameters of *Clarias gariepinus* fingerlings (Burchell, 1822) Aquaculture Reports, 10, 32–38.

Reitman, S., Frankel, S. (1957): A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases, American Journal of Clinical Pathology, 28, 56-63.

Secombes, C. J. (1990): Isolation of salmonid macrophages and analysis of their killing activity, Tech. Fish Immunology, 1, 137-154.

Shazali, H. S., El-Zubeir, E. A., Abdelhadi, O. M. A. (2013): The effects of feeding watermelon seed meal and full fat seed on broiler chicks growth. Iranian Journal of applied Animal Science, 2, 3, 279-282.

Sula, E., Aliko, V. (2017): Effects of stressors on hematological and immunological response in the fresh water crucian carp fish, *Carassius carassius,* Alban. Journal of Agricultural Science, 575-581.

Tiamiyu L. O., Okomoda V. T., Agbese, V. E. (2015): Growth performance of *Clarias gariepinus* fingerlings fed *Citrullus lanatus* seed meal as a replacement for soybean meal. Journal of Aquaculture Engineering and Fisheries Research, 1, 1, 49-56.

Tiamiyu, L. O., Ayuba, V. O., Okomoda, V. T., Umar S. (2014): Effect of various levels of raw *Citrullus lanatus* seed meal diets on growth performance of *Cyprinus carpio* fingerlings. Jordan Journal of Biological Sciences, 7, 4, 269-274.

Tiamiyu, L. O., Ayuba, V. O., Okomoda, V. T., Umar, S. (2016): Growth response of *Cyprinus carpio* fingerlings fed diets containing hydrothermally processed *Citrullus lanatus* seed meal. Journal of Aquaculture Engineering and Fisheries Research, 2, 1, 1-10.

Tiamiyu, L. O., Okomoda, V. F., Izundu, C.I. (2015): Nutritional value of hydrothermally processed *Citrullus lunatus* seeds in the diet of *Clarias gariepinus*. International Journal of Aquaculture, 5, 10, 1–4.

Tiamiyu, L. O., Solomon, S. G., Satimehin, S. P. D. (2014): Growth performance of *Clarias gariepinus* fingerlings fed varying levels of the seed of *Luffa cylindrica* meal in outdoor Hapas. Octa Journal of Bioscience, 2, 1, 8–12.

Tietz, N. W., Burtis, C. A., Duncan, P., Ervin, K., Petitclerc, C. J., Rinker, A. D., Shuey, D., Zygowicz, E.R. (1983): A reference method for measurement of alkaline phosphatase activity in human serum, Clinical Chemistry, 29, 751-761.

Trinder, P. (1969): Determination of blood glucose using 4-amino phenazone as oxygen acceptor, Journal of Clinical Pathology, 22, 240-246.

Vankampen, E. J., Ziglstra, W. G. (1961): Colorimetric determination of haemoglobin, Clinical Chemistry Acta, 6, 538.

WHO (1986). World Health Organisation. International Standards for drinking water, 3rd Edn. WHO, Geneva.