Harnessing the Phytochemical Attributes and Potentials of *Gongrenema latifolium* Leaf Meal Supplement in the Optimization of biochemical indices in Cultured African catfish (*Clarias garipinus*)

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Abstract

This study was conducted to determine the significant influence of *Gongronema latifolium* leaf meal on the biochemical indices of the African catfish (*Clarias gariepinus)* post fingerlings for thirty (30) weeks. Five treatments were used in all, made up of four levels of *G. latifolium* leaf meal (2.5, 5.0, 7.5 and 10.0) and the control diet (0g/kg, in triplicate. The experimental diets were fed to fish in fifteen tarpaulin tanks of 100 by 80 by 100 cm^3 capacity each and stocked randomly with twenty *C. gariepinus* post fingerlings of mean initial bulk body weight of 2.881± 0.00 g and mean total length of 5.552 ± 0.0cm. The fishes were fed twice daily at 8.00 am and 4.00 pm at 3% of their body weight. The proximate composition of five experimental diets contained crude protein, crude fibre, moisture, crude fat, ash and nitrogen-free extract within the recommended range for fish growth. The phytochemical attributes of G. latifolium evaluated included alkaloids, saponins, tannins, glycosides, flavonoids, polyphenols and reducing compounds. Biochemical indices of *C. gariepinus* includes total protein, albumin and globulin increased significantly ($P<0.05$), whereas glucose level, cholesterol, triglyceride and urea increased insignificantly ($p>0.05$). Water quality parameters such as pH, temperature and dissolved oxygen were maintained at recommended levels for optimal growth and good health of freshwater fishes. The inclusion of *G. latifolium* leaf meal in *C. gariepinus* feed led to improved and balanced (optimal levels of) biochemical parameters of the experimental fish required for optimal growth and development of the cultured fishes. Biochemical indices of *C. gariepinus* fed on *G. latifolium* leaf meal produced significant increase in protein, albumin, globulin but insignificant increase in Urea Cholesterol and Triglyceride levels as significantly influenced by flavonoids, alkaloids, saponins and tannins phytochemicals contained in G. latifolium leaves.

Keywords: Flavonoids, reducing compounds, total proteins, cholesterol, catfish, leaf meal.

INTRODUCTION

According to Tacon *et al* (2006) the introduction of locally available and cheaper plant protein source is proved to be very important for future development of aquaculture in Nigeria. Increase demand for fish protein and consumption is driven by many factors including increased in population (FMARD, 2015). Also, acceptability of fish in Nigerian diet as a substitute for meat is due to health and nutritional benefits of fish protein. It is a known fact that fish currently constitutes 41% of total animal protein intake by the

average Nigerian (FMARD, 2015). This demands a sustained fish production as well as a matching increase in fish feed production. In order to intensify aquaculture production, locally formulated plant protein feeds that must satisfy the nutritional requirements of fish to be cultured and with minimal cost should be investigated. Efforts aimed at reducing fish feeds production cost have resulted in an increased of cheap and easily affordable plant protein source.

Study of inventory of fish feeds marketers in Nigeria estimated that 4,000 tons of quality fish feeds were imported into the country yearly and that contributed to an increase in the cost of fish (AIFP, 2004). The experiment on African aquaculture suggested more research on how best plant production can be incorporated in fish feed for the development and management of fish feed as a major factor that determine the profitability of aquaculture incentive (Hetch, 2006). The x-ray of potentials for development of aquaculture in Africa reported by Jamu and Ayinla (2003) indicated that low quality of local fish feed and high cost of commercial feed were the major factors limiting the development of aquaculture. These are attributed to the fact that feed accounts for, at least, 60% of total cost of fish production in Africa which, to a large extent, determine the viability and profitability of fish farming enterprise (Jamu and Ayinla, 2003).

Gongronema latifolium is used as a leafy vegetable in Nigeria and a good source of vitamins, protein, iron and minerals. The medicinal importance of *G. latifolium* cannot be over emphasized. The plant plays a vital role in the treatment and prevention of varied health related problems including liver diseases, diabetes mellitus, high blood pressure, loss of appetite, dysentery, stomach pains, worm infectors, cough and malaria fever" (Agbo et al. 2005);. Medicinal importance of the plant is further elaborated by the presence of five bioactive compounds including alkaloids, saponins, tannins, flavonoids, and glycosides in leaves, which was suggested to proffer varied pharmacological effects on its specie

Biochemical investigations remains the easiest tools to diagnose the physiological status of higher animals including fishes (Joshi, 1979). Olusegun and Adedayo (2014) analyzed responses on serum biochemistry of *Clarias gariepinus* exposed to sub-lethal concentration of cold water extracts of *plumbago zeylanical* (leadwort) and observed that biochemical inices were significantly influenced by the plant treatment.

The African catfish, *C. gariepinus*, belonging to the family Clariidae dominates the cultured fish species in Nigeria. This is due to several culture characteristics exhibited by this species. Such culture characteristics include its ability to tolerate a varying range of environmental conditions, high stocking densities under culture conditions, fast growth rate, disease resistance, acceptability of artificial feed, high fecundity, good taste and meat quality, ease of artificial breeding and high market value (Eyo *et al* 2014).

G. latifolium commonly known as Utazi and Arokeke by the South-South and South-West inhabitants in Nigeria is found in Africa, Asia and Oceania. It is a tropical phyto-protein that is used as spice

(Ugochukwu *et al* 2003). Apart from the proteinous aspect of this plant, it is also used as a traditional medicinal plant due to it phytochemical composition for the treatment of various gastrointestinal disorders such as diarrhea, ulcers, dyspepsia and also in the management of diabetes mellitus (Okafor *et al* 1996; Nwing *et al* 2005). The use of G. *latifolium* to enhance fish, and its biochemical and growth indices value are particularly important, since fish contributes to the aquatic resources of socio-economic importance and sustenance for economic stability. Therefore, it is essential to study the growth performance in relation to gonadal development and biochemical characteristics through the application of different inclusion levels of *G.latifolium* leaf meal in the diets of C. *gariepinus.*

The use of G. *latifolium* to enhance fish biochemical indices and value are particularly important, since fish contributes to the aquatic resources of socio-economic importance and sustenance for economic stability. Therefore, it is essential to study the role of the phytochemistry of G. latifolium on the optimization of biochemical parameters which are directly influenced through the application of different inclusion levels of *G. latifolium* leaf meal in the diets of C. *gariepinus.*

MATERIALS AND METHODS

2.1 Determination of phytochemical attributes of leaf extracts G. latifolium leaves

Phytochemical analysis was carried out on the powdered and aqueous extract of samples of *G. latifolium* leaves using standard procedures to identify the constituents as described by AOAC (2020). The phytochemical analysis was carried out to determine the presence of the following chemicals in the plant extracts: tannins, saponins, flavonoids and alkaloids.

2.2.1 **Determination of Tannins in G. latifolium leaves**

About 0.5g of the dried powered sample was boiled in 20 .0 millimeters (ml) of water in test tube and then filtered. A few drops of 0.1 (%) ferric chloride were then added. Observation of a brownish green colour indicated the presence of tannins.

2.2.2 **Determination of Saponin in *G. latifolium* leaves**

A 2.0g of the powdered sample was boiled in 20.0ml of distilled water in a water bath and filtered. Ten ml. of the filtrate was mixed with 5.0ml of distilled water and shaken vigorously for a stable persistent froth to occur. The frothing was mixed with 3 drops of olive oil and shaken vigorously. Formation of emulsion indicated the presence of saponin.

2.2.3 **Determination of Flavonoids in *G. latifolium* leaves**

A one ml. of 10.0% lead acetate solution was added to 1.0ml.of aqueous extract of the plant. The formation of a yellow precipitate indicated a positive test for flavonoids.

2.2.4 **Determination of Alkaloids in *G. latifolium* leaves**

A Three ml. of aqueous extract was stirred in 3ml. of 1% Hcl on a steam bath.

Mayer's and Wagner's reagents were added to the mixture. Turbidity of the resulting precipitate indicated the presence of alkaloids.

2.3 Determination of biochemical parameters/indices of African catfish

2.3.1 Determination of Albumin

Three microlitres (ml) of the test serum were pipetted into a glass test tube. That was followed by addition of 1000ml 3,3,5,5 – tetrabromo-m- cresol sulphonephthalein (bromocresol green, BCG) indicator reagent to the test tube. Two tubes were them set up as that of the test sample, but to one of the tubes were added 3.0ml of de-ionized distilled water (ddH2^0) and 1000ml of BCG to serve as reagent blank while 3.0ml of Albumin standard solution of 4.6g/dl and 1000ml of BCG indicator reagent were added to the second tube. The test, standard and blank solutions were mixed and incubated at 20-25^{0}C for 20 minutes or 37^{0}C for 10 minutes. At the end of the incubation, spectrophotometer of 630nm wavelength was standardized and zeroed with the reagent blank that was poured into a cuvette of 1cm light path at wavelength of Hg578nm or Hg623nm. That was followed by the pouring of the test and standard solutions each into the cuvette, inserted into the spectrophotometer and the absorbance of the test and standard solutions recorded and read spectrophotometrically.

Calculation of Albumin concentration (g/l or g/dl):

$$= \frac{\text{Absorbance of test sample}}{\text{Absorbance of standard solution}} \text{ x}$$

Concentration of standard (g/dl)

2.3.2 **Determination of total protein**

Three tubes labeled reagent blank, standard, and sample were set up. To the corresponding labeled tubes were added 0.02ml of reagent blank, standard solution and sample. That was followed by addition of 1.0ml of Biuret reagent to each of the tubes. The tubes contents were mixed, and incubated for 30 minutes at 20 – 25^{0}C. The reagent blank standard and sample solutions were read spectrophotometrically at the wavelength of Hg546nm. The absorbance of the sample and standard solutions were measured against the absorbance of the reagent blank.

Calculation of Total Protein concentration (g/l or g/dl)

$$\text{Total protein conc.} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of standard solution}} \text{ x}$$

Concentration of standard (g/dl)

(Tiertz, 1994)

2.3.2 **Determination of urea**

Five microlitres (ml) of serum (test sample), standard serum, and distilled water were pipetted into respective labeled cuvettes. That was followed by addition of 50ml of Reagent `1 (EDTA, sodium nitroprusside and urease) to each of the cuvettes. They were then mixed and incubated at 37^{0}C for 10 minutes. Thereafter, 1.25ml of reagent 2 (dilute Phenol 120mmol/L) and Reagent 3 (Sodium Hypochlorite (27mmol/L), Na0H:0.14N) were successively added to each of the cuvettes. The solutions of each of the cuvettes were mixed and incubated at 37^{0}C for 15 minutes. They were inserted into

MONZA flow cell folder and press read. The readings were automatically posted on the screen.

Concentration of standard (mmol/L)

Calculation of urea concentration (mmol/L)

$$= \frac{\text{Absorbance of test sample}}{\text{Absorbance of standard solution}} \text{ x}$$

2.3.4 Determination of cholesterol by enzymatic Endpoint method

Three cuvettes were labeled Blank, standard and sample. Ten microlitres of reagent blank (Distilled $H_2{}^0$) standard, solution and sample were pipette into corresponding labeled cuvettes. That was followed by addition of 1000µl of Reagent to each of the cuvettes. The solutions were mixed and incubated at 20- 25^0C for 10minutes or 37^0C for 5 minutes. They were inserted into spectrophotometer and the values read at the wavelength of Hg546mm within 60 minutes. The absorbance of the sample was measured against the reagent blank.

Calculation of cholesterol concentration (mmol/L):

$$\frac{\text{Absorbance of test sample}}{\text{Absorbance of standard solution}} \text{ x}$$

Concentration of standard (5.33 mmol/L

Or 206mg/dl)

2.3.5 Determination of triglycerides concentration by colorimetric method

Three cuvettes were each labeled Reagent blank, standard and sample. To each of the corresponding labeled cuvettes was added 10.0µl of $ddH_2{}^0$, 5.0µl of standard solution and 5.0µl of sample. Thereafter, 500.0µl of Reagent 1 (Enzyme Reagent) were added to each of the cuvettes. The contents were mixed and incubated for 10 minutes at 20 – 25^0C or 37^0C for 5 minutes. They were then inserted into the RX Monza flowcell holder and pressed read within 60 minutes. The values were automatically posted on the screen.

Calculation of Triglyceride concentration (mmol/L or mg/dl) =

$$\frac{\text{Absorbance of test sample}}{\text{Absorbance of standard solution}} \text{ x}$$

Concentration of standard

(ie 2.16mmol/L or 191mg/dl) (Sidel *et al*, 1981)

3.1 RESULTS

Phytochemical analysis of *G.latifolium* leaf meal (Table 1) showed mean alkaloid (2.41 ± 0.06%), mean glycoside (2.25 ± 0.01%), mean saponin (2.08 ±0.01%), mean tannin (1.18 ± 0.01%), mean flavonoid (2.52 ±0.01%), mean polyphenol (4.61 ± 0.01%) and mean reducing compound (7.91 ± 0.01%).

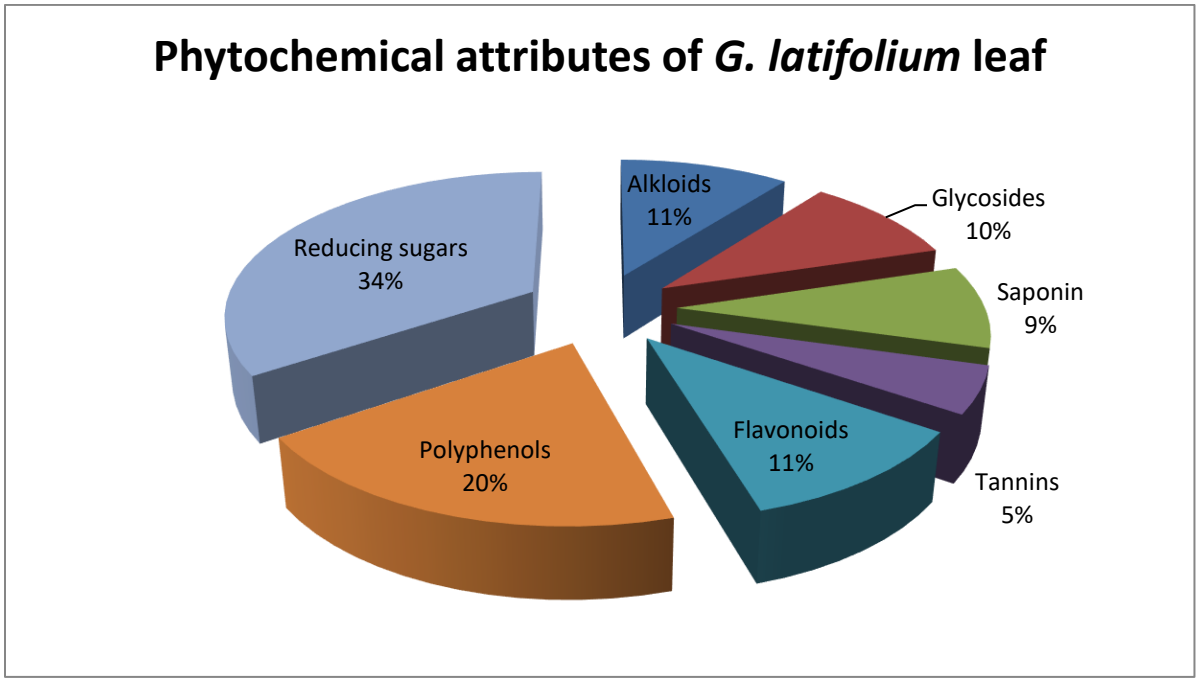
The phytochemical indices of the least 1.18± 0.01% and highest 7.91± 0.01% were constituted by tannins and reducing compounds, respectively.

TABLE 1

Phytochemical analysis of *G.latifolium* leaf meal and the experimental diets

Phytochemical Indices	Values
Alkaloids	2.41 ± 0.06%
Glycosides	2.25 ± 0.01%
Saponins	2.08 ±0.01%

Tannins	1.18 ± 0.01%
Flavonoids	2.54 ±0.01%
Polyphenol	4.61 ± 0.01%
Reducing Compounds	7.91 ± 0.01%



Mean effects of GLM and phytochemical attributes on biochemical indices of *C. gariepinus*

Table 2 shows the mean effects of GLM on biochemical indices of *C. gariepinus*. The highest glucose value 3.89± 0.026 was record for fishes in Treatment E (10.0g/kg GLM), followed by 3.69± 0.146 in Treatment D (7.5g/kg GLM) and least value 3.63± 0.048 in Treatment C (5.0g/kg GLM). The differences in the values obtained were statistically insignificant (P>0.05).

The highest, higher and least values of Cholesterol 3.03± 0.148, 2.43± 0.160 and 2.13± 0.202 were obtained from Treatments C (5.0g/kg GLM), D (7.5g/kg GLM) and A (control) respectively. The different values obtained were statistically insignificant (P>0.05).

The highest higher and least values of Triglyceride 0.78± 0.05, 0.49.1± 0.007 and 0.5± 0.023 were recorded for Treatments D (7.5g/kg GLM), A (Control) and C (5.0g/kg GLM) respectively. The differences in values obtained were statistically insignificant (P>0.05).

The records of protein values were highest (64.25± 3.210) higher (60.6± 0.427) and least (53.73± 0.895) from Treatments C (5.0g/kg GLM) E (10.0g/kg GLM) and A (control) respectively. The different values obtained were statistically significant (P<0.05). The highest, higher and least Albumin values of 39.25± 0.953, 36.77±

0.593 and 32.8±0.4 were recorded for Treatments C (5.0g/kg GLM), B (2.5g/kg GLM) and D (7.5g/kg GLM) respectively.

The differences in values obtained were statistically significant (P<0.05) for Globulin with highest and least values 31.97± 0.573, 25.75± 0.997 and 19.42 ± 0.259 for Treatments C (5.0g/kg GLM) E (10.0g/kg GLM) and B (2.5g/kg GLM). The value obtained were statistically significant (P <0.05). For urea values 1.72± 0.058 1.58± 0.06 and 1.25± 0.017 were highest, higher and least obtained from Treatment D (7.5g/kg GLM), A (control) and C (5.0g/kg GLM) each. The values obtained were statistically insignificant (P>0.05).

TABLE 2

Mean effects of *Gongronema latifolium* leaf meal on biochemical indices of *Clarias gariepinus*

Treatments	Glucose (mmol/l)	Cholesterol	Triglycerides	Protein	Albumin	Globubin (g)	Urea
A – 0/gkg	3.67±0.06	2.13±0.202	0.491±0.007	53.73±0.895	32.9±2.6	19.62±0.249	1.58±0.006
B - 2.5g/kg(GLM)	3.66±0.035	2.28±0.528	0.48±0.007	55.48±1.385	36.77±0.593	19.42±0.259	1.48±0.007
C – 5.0g/kg (GLM)	3.63±0.048	3.03±0.148	0.5±0.023	64.28±3.210	39.25±0.953	31.97±0.573	1.25±0.017
D – 7.5g/kg(GLM)	3.69±0.146	2.43±0.160	0.78±0.05	57.37±2.756	32.8±0.4	24.02±0.366	1.72±0.058
E – 10.0g/kg(GLM)	3.89±0.026	2.28±0.000	0.49±0.007	60.6+0.427	35.23±0.328	25.75±0997	1.28±0.003
LDS (P<0.05)				5.85	2.69	1.79	

Mean values having the same superscript are insignificant (P>0.05)

Statistical evaluation of the significant differences in biochemical indices influenced by phytochemical attributes of G. latifolium leaf meal fed to C. gariepinus

Analysis of variance (ANOVA) for determination of the significant differences in biochemical indices of of fish fed on experimental diets (Table 3) shows Glucose (p-value 0.131) Cholesterol (p-value

0.0002), Triglycerides (p-value 0.008), Protein (p-value 0.0199), Albumin (p-value 0.0025), Globulin (p-value 0.0000) and Urea (p-value 0.0002). The differences in values obtained from Protein, Albumin and Globulin were statistically significant ($p<0.05$), but the different values obtained from Glucose Cholesterol, Trigylcerides, Urea, were insignificant ($p>0.05$).

TABLE 3

Analysis of variance for determination of the significant differences of biochemical indices of fish fed on experimental diets

Indices	P-value	Inference
Glucose	0.130697	Insignificant (p>0.05)
Cholesterol	0.100165	Significant (P<0.05)
Triglycerides	0.100864	Significant (p<0.05)
Protein	0.019982	Significant (p<0.05)
Albumin	0.002508	Significant (p<0.05)
Globulin	0.00000119	Significant (p<0.05)
Urea	0.100165	Insignificant (p>0.05)

3.3 Discussion

Results obtained in this study shows significant variation in some biochemical indices of fish fed on diets with varying levels of *G. latifolium* leaf meal. Findings of this study shows that glucose which is a source of energy was insignificantly higher (P>0.05) in fish fed feed on E (10.0g/kg GLM) with value of 3.89± 0.026 mmol/L compared to feed B 2.5g/kg (3.66±0.35 mmol/L), Feed C (5.0g/kg GLM) 3.63±0.48 mmol/L, feed D (7.5g/kg) 3.47±0.09 mmol/L and feed A (control) 3.67±0.06 mmol/L. Results obtained in this study were not as high as values obtained from the findings of Egwu & Omeodu, (2010);Yekeen & Fawole, (2011) who recorded highest glucose values of 46.2±0.13mmol/l and 123.67± 6.49mmol/l respectively for *C. gariepinus*..

Cholesterol which is major component of bad lipid and culprit in cardiovascular disease recorded the highest insignificant value of 3.03± 0.0148 in fish fed with 5.0g/kg of *G. latifolium* leaf meal. Higher, high and least cholesterol values were obtained in those of 2.5g/kg, 7.5g/kg, 10.0g/kgGLM and control 0g/kg. They were inconsistent insignificant rise in cholesterol level with variable concentrations of GLM used. However, statistically decrease in cholesterol in fish fed on coppens and 10.0g/kg of *G. latifolium* indicated decrease in the lipid content in the blood. Therefore, increase in cholesterol found in fish fed on 5.0g/kg *G. latifolium* may be indication of

malmembrane function and structure disruption; thus affecting fluidity, permeability and transport system. The value obtained in this study is not as high as values reported by Yekeen and Fawole (2011) who attributed the increase in cholesterol level to increased lipid content in blood of fish exposed to heavy stress due to pesticide.

Triglyceride recorded in this study was insignificantly higher (P>0.05) in fish fed on control than those of 2.5g/kgn to 10.0g/kg of *G. latifolium* leaf meal.When concentration was increased from 7.5g/kg to 10.0g/kg, the higher value 0.78±0.005 was obtained in fish fed on 7.5g/kgGLM than 2.5g/kg, 5.0g/kg and 10.0g/kgGLM. The values were statistically insignificant (P>0.05). Statistically decrease in triglycerides found in GLM maintained activity of hepatic lipase enzyme responsible for lipid catabolism, whereas increase triglyceride level causes alteration of hepatic lipase.

Total protein which aids in the maintenance of tissue, growth or formation of new protein source as enzymes, defense against disease causing agents, among others was observed to be 65.67+4008 significantly higher (P>0.05) in fish fed on feed C (5.0g/kg of *G. latifolium* leaf meal) than those of control and other varying concentrations of 2.5g/kg,7.5g/kg and 10.0g/kgGLM. Successive increase in concentration of *G. latifolium* leaf meal (GLM) caused significant (p>0.05) increase in protein of *C. gariepinus.* Results obtained from this study were similar to the findings of Simeon and Madueke (2012) who reported high protein levels of *C. gariepinus* as a result of diminished feed and water intake

Similarly, albumin which is the most vital plasma protein produced by the liver and aids in maintaining pressure for proper distribution of body fluid between extra vascular compartments and body tissue in this study had the highest value of 39.25± 0.953 in 5.0g/kg of *G. latifolium* leaf meal. The higher value and irregular different values obtained from control and other varying concentrations of GLM were statistically significant (p.>0.5). The values obtained in this study are not as low as values recorded from the findings of Simeon and Madueke, (2012); Eguwu Omeodu 2010 & Yekeen and Fawole (2011) for *C. gariepinus* exposed to different treatments.

In this study fish fed on control 0g/kg had insignificantly (p>0.05) higher globulin than those fed on 2.5g/kg of *G. latifolium* leaf meal. However, those fed with 5.0g/kg of *G. latifolium* leaf meal, had highest value of 31.97±0.573g. Successive increase in concentration of *G. latitfolium* leaf meal from 2.5g/kg to 10.0g/kg caused significant (P>0.05) increase in globulin value of *C. gariepinus.* These findings were similar to that of Simeon and Madueke, (2012) who used 25% diet containing *Mytilusedulis* shell.

Urea which is the main by- product of protein metabolism in this study recorded low level of 1.25±0.017 in fish fed on 5.0g/kg GLM. There were no statistically insignificant differences in those values obtained (P>0.05). Changes in Urea suggest serious protein breakdown in animal tissues.

Conclusion

The phytochemical composition of G. latifolium used in the supplementation of

different levels of *G. latifiolium* leaf meal has shown beneficial effects on the biochemical indices of *C. gariepinus* when compared to the control, 0g/kg diet. The phytochemical attributes has significantly influenced the optimization and maximum utilization and enhanced the availability of total proteins,, albumin, globulin and also significantly reduced the bioaccumulation of cholesterol, triglycerides and urea in the tissues for optimal benefit of the catfish. Biochemical indices of *C. gariepinus* fed on *G. latifolium* leaf meal produced significant increase in protein, albumin, globulin but insignificant increase in Urea Cholesterol and Triglyceride levels as significantly influenced by flavonoids, alkaloids, saponins and tannins phytochemicals contained in *G. latifolium* leaves.

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