

# Individual and Combined Effects of Moringa Leaf and Garlic Powder on Growth and Plasma Biochemical Indices of *Clarias gariepinus* Juveniles

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**Abstract** The study was designed to compare the growth promoting and some plasma biochemical indices of moringa leaf powder (MLP) and garlic powder (GP) each at 15.00g/kg; treatments 1 and 2, treatments 3 and 4 contained a combination of 7.50g and 15.00g/kg each of the phytoadditives respectively and a control diet without additive. These five diets were fed to juveniles of *Clarias gariepinus* (46.45±0.12g). Relative to the juveniles in control diet, growth and nutrient utilization indices, (except survival percentage) of juveniles in phytoadditive diets increased. Plasma transaminase enzymes reduced significantly ( $p < 0.05$ ) except for juveniles in treatment 4, likewise there were significant ( $p < 0.05$ ) reduction in ALP activities, except for juveniles in treatments 1 and 3. Also, there was significant ( $p < 0.05$ ) reduction in plasma cholesterol of juveniles in the phytoadditive diets. However, juveniles in treatments 1 and 2 recorded significant ( $p < 0.05$ ) increase in plasma phospholipids and triglycerides concentration compared to juveniles in control diet. Likewise, all juveniles in phytoadditive diets had significant ( $p < 0.05$ ) increase concentration of total protein, creatinine increased in treatment 2 and bilirubin increased in juveniles in treatment 3 as well as an increase in urea concentration with juveniles in treatments 1 and 3. Contrarily, creatinine concentration of juveniles in treatments 1, 3 and 4, albumin of juveniles in treatment 3 and bilirubin of juveniles in treatments 1 and 4 significantly ( $p < 0.05$ ) reduced compared to the values obtained with juveniles fed the control diet. While the bilirubin of juveniles in treatment 3 increased significantly ( $p < 0.05$ ). The results of this study showed improvement in the performance of juveniles fed phytoadditive diets when compared to those in the control diet. However, the inclusion of garlic powder at 15.g/kg produced the best growth, nutrient utilization and improved plasma biochemical indices of *Clarias gariepinus* juveniles.

**Keywords:** Moringa leaf, garlic, growth performance, feed utilization, plasma biochemical indices

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## 1. Introduction

Globally, there is increasing emphasis on the ban on the use of chemically synthesized antimicrobial drugs and growth enhancers. This emanated from the need to reduce environmental pollution, eliminate the emergence of antimicrobial resistance diseases and the quest for safe food. Hence, nutritionists are employing the use of natural alternatives, thus the emergence of natural feed additives. Natural feed additives or phytoadditives comprise of plant materials which possess growth promoting, antimicrobial, immune modulating and anti-oxidative properties among others.

Garlic (*Allium sativa*) is a plant species in the onion family. It has been used in food flavouring, traditional

medicine and recently in animal nutrition. Garlic has antioxidant effects and useful in the control of pathogens; particularly bacteria and fungi. The allicin found in garlic has a broad range of chemical and biological properties [1]. Garlic also contains minerals (phosphorus and calcium), vitamins (A, C and B complexes), linolenic acid, carbohydrates [2] and many valuable compounds such as iodine, silicates and sulphur which have positive effects on circulatory, skeletal, cholesterolemia and control of liver diseases [3]. While *Moringa oleifera* is the most widely cultivated species of the genus Moringa. Its leaves possess nutritional and medicinal values. Nutritionally, it has a high exact protein content, an excellent source of B-vitamins, vitamin C and provitamin A as beta-carotene, vitamin k, minerals; manganese, iron and other essential nutrients. Medicinally, moringa has antioxidant, antibacterial and antifungal activities [4,5]. The leaves are

free from antinutrients except for phenols that are much below the toxic threshold for animals and saponins which are inactive as far as haemolytic properties are concerned [6]

The growth promoting activities of garlic powder has been reported in *Clarias gariepinus* fingerlings [7,8] and *Oreochromis niloticus* [9] fingerlings. Likewise, there are reports on the growth enhancing potentials of moringa leaf meal in the diet of *C. gariepinus* juveniles [10] and the fingerlings [11]. Few studies suggested a potential synergistic effect of garlic with other phytoadditives [12,13,14], this suggestion needs further investigation before its adoption in practice. Consequently, this study was designed to compare the growth promoting, liver and kidney as well as lipid enhancing activities of garlic and moringa leaf powder in the diets of *Clarias gariepinus* juveniles when fed individually or in combination. Thus, growth performance, nutrient utilization, survival and some blood plasma chemistry of the juveniles fed diets containing these phytoadditives were assessed.

## 2. Materials and Methods

### 2.1. Materials and Feed Preparation.

Two phytoadditives were used for the feeding trial. Flash dry moringa leaf powder (MLP) was purchased from the producer Julie Manny products limited Sagamu, Ogun State, Nigeria and garlic powder (GP) was purchased from a reputable Supermarket in Lagos, Nigeria. Five isoproteinous (35% crude protein) and isolipidic diets (2% palm oil) were formulated. The control contained no additive, treatment 1 contained 15.00g of MLP, treatment 2 contained 15.00g of GP, treatment 3 contained a mixture of 7.50g each of MLP and GP, while treatment 4 contained a mixture of 15.00g each of MLP and GP. Percentage gross composition of experimental diets is shown in Table 1. The feed preparation was done at a reputable commercial fish feed manufacturer where all other feed ingredients were purchased. The ingredients were weighed, ground into fine powder, thoroughly mixed and pelleted into 2mm with a 200kg/hr. table-top pelleting machine. The pellets were stored in air tight containers until when needed.

### 2.2. Fish Feeding and Management

The experiment was conducted at the indoor of Lagos State University Hatchery, Ojo Campus, Ojo, Lagos, Nigeria. Fifteen plastic aquaria tanks with 80L water capacity and 0.5m depth were filled with borehole water to three quarter capacity. The water in the experimental tanks was aerated by an electric air pump (Shining model; horsepower 50Hz). One hundred and fifty (150) *Clarias gariepinus* juveniles with a mean weight of  $46.45 \pm 0.12$ g were obtained from a reputable commercial farm in Iba, Lagos, Nigeria. The fish were transported early in the morning between 6.30 and 7.00 am to the hatchery and acclimatized for three days before the start of the experiment. The fish were randomly distributed to 15 plastic aquaria at the stocking rate of 30 juveniles per

experimental treatment and 10 per replicate. The fish were starved for 72hours to eliminate residual feed from the gut. Daily 50% of water in each tank was gently siphoned in exchange for fresh water. This was done to get rid of left over feed and faecal matter. Fish were fed to satiation twice daily between 7.00-8.00 and 17.00-18.00hours for a period of 12weeks. Feeding was done by hand and spread evenly across water surface of the aquarium.

### 2.3. Water Quality Analysis

Daily water temperature was measured using a mercury in-glass thermometer, hydrogen ions (pH) concentration measured using pH meter (Jenway Model 9060) and weekly dissolved oxygen (DO) concentration was measured by oxygen meter (Hanna Model HI-9142). Ammonia concentration was determined according to [15].

### 2.4. Growth Performance and Feed Utilization

Batch weighing of fish in each replicate aquarium was done at the beginning of the feeding trial, subsequently biweekly, using a Mettler 20110 top-loading balance. The evaluation of experimental diets for growth and feed utilization was according to [16].

### 2.5. Blood Collection

Blood samples were collected in triplicate following the procedure of [17,18]. The blood was collected using 5ml heparinise K3 vacuum tubes; the needle was run quite deep as much as possible through a middle line just below the sex organ in a dorsocranial direction till it strikes the vertebra column. The blood was immediately kept in the iced container after each collection. It was later taken to the laboratory and centrifuged for 10minutes. After that, the clear fluid which is the plasma was collected using a syringe and discharged into clean and sterilized tubes and kept in the freezer as described by [19] further analysis.

### 2.6. Biochemical Blood Analysis

#### 2.6.1. Measurement of Plasma Liver Function Test

The activities of both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to the methods before [20]. While, alkaline phosphatase (ALP) activity was assayed using the method of [21]. The albumin analysis was performed using the method of [22], bilirubin analysis was performed using the method of [23], urea analysis was performed using the method of [24], creatinine analysis was performed using the method of [25]. Triglycerides, cholesterol and phospholipids analyses were performed using the method of [26].

### 2.7. Statistical Analysis

All data collected were subjected to statistical analysis using GraphPad.

**Table 1. The composition of the experimental diets (g/kg)**

Ingredients	Treatments				
	Control	1	2	3	4
Maize	310.00	295.00	295.00	295.00	280.00
Soymeal	220.00	220.00	220.00	220.00	220.00
Groundnut cake	220.00	220.00	220.00	220.00	220.00
Fishmeal	150.00	150.00	150.00	150.00	150.00
Wheat offal	60.00	60.00	60.00	60.00	60.00
Palm oil	20.00	20.00	20.00	20.00	20.00
DCP	10.00	10.00	10.00	10.00	10.00
Vit/Min. P*	5.00	5.00	5.00	5.00	5.00
Vitamin C	2.50	2.50	2.50	2.50	2.50
Salt.	2.50	2.50	2.50	2.50	2.50
FDMP	0.00	15.0	0.00	7.50	15.00
GP	0.00	0.00	15.00	7.50	15.00
Gross Energy**	4469.22	4463.59	4464.01	4463.65	4458.47
Proximate Composition					
CP (N×6.25)9	34.97	35.01	34.95	35.02	34.99
Crude fat	6.10	6.01	6.03	5.95	6.03
Crude fiber	3.81	3.82	3.83	3.85	3.83
Total ash	5.02	4.98	4.98	4.96	4.96

\*Note: Each kg of the vitamin and minerals premix contained 2,000IU vitamin A; 4,000IU vit. D<sub>3</sub>; 2,000 vit. E; 1,200mg vit.K; 10,000mg vit. B; 30,000mg vit. B<sub>2</sub>; 19,000 mg vit. B<sub>6</sub>; 100mg Mn; 40mg Zn; 40mg Fe; 4gm Cu; 5gm I<sub>2</sub>; 0.2mg Co; 600gm calcium; 400mg choline chloride; 40mg biotin; 400,000mg phosphorus; 100,000mg glycine; 400gm methionine and 125IU antioxidant. Gross energy\*\* (Kcal kg<sup>-1</sup>) calorific value of protein 5.65; nitrogen free extract 4.1 and lipid 9.45.

### 3. Results

The water quality and the summary of results of the growth performance, feed utilization and percentage survival of *Clarias gariepinus* juveniles fed diets fortified with flash dry moringa leaf powder, garlic powder and a combination of the two are presented in [Table 2](#) and [Table 3](#) respectively. While the results of plasma blood

biomarkers measured are presented in [Figure 1](#) to [Figure 3](#).

#### 3.1. Water Quality Analysis.

The water quality parameters of *Clarias gariepinus* juveniles fed the untreated and diets containing MLP and GP (treatments 1-4) individually or combined MLP and GP (treatments 1-4) were not significantly ( $p>0.05$ ) different.

**Table 2. Water quality analysis of *Clarias gariepinus* juveniles fed the experimental diets.**

Water quality parameters	Treatments				
	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Ph	6.57±0.02	6.63±0.03	6.39±0.22	6.65±0.02	6.68±0.02
DO (mg/L)	8.45±0.09	8.39±0.13	8.43±0.10	8.41±0.11	8.41±0.12
Temperature	28.83±0.16	28.66±0.13	28.72±0.13	28.77±0.15	28.60±0.13
Ammonia (µmol/L)	0.07±0.01	0.07±0.01	0.07±0.01	0.06±0.01	0.06±0.01

**Table 3. Growth, feed utilization and percentage survival of *Clarias gariepinus* juveniles fed the experimental diets**

Growth Parameters	TREATMENTS				
	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Initial wt./fish (g)	46.41±0.07 <sup>a</sup>	46.70±0.09 <sup>a</sup>	46.34±0.23 <sup>a</sup>	46.41±0.08 <sup>a</sup>	46.41±0.13 <sup>a</sup>
Final wt./fish (g)	165.34±2.8 <sup>d</sup>	187.93±6.82 <sup>bc</sup>	210.59±14.75 <sup>a</sup>	175.39±16.71 <sup>bc</sup>	183.36±8.02 <sup>bc</sup>
Wt. gain/fish (g)	118.93±2.77 <sup>d</sup>	141.23±6.91 <sup>bc</sup>	164.25±14.88 <sup>a</sup>	128.99±16.69 <sup>bc</sup>	136.96±8.15 <sup>bc</sup>
Feed intake/ fish (g)	227.92±12.89 <sup>b</sup>	263.4±10.97 <sup>ab</sup>	290.64±25.05 <sup>a</sup>	229.42±21.04 <sup>b</sup>	248.30±4.60 <sup>ab</sup>
FCR	1.92±0.10 <sup>a</sup>	1.87±0.02 <sup>a</sup>	1.77±0.01 <sup>a</sup>	1.80±0.08 <sup>a</sup>	1.82±0.10 <sup>a</sup>
Protein intake/fish (g)	79.77±4.51 <sup>b</sup>	92.24±3.84 <sup>ab</sup>	101.72±8.77 <sup>a</sup>	80.30±7.37 <sup>b</sup>	86.91±1.61 <sup>ab</sup>
PER	1.50±0.09 <sup>a</sup>	1.53±0.01 <sup>a</sup>	1.61±0.01 <sup>a</sup>	1.60±0.08 <sup>a</sup>	1.57±0.08 <sup>a</sup>
SGR	0.76±0.01 <sup>b</sup>	0.83±0.02 <sup>ab</sup>	0.90±0.04 <sup>a</sup>	0.79±0.06 <sup>ab</sup>	0.82±0.03 <sup>ab</sup>
Survival %	93.33±3.33 <sup>a</sup>	93.33±3.33 <sup>a</sup>	96.67±3.33 <sup>a</sup>	90.00±5.77 <sup>a</sup>	96.67±3.33 <sup>a</sup>

\*Means with the same superscripts in the same row are not significantly ( $P>0.05$ ). Where wt. represent weight, WG represent weight gain, FI is feed intake, SGR specific growth rate, FCR feed conversion ratio and PER protein efficiency ratio.

### Liver Biomarkers

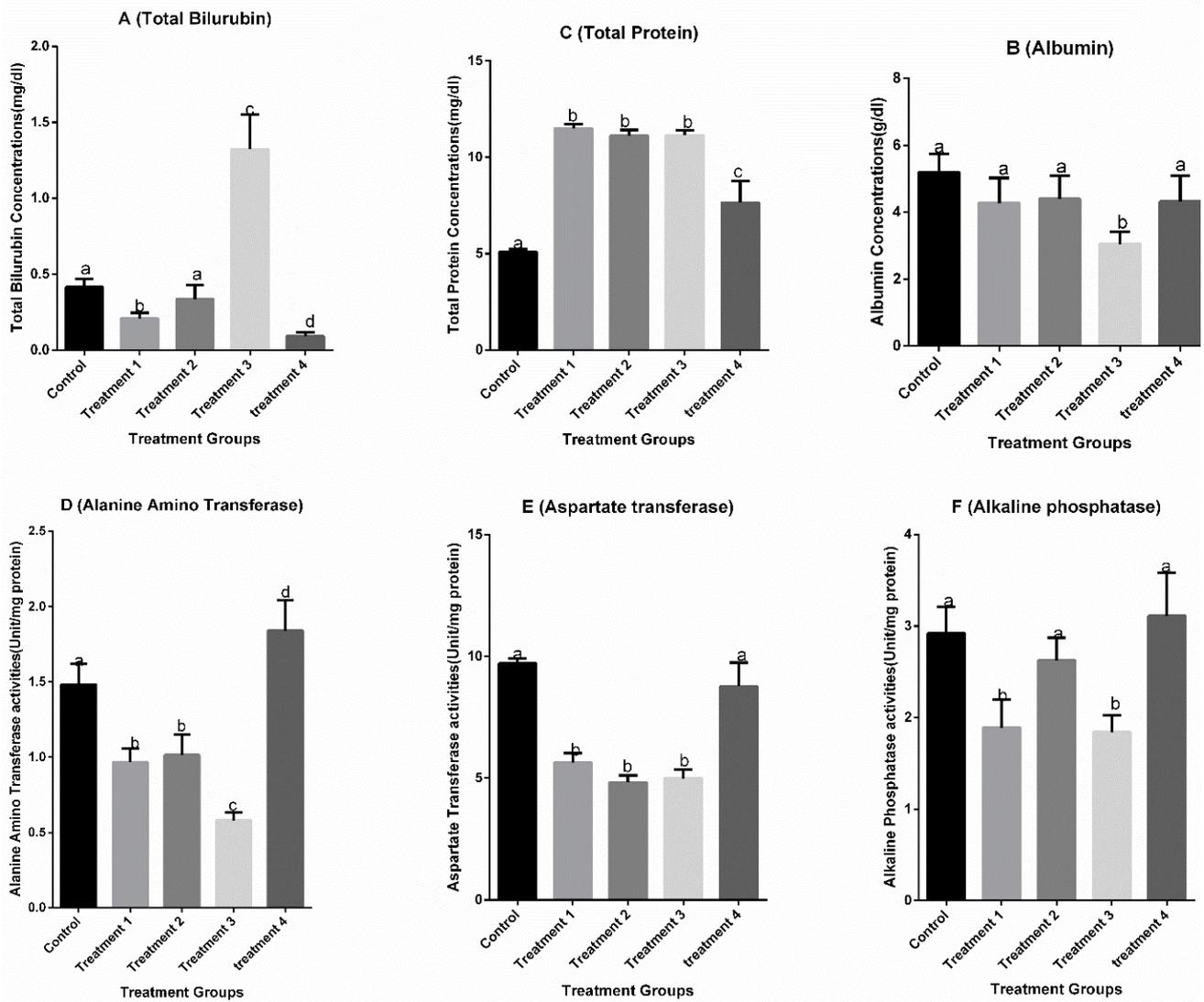


Figure 1. Liver Biomakers

### Kidney Biomarkers

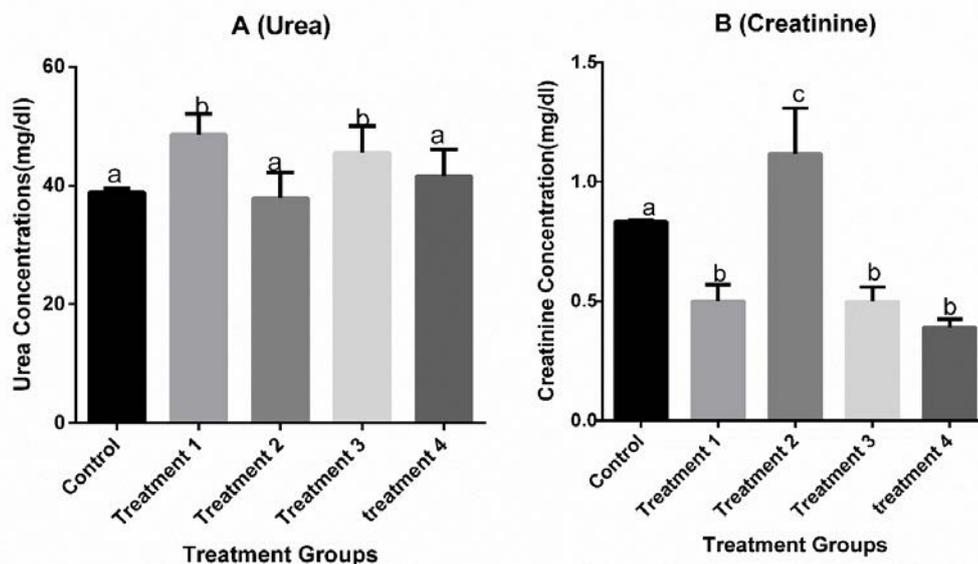


Figure 2. Kidney Biomarkers

## Lipids Profile

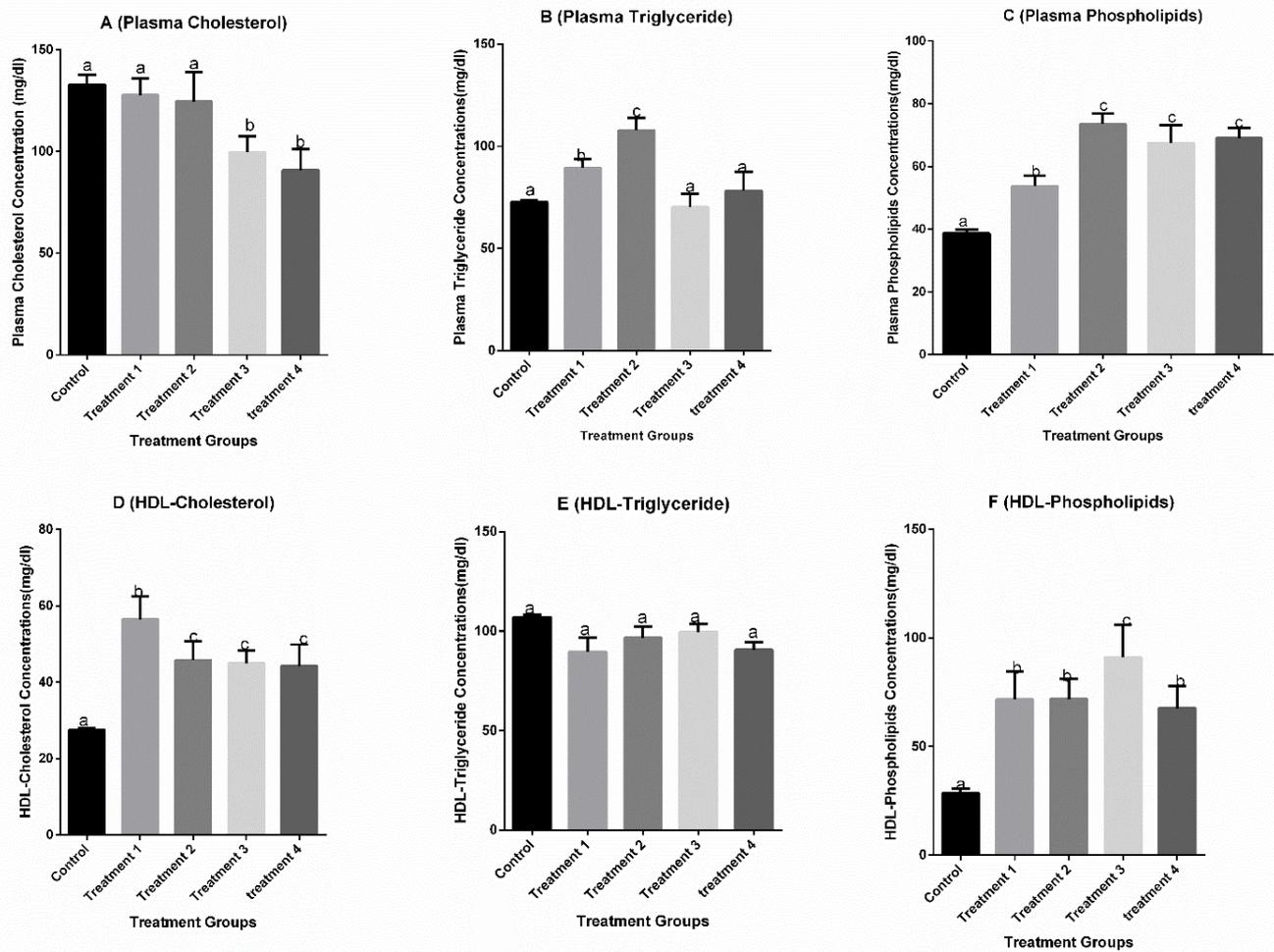


Figure 3. Lipid Profile

### 3.2. Growth Performance and Nutrient Utilization

The addition of flash dry moringa leaf powder (MLP) and garlic powder (GP) individually or in combination to the diets of *Clarias gariepinus* juveniles did not significantly ( $p > 0.05$ ) affect feed conversion ratio, protein efficiency ratio and the survival rate throughout the 12 weeks of the experiment.

However, *Clarias gariepinus* juveniles fed the control diet had significantly ( $p < 0.05$ ) lowest final weight ( $165.34 \pm 2.80$ ) and weight gain ( $118.93 \pm 2.77$ ), juveniles on diet containing 15.00 g/kg GP (treatment 2) had significantly ( $p < 0.05$ ) highest final weight ( $210.93 \pm 14.75$ ) and weight gain ( $164.25 \pm 14.88$ ) while the final weight and weight gain of juveniles in other treatments did not differ ( $p > 0.05$ ) significantly from each other. Also, the lowest specific growth rate ( $0.76 \pm 0.01$ ) was observed among juveniles in the control, while the significant ( $p < 0.05$ ) highest specific growth rate ( $0.90 \pm 0.40$ ) with significant difference ( $p < 0.05$ ) significantly from values obtained with juveniles in other phytoadditives diets.

Feeding juveniles of African catfish (*Clarias gariepinus*) with 15.00 g/kg GP (treatment 2) appeared to promote best growth performance and nutrient utilization. However, the mixture of MLP and GP did not improve growth performance and feed utilization of juveniles beyond the

values obtained with juveniles fed either 15.00 g/kg MLP or GP.

### 3.3. Plasma Biochemical Analysis

Blood plasma biochemical analysis of *Clarias gariepinus* juveniles fed the experimental diets for 12 weeks are presented in Figure 1 to Figure 3.

### 3.4. Liver Biomarkers

The inclusion of the phytoadditives to the diets of *Clarias gariepinus* juveniles resulted in significant ( $p < 0.05$ ) reduction in the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in juveniles fed treatments 1, 2 and 3 and alkaline phosphate (ALP) activities were also significantly ( $p < 0.05$ ) reduced in the plasma of juveniles fed treatments 1 and 3. However, the addition of 15.00 g/kg<sup>-1</sup> each of MLP and GP (treatment 4) increased significantly ( $p < 0.05$ ) ALT activities of juveniles of *Clarias gariepinus* fed with this diet. Feeding of *Clarias gariepinus* juveniles with treatment 3 (7.50 g/kg<sup>-1</sup> each of MLP and GP) resulted in significant ( $p < 0.05$ ) reduction in albumin concentrations compared with values obtained from juveniles on the control and other diets. Likewise, significant reduction ( $p < 0.05$ ) was observed with bilirubin concentration of

juveniles fed with treatments 1 and 4 (15.00 MLP and 15.00g/kg<sup>-1</sup> of MLP and GP respectively). Contrarily, fortification of the juveniles' diets with the phytoadditives resulted in significantly ( $p < 0.05$ ) increase in total protein concentration (though, lower value was obtained with juveniles fed with treatment 4) as well as in bilirubin concentration of juveniles fed with treatment 3 (7.50g/kg<sup>-1</sup> each of MLP and GP).

### 3.5. Kidney Biomarkers

Feeding juveniles of African catfish with treatment 2 (15.00g/kg<sup>-1</sup> of GP) resulted in significant ( $p < 0.05$ ) increase in creatinine concentration when compared with juveniles fed the control diet. While significant ( $p < 0.05$ ) reduction in creatinine concentrations were observed with *Clarias gariepinus* juveniles fed other treatments when compared with the values obtained with the juveniles in control diet

### 3.6. Lipid Profile

The combination of MLP and GP at 7.50 and 15.00g/kg<sup>-1</sup> each (treatments 3 and 4) in the diets of *Clarias gariepinus* juveniles significantly ( $p < 0.05$ ) reduced the plasma cholesterol when compared with the values obtained from other treatment diets and the control. However, the HDL-Cholesterol, HDL-phospholipids and triglycerides concentrations of juveniles on treatments 1 and 2 were significantly ( $p < 0.05$ ) higher than values obtained from juveniles in the control. Also, juveniles fed treatment 1 (15.00g/kg<sup>-1</sup> MLP) had significant ( $p < 0.05$ ) higher HDL-Cholesterol among the phytoadditive fortified diets. In contrast, HDL triglycerides were not significantly ( $p > 0.05$ ) affected by the inclusion of the phytoadditives.

## 4. Discussion

### 4.1. Growth Performance and Nutrient Utilization

Despite the global shift to the use of phytoadditives, there still exist dearth of information on the comparative use of moringa leaf powder and garlic powder individually or their combination as additives in the diets of African catfish; *Clarias gariepinus* juveniles. The results of parallel use of moringa, garlic and combination of MLP and GP showed significant improvement in growth performance and nutrient utilization of the *Clarias gariepinus* juveniles. Earlier work has reported the individual abilities of moringa and garlic powder to improve growth, efficiencies of feed and protein utilization, [9,27,28] in the diets of fish. The results of this study further attest to this claim, as juveniles on the phytoadditives showed better performance in feed utilization and growth. However, the improvement observed in feed utilization and growth performance parameters measured was highest when 15.00g/kg of garlic powder (treatment 2) was included in the diets of African catfish juveniles. Juveniles fed this diet consumed the highest amount of feed and protein, which were translated to best feed and protein efficiencies, cumulating

in highest final weight and weight gain. This result agrees with the findings of [29,30,31] as well as that of [32] when garlic powder was included in the diets of *Sterlet surgeon* fingerlings, *Dicentrarchus labrax* fry, *Tilapia zillii* and *Dicentrarchus labrax* juveniles respectively. The improvement in growth and utilization of nutrients of African catfish juvenile induced by garlic inclusion over the performance of juveniles fed 15.00g/kg<sup>-1</sup> moringa could have emanated from the presence of allicin and other biological organo-sulphur compounds found in garlic. Allicin is known to promote feed intake, the performance of intestinal flora, improve digestion and enhances energy utilization [14,33]. These could have boosted nutrient utilization, resulting in higher weight gain than juveniles in treatment 1 (15.00g/kg<sup>-1</sup> MLP).

The lower performance observed with juveniles fed with diets containing the mixture of the two phytoadditives; moringa and garlic powder seems not to support the synergetic potentials of garlic with other phytoadditives suggested by previous studies [12,13,14,34]. Rather the results of this study showed lower growth performance, feed and protein intake when garlic and moringa leaf powder mixture was added to the diets of *C. gariepinus* juveniles. The low performance was more pronounced at higher inclusion (15.00g/kg each of MLP and GP) treatment 4. Consequently, there might be the need to adjust the quantity of inclusion of moringa leaf and garlic powder, when used individually and when combined with other phytoadditives. Also, there is need to consider the intended fish to be cultured. This suggestion is borne out of the fact that the two mixtures (7.50 and 15.00g/kg<sup>-1</sup> each of MLP and GP, though with similar ratios; 1:1 in the diets of the *C. gariepinus* juveniles) had different feed utilization and growth performance. The discrepancy observed in this study may have resulted from the quantity and type of phytoadditives (among other factors) accompanying garlic in the diet used [32]. Hence, there might be the need to establish moringa leaf powder and garlic powder ratio when combined with the diets of *Clarias gariepinus* juveniles. This suggestion is buttressed by the findings of [13] who concluded that favourable health status of *Cyprinus carpio* L was only observed when garlic powder was used at 10g/kg diet or 7.5g and 2.5g/kg<sup>-1</sup> of garlic and ginger respectively as against other inclusion mixtures.

### 4.2. Plasma Biochemical Indices

Physiological stress indication such as biochemical variables can be useful in assessing nutritional effects of phytoadditives in fish. They are also useful biomarkers of liver and kidney physiological status.

### 4.3. Liver Biomarkers

ALT, AST and ALP are non-plasma specific enzymes that are localized in tissue cells of liver, kidney, heart, gills, muscles and other organs. Their presence in the blood may give specific information about organ dysfunction [35]. Transamination represents one of the pathways for the synthesis and deamination of amino acids. Thus, allowing interplay between carbohydrate and protein metabolism during fluctuating energy demands in

various adaptive situations. It is considered necessary in assessing the state of the liver, kidney, heart and other organs. Therefore, attention has been focused on changes in these enzymes activities, their levels have been proposed to be useful indicator of extreme stress and provide information on organ dysfunction [36,37].

The decrease activities of the amino transaminases enzymes of phytoadditives fortified diets compared with the values obtained with juveniles on control diet may be attributed to moringa and garlic; which may have stabilized cell membrane and protect the liver and other organs [14,38] against deleterious agents and free radicals. This agreed with the findings of many studies when garlic was included in the diets of Nile tilapia and common carp [9,13,39]. ALT plays a vital role in synthesis and deamination of amino acids during stress-imposed conditions for meeting high energy demand of organisms [40,41,42]. Therefore, the elevated ALT activities at 15.00g each of MLP and GP (treatment 4) may suggest liver damage, which may be due to a disturbance in normal physiological and biochemical processes of *C. gariepinus* juveniles as fed in this study. This suggestion is borne out of the submission of [9] who reported that an alteration in a variety of chemical, biological and physiological factors or a disturbance in Krebb's cycle might alter ALT activities. Consequently, the increased ALT activities may have arisen from any of these factors, which may have imposed some stress on the liver of the *C. gariepinus* juveniles in treatment 4. Alkaline phosphatase is membrane-bound primarily found in liver and bone, to a lesser amount in cell lining of the intestine, placenta and kidney. Whatever is measured in the blood is the total amount of ALP released from these tissues, which indicate the possibility of bone or liver diseases [43]. The level of ALP is a reliable biomarker for other fish organs but not in plasma and muscles of *Clarias gariepinus* [42]. The reduced levels of ALP in treatments 1 and 3 compared with levels recorded with fish on other treatments and control diet may indicate an interruption in the secretion and flow of bile in the liver as reported by [44].

The elevated total protein concentration of juveniles of *Clarias gariepinus* fed the phytoadditive fortified diets as against the control may have arisen from the high feed and protein intake of juveniles fed with these diets [45]. Also, the additives may have repairing effects on the liver due to their nutritional properties [46,47]. Albumin is used as an indicator of liver function, reduced absorption or protein loss [48]. There were reductions in the concentrations of albumin in treatments 3 and 4 as well as in bilirubin concentration of juveniles in treatments 4. Conversely, higher bilirubin levels were observed with juveniles fed with treatment 3. Albumin and bilirubin are some of the biochemical indices for monitoring liver function in the blood. Abnormal levels of these proteins have been associated with liver damage [49]. Consequently, the reduced albumin concentrations observed in treatments 3 and 4 seem to indicate that the livers of the juveniles fed with these treatments were negatively affected. Also, the higher bilirubin concentration of juveniles on treatment 3 seems to lend credence to liver damage. The results observed seem to point to the probability of an inappropriate combination of moringa leaf meal and garlic powder, or perhaps improper mixing ratios of moringa

leaf powder and garlic (7.50 and 15.00g/kg each) for the juveniles of *Clarias gariepinus* in this study. This could have exerted some level of stress on the liver resulting perhaps in reduced protein absorption by juveniles fed these treatment diets when compared with juveniles on other phytoadditive diets. These may have been responsible for the lower weight gained by juveniles of *Clarias gariepinus* fed with treatments 3 and 4 when compared with juvenile in other phytoadditive diets (treatments 1 and 2). However, the reduced bilirubin concentration recorded with *Clarias gariepinus* juveniles on 15.00g/kg MLP may be attributed to the inappropriate level of inclusion (rather than improper use) of the phytoadditives in the diet of the juveniles of *Clarias gariepinus*. This is because moringa leaf powder is known to possess nutritional qualities that enhance liver function [5,46] and protein absorption, which is evident in higher growth performance next to the performance recorded with juveniles on 15.00g/kg garlic diet. Hence, suggesting the need to further investigate this abnormality or establish their level of inclusion in the diet of *Clarias gariepinus* juveniles.

#### 4.4. Kidney Biomarkers

The high creatinine concentration of juveniles fed treatment 2 (15.0g of garlic) may have resulted from the high muscle mass of juveniles in this diet [47]. While the reduced levels of creatinine observed with juveniles in other phytoadditives diets (treatments 1, 3 and 4) relative to juveniles in the control diet may be suggestive of the ability of the kidney in filtering the metabolic end products of the diets [50,51], rather than low muscle mass. This is because juveniles in these diets gained higher body weights when compared with juveniles fed the control diet. As earlier explained, the increased urea concentrations of juveniles fed treatments 1 and 3 (15.00g MLP, 7.50g each of MLP and GP) may have resulted from increased activity of the kidney [47,51].

#### 4.5. Lipids Profile

Contrary to earlier reports the inclusion of moringa and garlic powder at 15.00g/kg each in treatments 1 and 2 did not have a hypolipidemic effect on *Clarias gariepinus* juveniles as fed in this study. Likewise, the increased plasma concentration of phospholipids and triglycerides found with all moringa and garlic supplemented diets. The discrepancy observed in this study may have emanated from the types and preparation adopted for the phytoadditives, time and dosage of application, species and stage of fish development as reported by many authors [32,52,53]. Also, since the diets fed were not high-fat diets, the hypolipidemic effect might not come into play [52,53]. Also relative to control and other phytoadditives diets, the inclusion of a mixture of MLP and GP at 7.5 and 15.0g/kg each (treatments 3 and 4) reduced plasma cholesterol concentration in the diets of African catfish juveniles. It can be inferred that the reduced cholesterol concentration observed with the juveniles of African catfish tend to suggest that the mixture of the phytoadditives as used in this study possess a synergetic ability that tend to enhance their hypolipidemic properties.

However, of importance in the moringa and garlic fortified diets are the increased plasma concentrations of HDL-cholesterol and HDL-phospholipids in the plasma of juveniles fed with these diets. These compounds are known to transfer fat away from cells, arteries and tissues. Increasing HDL particles are associated with decreasing accumulation of atherosclerosis, thus of significance in the production of fish for human consumption.

## 5. Conclusion

In conclusion, judging by the growth performance, nutrient utilization and plasma biochemical parameters (which are indices of the state of liver and kidney's health status) assessed in this study, the inclusion of garlic at 15.00g/kg<sup>-1</sup> in the diets of *Clarias gariepinus* juveniles produced best results. While the mixture of moringa and garlic did not improve the performance observed with the individual use of either of the phytoadditives in the diets of *Clarias gariepinus* juveniles. Consequently, the potential synergic effect of garlic with other phytoadditives appear not to hold for moringa leaf powder as used in the diets of *Clarias gariepinus* juveniles in this study. There might also be the need to further investigate the level of the phytoadditives inclusion individually or in combination to establish their level (s) of inclusion in the diet of juveniles of *Clarias gariepinus*. However, of importance is the reduced tendency of atherosclerosis in man consuming catfish fed with these phytoadditives diets.

## Statement of Competing Interest

The authors have no competing interests.

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