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Growth performance and serum composition of Heteroclarias fed *Moringa oleifera* leaf meal based diet

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Abstract
An experiment was carried out to determine the growth performance and serum composition of hybrid catfish Heteroclarias fed *Moringa oleifera* leaf meal based diet as a feed additive.
At the start of the experiment, twelve glass aquaria of size 70cm x 45cmx 40cm each filled with well water up to 70% of its volume were stocked with 120 fingerlings (mean weight 2.2 ± 0.04) at 30 fish per treatment, replicated thrice in a completely randomized design. Four experimental diets (D1–D4) including the control were formulated to be isocalorific (12.2kcal/kg) and isonitrogenous (40% crude protein) the fish were fed twice daily at 8:00 – 9:00 and 18:00 – 19:00 hours at 5% of their body weight. The body weights were determined weekly for a period of ten weeks. About 2ml of blood were collected by direct cardiac puncture before and after the experiment into the sterile plastic test tubes without anticoagulant. The fish weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), protein intake (PI), survival and mortality were determined. After 70 days of the experiment, the highest fish weight gain and best results in food conversion ratio (FCR) were obtained in D2 formulated with 10% *Moringa oleifera* leaf meal (8.26 ± 0.13g, 2.45 ± 0.04) respectively. The final weight gain, feed intake and specific growth rate were significantly higher ($P< 0.05$) in fish fed D2 compare to other treatments. There were no significant differences ($P< 0.05$) in the values of glucose and albumin among treatments. However, cholesterol, total protein and urea were significantly different ($P< 0.05$), with the highest values obtained in D4. The *Moringa oleifera* is best included at 10% in the diets of Heteroclarias fingerlings.

Keywords: Heteroclarias, *Moringa oleifera* leaf meal, growth performance, serum composition

1. Introduction
Aquaculture is the most rapidly expanding sector of agricultural business nowadays and it has shown continuous growth for the last 20 years, substantially to the global fisheries production [1]. With the increasing demand for fish food and the decline in capture fisheries production, aquaculture in Nigeria is heading towards intensification. This shift from low density to high density culture is leading to an unprecedented rise in the demand for feed for fish production [2]. *Heteroclarias* fish culture in ponds combines the fast growth traits of *Heterobranchus species* and early maturity traits of *Clarias gariepinus* [3]. The *Heteroclarias species* constitutes an excellent food fish of high commercial value in Nigeria and some other countries of Africa. The species is able to withstand unfavourable conditions; efficient in utilizing various types of locally formulated fish feed [4], resistance to diseases [5]. Lack of readily available nutritive fish feed ingredients has continued to be a major constraint to the survival of aquaculture in the competitive global food production system [6, 7]. Consequently fish nutrition experts have considered the recruitment of alternative protein feed ingredients necessary for inclusion in fish diet. Fish nutrition has advanced dramatically in recent years with the development of new, balanced commercial diets that promotes optimal fish growth and health. Production of Heteroclarias can be sustainable and economical only when both qualitative and quantitative feed requirements are known and established. This could be made possible by preparing nutritionally balanced and low cost diets for fishes at different stages of life. Protein is usually the most expensive component in the fish feed formulation; therefore different protein sources are used to formulate the diet. Recent years have witnessed advances in replacing or supplementing fish meal in aquaculture feeds with other protein sources. These involve the use of conventional and non-conventional protein sources of both animal and plant origins [8].

The use of *Moringa oleifera* leaf meal is rare in Nigeria because of less availability in the past. Moringa is the sole genus in the flowering plant of the family *Moringaceae.* The name was derived from the family word "*murunggai*"or the Malayalam word "*muringa*" both of which refer to *Moringa oleifera* [9]. It contains 13 species from tropical and sub – tropical climate that range in size from tiny herbs to massive trees. The most widely cultivated species is the *Moringa oleifera*, a multi-purpose tree native to the foot hills of the Himalayas in the North western India and cultivated throughout the tropics [10]. *Moringa oleifera* has vital essential amino acids, rich in protein, vitamins (A, B and C) and minerals [10, 11, 12, 13]. About 30% substitution of *Moringa oleifera* leaf meal for fish meal has been recommended for the diet of Nile tilapia *Oreochromis niloticus* [14]. Recently, researchers have increasingly been paying attention to Moringa (*Moringa oleifera* Lam.) which is a widespread, drought–tolerant tree. Its fresh foliage has been included into the diet of different animals. Researches in Heteroclarias culture have been focused on different aspects of nutrition where much importance is on natural foods and source of protein. According to [2], feed management determines the viability of fish culture. Different fish meals given to Heteroclarias have proven successful but do not contribute immensely to its expected fast growth and optimum healthy performance.

Therefore, it is assumed that fish diets fortified with *Moringa oleifera* leaf meal will boost and improve the performance and increase the growth rate of *Heteroclarias*. It might be expected that the serum composition of other related fish spp or animals would show differences from that of *Heteroclarias spp*. Fish haematology and serum biochemistry analysis is gaining increasing importance in fish culture because of its importance in monitoring the health status and nutrition of fish [15]. Haematological characteristics of most fish have been studied with the aim of establishing normal value range and deviation from it which may indicate a physiological disturbance in fish [16]. This research aimed to evaluate the proximate composition and the effect of *Moringa oleifera* (leaf meal diet) on the growth performance, survival and serum composition of Heteroclarias spp.

2. Materials and Methods

2.1 Study site

The study was carried out at the Department of Fisheries and Aquaculture Management research laboratory, Ekiti State University, Ado-Ekiti. 120 fingerlings of Heteroclarias (mean weight of 2.2 gram) were purchased from Negro Farms in Ido- Ekiti, Ekiti state, Nigeria. The fish were transported to the research laboratory in a plastic bowl and were acclimatised in a well aerated tanks containing preconditioned water for one week before the experiment.

2.2 Experimental diets

The feed ingredients were purchased from Metrovet Feed Mill in Ado-Ekiti. *Moringa oleifera* leaf meal was processed by collecting fresh wet leaves from Moringa plantation in Ekiti State University, allowed to dry under room temperature and ground to form the meal. The ingredients were mixed together thoroughly and pelleted using Hobart A-200 pellet machine with 2.0mm diameter die hole (Hobart Manufacturing Ltd, UK). The pellets were immediately sun-dried and broken mechanically into small sizes, and later kept in air-tight container prior to use. The percentage composition of the experimental diets is presented in Table 1.

Table 1: The percentage composition of the experimental diets

Ingredients	D1	D2	D3	D4
Fish meal	21.7	22.3	22.8	23.2
Soybean meal	21.7	22.3	22.8	23.2
Groundnut cake meal	21.7	22.3	22.8	23.2
Maize	16.0	12.6	9.4	6.2
Rice bran	16.0	12.6	9.4	6.2
M. oleifera leaf meal	0.0	5.0	10.0	15.0
Cod liver oil	0.5	0.5	0.5	0.5
Mineral salt	0.5	0.5	0.5	0.5
Mineral/vitamin premix	1.5	1.5	1.5	1.5
Oyster shell	0.5	0.5	0.5	0.5

2.3 Experimental design

Twelve plastic aquarium tanks of size 70cm x 45cm x 40cm filled with 30 litres of water each were used for the experiment. Four isonitrogenous diets were formulated with 40% crude protein. Each treatment had three replicates and arranged in a complete randomized design. During the acclimatization process, the fish were fed with a commercial diet of 35% crude protein. After which, the fingerlings were starved for 24 hours before the commencement of the experiment in order to maintain a uniform stomach condition of fish and to induce their appetite for the feeding trial. The experimental diets were fed to fish twice daily between 8:00 am - 9:00 am and 6:00 pm – 7:00 pm at 5% of their body weight throughout the experimental period. The rations were adjusted every week after the new weights were determined. The left over feed and faeces were siphoned out on daily basis to reduce water pollution.

2.4 Chemical analysis

The proximate analysis *Moringa oleifera* leaf meal, the diets and fish carcasses used in the experiment were determined according to the method of. The dry matter was determined according to the method described by.

2.5 Water quality parameters

Water quality parameters including dissolved oxygen, temperature, pH, water hardness and ammonia were measured on weekly basis. Temperature was measured using mercury in glass thermometer, pH was measured by Jenway pH meter (Model E 512) and dissolved oxygen was determined by the method described by.

2.6 Determination of fish growth performance

Data on feed utilisation and growth performances were collected on weekly basis using electronic kitchen Salter Scale- 1036, UK. The growth parameters were calculated following the method described by as follows:

2.6.1 Protein efficiency ratio (PER)

$$PER = \frac{\text{Wet weight gain (g)}}{\text{Crude protein fed}}$$

2.6.2 Specific growth rate (SGR)

$$SGR = \frac{\ln(wt_2) - \ln(wt_1)}{t_2 - t_1} \times \frac{100}{1}$$

Ln (wt_1) = natural log of the weight of the fish at the initial stage (t_1).

Ln (wt_2) = natural log of the weight of the fish at the final stage (t_2).

2.6.3 Feed conversion ratio

$$(FCR) = \frac{\text{Weight of food consumed by fish (g)}}{\text{Weight gain by fish (g)}} \times \frac{100}{1}$$

2.6.4 Total weight gain (TWG) and Mean weight gain (MWG)

TWG = $(Wt_2) – (Wt_1)$ where (Wt_2) = Final weight (g) of fish and (Wt_1) = Initial weight (g) of fish.

MWG = Total weight gain (g) / number of fish/days

2.6.5 Total Protein intake (PI)

This was computed from the relationship between the protein intake and the number of days of the experiment.

$$TPI = \frac{\text{protein intake}}{\text{Number of days.}}$$

Where

$$\text{Protein Intake (PI)} = \frac{\text{food consumed} \times \text{total crude protein}}{100}$$

2.6.6 Mortality (M) = $(N_o – N_1) / No \times 100\%$

Where

N_o = number of fish at the start of the experiment

N_1= Number of fish at the end of the experiment.

2.7 Blood Serum collection and biochemical analysis

Blood samples were collected in triplicate from the fish at the beginning and the end of the feeding trial following the procedure of and. About 2ml of blood was collected by direct cardiac puncture using a 2ml sterile plastic disposable syringe fitted with 0.8 x 38mm hypodermic needles and transferred into sterile plastic test tubes without anticoagulant. The tubes were kept in a slanting wooding rack at room temperature to allow the blood to clot. The clotted blood was centrifuged for 15 minutes at 3500 revolution per minute (rpm). The serum as a clear fluid was pipetted out into a clean and sterilized bottle for serum composition analysis as described by. The concentration of total serum proteins, cholesterol, glucose, albumin and blood urea were estimated using the standard methods described by and.

2.8 Statistical analysis

The data collected were subjected to Normality test. Data that were not significantly different from normal distribution were subjected to one way analysis of variance (ANOVA). Comparisons among diet means were carried out by using Duncan Multiple Range Test at a significant level of 0.05. All computations were performed using statistical package SPSS 16.0 (SPSS Inc., Chicago, IL, U.S.A.).

3. Results and Discussion

The results of the proximate composition of *Moringa oleifera* leaf meal presented in Table 2 showed that the leaf meal contain 26.20% crude protein, 7.04% crude fat, 8.42%crude fibre, and 44.14% total nitrogen free extract. These value are within the range reported by; however, the variation observed in this study compared to previous study could be attributed to difference in environmental conditions and method of preparation of samples and the analytical procedure as well as the species of *Moringa oleifera* used. It is pertinent to mention that the application of Moringa either as additive or feed substitutes is receiving increasing attention due to its minerals and vitamins composition with overall usefulness in improving the quality of reproduction and growth promoter in animal. Previous report showed that *Moringa oleifera* supplementation into animal diets enhances growth and reproductive outcomes. However, it is important that the selected protein sources in practical applications do not conflict with or affects human food security interest as noted by.

Table 2: Proximate composition of *Moringa oleifera* leaf meal used for diets formulation

Nutrients	Percentage composition
Dry matter	92.97 ± 1.21
Moisture content	7.03 ± 0.01
Crude protein	26.20 ± 0.48
Ether extract	7.04 ± 0.12
Crude fibre	8.42 ± 0.23
Ash	7.17 ± 0.04
Nitrogen Free Extract	44.14 ± 0.62

The results of the proximate composition of the experimental diets are presented in Table 3.

The crude protein levels of the four diets (D1 – D4) ranged from 39.89% to 40.21%. The lowest value of fat (ether extract) was recorded in D4 (10.02%) with an insignificant increase to 12.73% in the control diet (D1. The Ash contents ranged between 13.07% in D1 and 15.15% in D4 respectively, while the percentage fibre was lowest in D3 (5.13%) and insignificantly highest in D4 (5.52%). The nitrogen free extract (NFE) content was highest in D1 (7.42%) and lowest D4 (6.82%). These values are within the range reported by: as good for raising fish in aquaculture.

Table 3: Proximate composition of the experimental diets with varying levels of Moringa leaf meal

Nutrient (%)	D1 (0%)	D2 (5%)	D3 (10%)	D4 (15%)
Dry Matter	78.45	77.70	77.09	77.45
Moisture	21.55	22.30	22.91	22.55
Crude protein	40.02	39.89	39.96	40.21
Fat	12.73^a	11.51^b	11.61^b	10.02^c
Ash	13.07^b	13.68^b	14.42^b	15.15^a
Fibre	5.44^a	5.42^a	5.13^b	5.52^a
NFE	7.42^a	7.29^a	7.08^b	6.82^c

Note: NFE = nitrogen free extract. The mean values with the same superscript are not significantly different (0<0.05).

3.1 The Water Quality Parameters

The results of the water quality parameters showed that the mean dissolved oxygen (DO) was 6.0 ± 0.5 mg/l, mean temperature 27.6 ± 0.2 ºC, mean pH of the water 7.2 ± 0.6, while the mean ammonia content of the water was 0.5 ± 0.03 mg/l, water hardness was 220 ± 32 mg/l while alkalinity was 135 ± 47 mg/l and mean water conductivity was 79.4 ± 4 µs/cm. The water parameters were within the range recommended for fresh water fish culture. A good water condition is a necessity for the survival and growth of fish since the entire life process of the fish is wholly dependent on the quality of its environment.

3.2 Proximate Composition of the Experimental Fish

The proximate composition of the experimental fish before and after the feeding trial is presented in Table 4. Generally it was observed that the crude protein level of the fish reduced and the crude fibre increased as the *Moringa* inclusion increased from D1 to D4, this could be attributed to the effect of the anti-nutritional factor present in *Moringa oleifera* (though not evaluated in this study) which makes the fibre to increase and reduces the crude protein and lower the growth performance in Nile Tilapia as reported by. Though, there were no significant differences observed in the crude protein of the diets with varying levels of *Moringa oleifera*, the fish fed control diet (D1) has the significant highest crude protein value (34.91%) ($P< 0.05$), the lowest value obtained in D2 (33.15%). The low level of fat with increasing levels of *Moringa oleifera* could be attributed to poor food intake, which caused reduced growth and mobilisation of body lipid reserves to meet energy requirement for metabolism and other vital body functions as reported by. The ash content was highest in D1 (1.61%) and lowest D3 (1.36%). This trend of reduction in the ash content with increasing level of *Moringa oleifera* was different from the previous reports when *Moringa oleifera* was used as a substitute in the diets of *Clarias gariepinus*. The highest value of moisture content was recorded in D1 (16.84%) while the value was lowest in D1 (13.17%), the percentage crude fibre was lowest in D1 (0.58%) and highest in D2 (0.74%). Also the fat contents was found lowest in D2 (23.97%) but highest in the control diet. The decrease in values of the body compositions with increasing inclusion levels of *Moringa oleifera* leaf meal agreed with previous study with *Clarias gariepinus*;;, *Oreochromis niloticus*.

Table 4: Proximate composition of fish before and after the experiment

Composition (%)	Before the experiment	After the experiment			
		D1 (0%)	D2 (5%)	D3 (10%)	D4 (15%)
Moisture	11.51 ± 0.35^{d}	13.17± 0.13^{c}	16.84 ± 0.30^{a}	13.28 ± 0.10^{c}	15.47 ±0.20^{b}
CP	42.52 ± 0.50^{a}	34.91± 0.31^{b}	33.65 ± 0.31^{b}	33.72 ± 0.31^{b}	33.67 ± 0.11^{b}
Fat	30.15 ± 0.53^{a}	26.26 ± 0.20^{b}	23.97 ± 0.42^{c}	24.16 ± 0.10^{c}	24.07 ± 0.20^{c}
Ash	1.99 ± 0.06^{a}	1.43 ± 0.11^{c}	1.61 ± 0.22^{b}	1.59 ± 0.04^{b}	1.36 ± 0.04^{c}
Fibre	0.90 ± 0.05^{a}	0.58 ± 0.03^{c}	0.74 ± 0.03^{b}	0.59 ± 0.03^{c}	0.67 ± 0.01^{bc}
NFE	13.14 ± 0.40^{c}	23.65 ± 0.61^{b}	23.47 ± 0.50^{b}	25.71 ± 0.50^{a}	24.88 ± 0.50^{a}

Note: MLM = *Moringa oleifera* leaf meal, CP= crude protein and NFE = nitrogen free extract. The mean values with the same superscript in the same row were not significantly different ($p> 0.05$)

3.3 Growth performance

The results of the growth performance, nutrients utilisation, and survival of *Heteroclarias* fed *Moringa oleifera* leaf meal based diets are presented in Table 5, while the final weight observed in the fish during the experiment are presented in Fig. 1. The weight gain was significantly ($P< 0.05$) highest in fish fed D3 meal (8.26 ± 0.13g) while the least weight gain was observed in fish fed D4 (6.69 ± 0.12g). However there were no significant differences ($P< 0.05$) in the total weight gain of the fish fed the control diet (D1) and D2. These results are in agreement with who reported that aqueous extracted and heat treated *Moringa oleifera* leaf meal at 10% inclusion in the diet of *Oreochromis niloticus* recorded higher weight gain than other diets with Moringa inclusion above 10% with the exception of the control diet.

Table 5: Growth performance and nutrient utilisation of Heteroclarias fed *Moringa oleifera* leaf based diets

Parameters	D1 (0%)	D2 (5%)	D3 (10%)	D4 (15%)
Initial weight (g)	2.26 ± 0.19^{a}	2.89 ± 0.13^{b}	2.32 ± 0.4^{b}	2.24 ± 0.3^{b}
Final weight (g)	9.50 ± 0.21^{b}	10.35± 0.19^{a}	10.58 ± 0.2^{a}	8.94 ± 0.22^{c}
Total weight gain(g)	7.24 ± 0.16^{b}	7.46 ± 0.07^{b}	8.26 ± 0.13^{a}	6.69 ± 0.12^{c}
Feed intake (g)	21.70 ± 0.03^{a}	14.80 ± 0.04^{c}	21.98 ± 0.01^{a}	17.01 ± 0.04^{b}
Specific growth rate	0.89 ± 0.04^{b}	0.79 ± 0.02^{c}	0.94 ± 0.00^{a}	0.86 ± 0.02^{b}
Protein efficiency ratio	0.21 ± 0.01^{c}	0.26 ± 0.00^{a}	0.26 ± 0.01^{a}	0.22 ± 0.01^{b}
Food conversion ratio	3.00 ± 0.1^{a}	2.62 ± 0.02^{b}	2.45 ± 0.04^{c}	2.97 ± 0.04^{a}
Total protein intake	0.11 ± 0.00^{a}	0.08 ± 0.00^{c}	0.09 ± 0.00^{b}	0.09 ± 0.00^{b}
% Survival	73	57	50	50

Note: Mean values with similar superscript are not significantly different ($P> 0.05$) across the row. MLM = *Moringa oleifera* leaf meal. The mean values with the same superscript in the same row are not significantly different ($p> 0.05$)

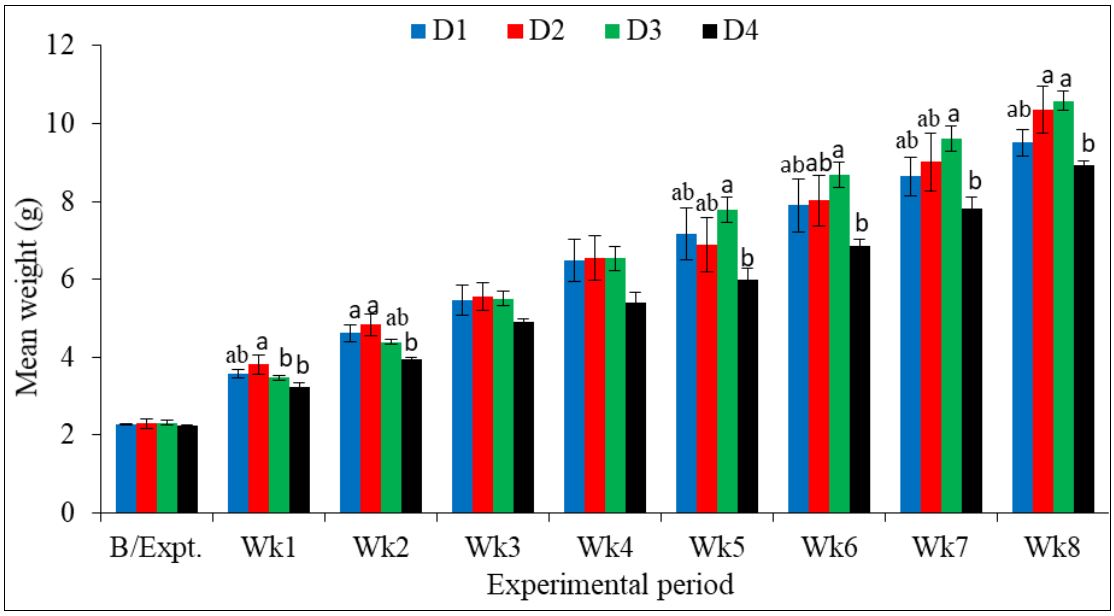

Fig 1: Weekly growth measurement of Heteroclarias fed experimental diets. Value are mean + SEM. WK = week, Bars with the same letter are not significantly different ($P< 0.05$)

The feed intake (21.98±0.01[a]g) and specific growth rate (0.94 ± 0.00[a]) (Table 5) were significantly highest ($P< 0.05$) in D3 than other dietary treatments. The high feed intake also reflected on the total weight gain which was found to be highest in D3. However there were no significant differences in feed intake between the control diet (D1) and D3. The lower feed intake recorded in the other diets could be attributed to high fibre contents in diets which might have caused dilution of nutrients, reduced digestibility, caused bulkiness of feed in gut, reduced feed consumption and depressed utilisation of energy. The food conversion ratio (FCR) was significantly highest ($P< 0.05$) in the D1 and D4 compared to other dietary treatments (Table 5). The total protein intake was not significantly different ($P< 0.05$) in D1 and D3. However, D3 produced the best result in protein efficiency ratio while food conversion ratio was significantly highest in D4. The results showed that the growth and nutrient utilisation by the fish decreased as the level of *Moringa oleifera* leaf meal inclusion increases above 10% in the diets. This result is similar to the report of and. The results of the percentage survival (Table 5) of the fish showed that the rate of survival reduced as the level of *Moringa oleifera* increases. The plant was reported to reduce survivorship, growth and reproductive success when fed as sole source of nutrition for zebra fish.

3.4 Serum biochemistry of Heteroclarias Fed the Experimental Diet

Environmental and physiological factors are known to influence fish haematology and serum chemistry, these include stress due to capturing, transportation, sampling, age and sex. Certain serum chemistry was used to identify tissue damage; Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) are normally found within the cells of the liver, heart, gills, kidneys, muscles and organs as reported by. Their increase in the plasma indicated tissue injury or organ dysfunction. The serum biochemical results of Heteroclarias fed *Moringa oleifera* based diets are presented in Table 6. The serum biochemical parameters were lower in all the fish fed the experimental diets compared to the initial result obtained before the experiment. This showed that *Moringa oleifera* has effects on the serum of the fish. The Cholesterol concentration, total protein, urea and glucose were most significant higher ($P< 0.05$) in the fish fed D4 (73.81mg/dl) while the lowest value of cholesterol (52.06 mg/dl) was observed in fish fed control diet (D1). Cholesterol is the principal sterol synthesised by animal which is formed predominantly in the liver of vertebrates.

Table 6: Serum composition of Heteroclarias fed *Moringa oleifera* leaf meal based diets

Parameters mg/dl	Before the expt.	After the experiment			
		D1 (0%)	D2 (5%)	D3 (10%)	D4 (15%)
Cholesterol	87.30	52.06 ± 7.95[b]	63.81 ± 6.41[ab]	65.05 ± 14.6[ab]	73.81 ± 12.7[a]
Total protein	49.75	43.31 ± 1.09[c]	44.23 ± 0.98[ab]	42.37 ± 1.17[c]	47.22 ± 3.09[a]
Urea	18.93	3.46 ± 0.66[b]	6.47 ± 1.83[ab]	10.20 ±5.14[ab]	13.58 ± 4.92[a]
Glucose	39.92	35.37 ± 1.45[a]	36.11 ± 1.56[a]	31.43 ± 4.19[a]	36.94 ± 3.65[a]
Albumin	33.03	29.61 ± 0.83[a]	30.82 ± 0.76[a]	30.18 ± 0.81[a]	31.55 ± 2.22[a]

Note: Mean values on the same row with the same superscript are not significantly different ($P> 0.05$). MLM = *Moringa oleifera* leaf meal

Cholesterol is a vital nutrient in fish and shrimp diets, important for membrane function, lipoprotein transport, hormone production and absorption/transport of fatty acids. Its uptake and distribution depends on the availability of phospholipids. Ahmed reported a hypercholesterolemia in catfish attributed to increased plant leaf dust, which may equally result from stress or water pollution. According to, haemo-concentration may be initiated by increase of plant leaves which increased liver protein. The findings of this study agreed with the report of that increase in *Moringa oleifera* in the diets caused increased level of albumin. Total protein results revealed that fish fed D4 had the highest value

of 47.22 mg/dl which was significantly different ($P< 0.05$) from values of 43.31 ± 1.09mg/dl, 44.23mg/dl and 42.37 mg/dl obtained in fish fed control diet (D1), D2 and D3 respectively. Urea concentration was observed to be lowest in fish fed control diet (3.46mg/dl). Glucose and albumin were not significantly different ($P< 0.05$) in the fish fed both the control diet and other dietary treatments with varying levels of *Moringa oleifera*. However, glucose concentration was observed to be lowest in the fish fed D3 *(*31.43 mg/dl) while albumin recorded the lowest value in fish fed control diet (29.61 mg/dl) during the experiment. The content of serum glucose in culture is dependent type of carbohydrate in the diet. Glucose is transported from the liver to body cell through the bloodstream from where it is available for cell absorption via the hormone insulin. Albumin functions in serum water balance maintenance, transportation and storage of varieties of compound in the body of animals. It is responsible for almost 80% of total osmotic regulation. The results showed that the serum biochemical components increased as the level of *Moringa oleifera* inclusion was increased in the diets. This report is in agreement with the study of who reported significant increase in the activities of serum enzyme (ALT, AST and ALP) as the level of *M. oleifera* increased in the diets of *Clarias gariepinus.*

The similarities in chemical compositions of this study with the other studies may be an indication that environmental factors such as season, geographical location and stage of maturity play a major role in determining the nutritive value of *Moringa oleifera* leaf meal. Values of chemical composition were comparable with those reported in other leaf meals such as *Leucaena leucocephala*, and *Ipomoea batatas*; Thus, from nutritional point of view, these reports suggest the potential of *Moringa oleifera* leaf meal as animal feed. If the research in dietary requirement, diet delivery, nutrients interactions, alternatives to fish meal and oil; diet formulation and processing, feed additives and the effects of nutrition on gene expression and metabolism are well addressed, the results is expected to improve the health and welfare of fish, nutritional quality of fish and economic aspects of aquaculture production.

4. Conclusion

This study showed that *Moringa oleifera* leaf meal was best at 10% inclusion in the diet of Heterclarias, with the weight gain. The study further affirmed that inclusion of this Moringa leaf meal above 10% may result in hyperglycaemia, hypercholesterolemia which may have debilitating effects on the general health status of the fish. Further study is recommended to determine the best ways to process plant leaves in order to reduce any anti-nutritional factors that might inhibit the nutrient utilisation of the leaf meal above 10%. This studies shows that *Moringa oleifera* leaves contained high protein and valuable minerals and vitamins useful for fish health, growth and healthy blood system.

5. Disclosure Statement: No conflict of interest exists.

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