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RESEARCH ARTICLE

SUBSTITUTION OF SOY BEAN MEAL WITH *GOMPHRAENA CELOSIODES* LEAF MEAL IN THE DIET OF FINGERLINGS OF *CLARIAS GARIEPINUS*: EFFECT ON HAEMATOLOGICAL INDICES AND BLOOD (SERUM) CHEMISTRY

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ABSTRACT

This paper aimed at evaluating the haematological responses of fingerlings of *Clarias gariepinus* fed experimental diets containing *Gomphrena celosiodes*leaf meal as plant protein source. *Gomphrena celosiodes* leaf meal was incorporated in diets at 0, 25, 50, 75 and 100% of experimental diets. All diets were iso-nitrogenous (41-42% Crude Protein). Fingerlings of *Clarias gariepinus* with initial mean weight of 10.15±1.54g were fed *ad lib* on allotted diets twice per day at 08.00 hrs and 18.00hrs for 60 days. The experiment was a complete randomized design (CRD). The results of fish fed test diets showed significant ($p>0.05$) differences with respect to RBC, WBC, MCH when compared with control. Results indicate a decreasing trend in Hb value of blood with increase in the quantity of *Gomphrena celosiodes* Leaf Meal (GCLM) in the diet. There were no significant different ($p<0.05$) in amongst all the diets with respect to Eosinophils, Basophils and MCHC. Blood sugar levels in fish fed on diets containing 75-100% GCLM was 72.73± 0.64 mg/dl and significantly lower ($p>0.05$) than fishes fed on 0-50% GCLM. The concentration of cholesterol in blood of fishes fed experimental diets decreased significantly ($p>0.05$) with increase in the quantity of GCLM in the diet. There was no significant difference ($p>0.05$) in creatinine, and total protein concentration amongst the various treatments. There were significant ($p>0.05$) increase in aspertate transaminase, alkaline phosphate and alanine transaminase concentrations in the blood of fishes among the treatment groups.

INTRODUCTION

Inadequacy in supply of protein especially those of animal origin is prevalent in all parts of Africa where it is estimated that an average of 10gms of animal protein is consumed per day compared with a recommended intake of 35gms (FAO, 2002). Globally, fishes account for about 16% of animal protein consumed (Matthews, 1999). Between 1986 and 1998, this category of food item contributed as much as 29-35% of the animal protein in the diet of people in the West African region of Africa, including Nigeria. In Nigeria, per capital, fish consumption has been estimated at 8.8kg/caput/annum (FAO, 2002) while, consumption for the same commodity for the rest of the Africa is estimated at 7.1kg/cap/year, (Matthews, 1999).
The low level of animal protein intake by Nigerians has been generally attributed to the short fall in its production which encourages scarcity and high prices, (Ekpo and Etim, 1989). Part of the solution to this problem of shortage of protein supply is to increase the production of animals that have a high reproductive and growth rate of which fish is one of such animal. Fish contributes to the economy of the Nigerian Nation and contributes 4.5% of Gross Domestic product (GDP), with a growth rate higher than for other extractive use of the Nigerian environment such as livestock and forestry, and contributes about 0.5% of Nigeria's export earnings (Ekpo and Etim, 1989; Moses, 2000).

However, fish feed is a major problem in fish production. Feed is known to be the single most expensive factor in animal and aquaculture production of which protein is the feed constituent with the highest cost implications (Aniebo *et al.*, 2008). Consequent to this, the development of leaf meals as alternative non-conventional protein sources for fish feed has intensified over the years. Cassava and alfalfa leaf meals have been used with varying degrees of success in the culture of *Orechromis niloticus* (Ng and Wee,1989, Yousif *et al.*, 1994). *Carica papaya, Morienga oleifera*, sunflower and sesame leaf meal have been used in feeding *Heterobranchus bidorsalis* and *Clarias gariepinus* (Anyanwu *et al.*, 2011, Ayotunde *et al.*, 2011; Fagbenro *et al* 2013; Ozovehe, 2013). It has been demonstrated that leaves of broad bean could replace 25% of soybean meal protein of Nile tilapia (*Oreochromis niloticus*) diet to improve growth performance and feed utilization. Ali *et al*. (2003) concluded that alfalfa meal could be included only up to 5% in 36% crude protein diet without reducing growth

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performance. Leaves of *Amaranthus spinosus* could be included in the practical diet of *C. gariepinus* without affecting growth and feed utilization (Adewolu and Adamson, 2011).The parameters for the assessment of the quality and efficiency of these leaf meals in fish production has spanned through growth responses (mean weight gain, specific growth rate), feed conversion ratio, haematological and biochemical indices of blood status.

Haematological indices are important indicators of the health status of the fish. The study of the physiological and haematological characteristics of cultured fish species has been an important tool in the development of aquaculture system, particularly in regard to differentiation of healthy from diseased or stressed animal (Rainza-Paiva *et al*., 2000; O'Neal and Weirich, 2001). It is used to assess the effect of feed ingredients and to assess extent at which the feed materials have been assimilated into fish tissue. Haematological indices of blood include Red blood cells (Erythrocyte) count, White blood cell (Leucocytes count) Haemoglobin and packed cell volume (PCV). Their values are used to calculate Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCHC). These constituents of the blood especially RBC are affected by age, sex, hormones hypoxia and ambient temperature. Myburgh *et al*. (2008) also demonstrated that quality and quantity of the food consumed by the animal can also influence blood parameters. *Gomphrena celosioides* Mart belongs to the family Amaranthaceae, it is an annual or short-lived perennial weed species, first discovered at Queensland in 1930, which has now spread throughout the old world tropics (Myers *et al*. 2000). *Gomphrena celosioides* weed occupies an important position in agro ecosystem as a source of food for invertebrates, higher trophic groups, as well as having an intrinsic biodiversity value (Marshall et. al 2003). It grows along roadsides, river banks, rail way and on fallow land, occasionally invades pastures. It is well distributed in South America, Asia, East and West Africa. Its presence in Ghana and Nigeria is recently recorded (Onocha *et al*. 2005).
This study was carried out to assess the potentials of *Gomphrena celosiodes* to serve as protein source in the experimental diets of *Clarias gariepinus*, a species of fish that is commonly cultured in Nigeria. To achieve these objectives fingerlings of *C. gariepinus* were fed for 60 days with *Gomphrena celosiodes* based diets replacing soybean meal as a protein source. Haematological and biochemistry indices were used for assessment.

MATERIALS AND METHODS

The experiment was conducted inside the Animal Nutrition Laboratory of the Department of Animal Science and Fisheries, Abia State University, Umuahia Campus. Two hundred (200) fingerlings of *C. gariepinus* fish were obtained from a private fish farm in Umudike, Abia State, Nigeria. They were then transported in three 50 liter plastic containers to the Laboratory. They were acclimatized for 23 days during which period they were fed ad-lib on "Coppes" a commercial feed. Mortality during this period was 11%. *Gomphrena celosiodes* Leaf Meal

Processing of Gomphrena celosiodes Leaf Meal

Leaves of *Gomphrena celosiodes* |Mart were harvested from wallowing farms of Umuahia Campus of Abia State University. They were chopped to pieces with a knife to facilitate drying under the sun and spread on a clean mat in a well-ventilated room for seven (7) days until the leaves became crispy. This approach was taken to prevent leaching and to maintain the reddish colouration of the leaves. The dried leaves were blended into a smooth paste in a 3.8 L kitchen-type blender (Warning Products, New Hartford, CT) with a sieve size of 3.15mm. The blender was thoroughly cleaned and dried between samples. Triplicate determination was made for each treatment to produce the *Gomphrena celosiodes* leaf meal (GCLM). The leaf meal was used to formulate 5 iso-nitrogenous (41-42% crude protein) *C. gariepinus* diets replacing soya bean at 0, 25, 50, 75 and 100% inclusion levels. All experimental diets were chemically analysed for crude protein, crude fiber, moisture and ash according to methods described by (AOAC, 2000).

Stocking/Feeding regime

All fish were starved for 24 hours prior to the commencement of the experiment. The feeding trial was carried out in fifteen transparent plastic tanks of 40L capacity with water depth of 0.40m. The tanks were placed on wooden table with a height of 1.4m. Experimental fish (fingerlings of *C. gariepinus*) were randomly distributed into five treatment groups and each group had 15 replicates. The experiment was a completely randomized design (CRD). The fish were fed with five iso-nitrogenous (41-42% crude protein) diets formulated with graded levels of inclusion of (0, 25, 50, 75 and 100%) *Gomphrena celesiodes* leaf meal (GCLM). Each diet was fed to apparent satiation to triplicate groups of ten fingerlings of *C. gariepinus* (mean weight=10.15±1.54g) for 60 days in a laboratory. All fishes were subjected to a 12:12 light and dark cycle using a natural day and night regime.

Water Management

Unprocessed borehole water used for the study was temporally stored in 500L plastic containers from where it was transferred into the experimental tanks every morning. Dead fish were removed and recorded daily. Mortality during the experiment was 5%. Unconsumed feed and excreta were siphoned out twice daily (one hour after feeding) after which water level was topped to maintain the same water level. There was 50% exchange of water in all the tanks.

Collection of Blood Samples from Fish

At the expiration of the experimental period, three fishes from each of the replicates were selected for blood samples. About 2ml of blood were collected into labeled sterile universal bottle containing a drop of ethylene diamineteracetate (EDTA). A second sample of 3ml were collected with a sterile labeled syringe without EDTA and then allowed to coagulate. Blood samples were taken from fish in triplicates on the last day of the experiment. The blood samples that were collected with EDTA were used to determine the hematological indices (Hb, RBC, WBC, and PCV) while the coagulated blood were used for the blood serum (aspertate transaminase, alanine transminase, alkaline phosphate, serum protein, creatinine, blood sugar). The haematological indices of mean cell haemoglobin concentration (MCHC), mean cell volume (MCV), mean cell haemoglobin (MCH) were calculated using the total red blood cell count (RBC), haemoglobin concentration (Hb) and haematocrit (Hct) using the formulae by Sotolu and Faturoti (2011)

MCHC (%) = (Hb/PCV) x 10
MCH (pg) = (Hb/RBC) x 10
MCV (fl) = PCV/RBC x 100

In all cases, blood was collected from the caudal peduncle using plastic disposable syringes

Statistical Analysis

Data on haematological and serum biochemical parameters of were subjected to analysis of variance (ANOVA) using the technique of Obi (1990) and significantly different means were elucidated using Duncan New Multiple range test as outlined by Obi (1990). Differences were considered significant at 5% probability levels ($p<0.05$).

RESULTS

The results in Table 1 show that GCLM contain 10.21% moisture, 21.23% crude protein, 13.77% crude fiber and 5.37% ash. Table 2 shows the percentage composition of the various ingredients used in the feed formulation of the experimental diets. Treatment T_1 contained 0% GCLM and treatments T_2, T_3, T_4 and T_5 contained 4, 14.25, 24.5 and 28.5% GCLM respectively. These represented 0, 25, 50, 75 and 100% of the total protein content in the experimental diets respectively. The proximate composition of experimental diets is presented in Table 2. The result revealed diets were generally iso-nitrogenous. The protein content ranged from 41.13±0.11 to 42.13±0.07% and were not significantly different ($p>0.05$). Similarly, there were no significant difference in NFE in the experimental diets and this parameter ranged from 49.35±2.19 to 50.98±3.55%. The results also show that there was a significant difference ($p>0.05$) in moisture, fat and carbohydrates of the experimental diets.

Table 1. Proximate Composition of *Gomphrena celosiodes* Leaf used in the experiment

Constituents	% Composition
Moisture content	10.21
Crude Protein	21.23
Crude fibre	13.77
Ash	5.37
Fat	4.66
Carbohydrate	44.76

Means and Standard Deviation (SD) were obtained from triplicate samples

Red Blood cell (10^6/ml)

The highest value of red blood cell counts were 6.73 x 10^6/ml for fishes fed with diet containing 0% GCLM and was significantly higher ($p<0.05$) than the red blood cell count in fishes fed diets containing 25, 50, 75 and 100% GCLM. There were no significant differences ($p<0.05$) in red blood cell counts of fishes fed diets containing 25, 50, 75 and 100% GCLM.

Haemoglobin (%)

Hb levels in experimental fish decreased significantly ($p<0.05$) from a value of 10.10±0.15% in blood of fishes fed 0% GCLM to 6.83±0.76% in those fed 100% GCLM. The results of the effect of feeding *Gomphrena celesiodes* leaf meal on haemotological parameters of *Clarias gariepinus* fingerlings fish were presented in Table 3. The effect of feeding *Gomphrena celesiodes* leaf meal on the haemoglobin concentration of fingerlings of *Clarias gariepinus* showed that fishes fed on 25, 50 and 75% GCLM had the following values of 9.60, 8.73 and 8.23% respectively and these values were significantly different ($p<0.05$). These indicate a decreasing trend in Hb value of blood with increase in the quantity of GCLM in the diet.

White Blood Cell (10^6/ml)

Results for the white blood cell counts of fingerlings of *Clarias gariepinus* fed varying levels of GCLM showed that there were significant differences ($p<0.05$) in the white blood cell counts. This result shows that fishes fed 100% GCLM had the highest WBC values of 18.70±0.55x10^6/ml while the lowest value of 13.03±0.02 x10^6/ml was recorded in fishes that were fed the diet containing 0% GCLM. The general trend is that WBC increased in number with increase in the inclusion level of GCLM. However there were no significant differences ($p<0.05$) in WBC in fishes fed 25% and 50% GCLM.

Means and Standard Deviation (SD) were obtained from triplicate samples. Means in the same row with different superscripts are significantly different ($P< 0.05$). Hb = Hemoglobin concentration, PCV = Packed Cell volume, WBC = White Blood Cell, RBC = Red Blood cell, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration Lymphocytes in WBC of experimental fish increased significantly from 2.10±0.21% in control diet to 7.37±5.82% in diet containing 50% GCLM and thereafter decreased significantly to 3.43±0.09% in fish fed on diet T_4 (75% GCLM). Neutrophils in the experimental fish also assumed the same trend as lymphocytes, peaking significantly in fish fed diets containing 50% GCLM. Results showed that there were significant differences ($p<0.05$) in the monocytes of experimental fish.

Monocytes in fish fed 50, 75 and 100% GCLM were not significantly different ($p<0.05$) amongst these diets but was significantly higher ($p<0.05$) than in fishes fed on diets containing 0 and 25% GCLM. Eosinophils and Basophils were not significantly different ($p<0.05$) amongst all the diets.

Packed Cell Volume (PCV%)

The results for the packed cell volume were 30.97% for T_1, 30.00% for T_2, 27.47% for T_3, 25.93% for T_4 and 21.17% for T_5.

Table 2. Percentage (%) composition of the experimental diets

Ingredients	$T_1$0% GCLM	$T_2$25% GCLM	$T_3$50% GCLM	$T_4$75% GCLM	$T_5$100% GCLM
Maize	49.8	49.8	49.8	49.8	49.8
Soybean	28.5	24.5	14.25	4	0
G. C. L. M	0	4	14.25	24.5	28.5
Wheat Offal	11	11	11	11	11
Palm Kernel Cake	5.1	5.1	5.1	5.1	5.1
Fish meal	3	3	3	3	3
Bone meal	1	1	1	1	1
Vitamin Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.1	0.1	0.1	0.1	0.1
Salt	0.25	0.25	0.25	0.25	0.25
Palm oil	1	1	1	1	1
Chemical Composition of diets					
Moisture	$4.67^{b}\pm0.11$	$4.89^{a}\pm0.25$	$4.66^{b}\pm0.65$	$4.91^{a}\pm0.98$	$4.12^{c}\pm0.41$
Crude fiber	$2.04^{b}\pm0.32$	$1.99^{b}\pm0.65$	$2.21^{a}\pm0.11$	$1.14^{c}\pm0.76$	$1.17^{c}\pm0.56$
Crude protein	$41.13^{a}\pm0.11$	$41.17^{a}\pm0.15$	$42.19^{a}\pm0.08$	$41.99^{a}\pm0.09$	$42.13^{a}\pm0.07$
Ash	$2.66^{a}\pm0.98$	$1.36^{c}\pm0.56$	$1.14^{c}\pm0.88$	$0.86^{d}\pm0.45$	$1.91^{b}\pm0.66$

Means and Standard Deviation (SD) were obtained from triplicate samples. Means in the same row with different superscripts are significantly different (P< 0.05).

Table 3. Haematological responses of *Clarias gariepinus* fingerlings fed on varied levels of *Gomphrena celesiodes*

PARAMETERS	T_1 0% (GCLM)	T_2 25% (GCLM)	T_3 50% (GCLM)	T_4 75% (GCLM)	T_5 100% (GCLM)
RBC ($x10^6$/ml)	$6.73^{a} \pm 0.35$	$5.20^{b}\pm 0.15$	$5.87^{b}\pm 0.29$	$5.97^{b}\pm 0.29$	$5.47^{b}\pm 0.18$
WBC ($x10^6$/ml)	$13.03^{d}\pm0.02$	$15.67^{bc}\pm0.13$	$15.63^{b}\pm0.75$	$16.27^{ab}\pm1.44$	$18.70^{a}\pm0.55$
Hb (%)	$10.10^{a}\pm0.15$	$9.60^{ab}\pm0.23$	$8.73^{bc}\pm0.48$	$8.23^{c}\pm0.19$	$6.83^{d}\pm0.76$
Lymph (%)	$2.10^{d}\pm0.21$	$2.50^{d}\pm0.89$	$3.87^{a}\pm5.82$	$3.43^{c}\pm0.09$	$4.80^{b}\pm0.76$
Neutro (%)	$6.97^{d}\pm0.09$	$8.33^{b}\pm0.54$	$9.10^{a}\pm0.86$	$8.27^{b}\pm0.90$	$8.67^{b}\pm0.33$
Mono (%)	$1.80^{b}\pm0.06$	$1.93^{b}\pm0.09$	$2.47^{a}\pm0.26$	$2.40^{a}\pm0.43$	$2.83^{a}\pm0.33$
Eosin (%)	$1.37^{a}\pm0.09$	$1.43^{a}\pm0.23$	$1.43^{a}\pm0.22$	$1.37^{a}\pm0.12$	$1.23^{a}\pm0.03$
Baso (%)	$0.80^{a}\pm0.56$	$1.00^{a}\pm0.12$	$0.97^{a}\pm0.09$	$0.80^{a}\pm0.06$	$0.90^{a}\pm0.06$
PCV (%)	$30.97^{a}\pm0.99$	$30.00^{ab}\pm0.58$	$27.47^{bc}\pm1.57$	$25.93^{c}\pm0.41$	$21.17^{d}\pm0.76$
MCV (%)	$46.13^{b}\pm1.67$	$57.85^{a}\pm2.72$	$46.88^{b}\pm3.04$	$43.73^{b}\pm2.78$	$38.78^{c}\pm1.69$
MCH (%)	$15.07^{b}\pm0.65$	$18.52^{a}\pm0.96$	$14.91^{a}\pm0.93$	$13.89^{b}\pm0.96$	$12.51^{b}\pm0.60$
MCHC (%)	$32.65^{a}\pm0.57$	$31.99^{a}\pm0.16$	$31.80^{a}\pm0.06$	$31.74^{a}\pm0.24$	$32.26^{a}\pm0.38$
FWT (%)	$43.80^{a}\pm4.26$	$26.07^{b}\pm1.39$	$26.30^{b}\pm3.26$	$24.60^{b}\pm0.38$	$22.37^{a}\pm0.48$

Means and Standard Deviation (SD) were obtained from triplicate samples. Means in the same row with different superscripts are significantly different (P< 0.05). Hb = Hemoglobin concentration, PCV = Packed Cell volume, WBC = White Blood Cell, RBC = Red Blood cell, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration.

There was a significant difference ($p<0.05$) among the treatment means. Treatments containing 0% GCLM (T_1 control diet) inclusion had the highest value of PCV while treatment 5 containing (100% GCLM) inclusion had the lowest value of PCV thus showing a significant decrease ($p<0.05$) in PCV with increase the GCLM in the experimental diet.

***Mean Corpuscular Volume* (MCV %)**

The MCV value of 57.85±2.72% recorded in fish fed diet containing 25% GCLM was significantly higher ($p<0.05$) than in the other treatments. MCV values for fish fed 0, 50 and 75% GCLM recorded MCV values that were significantly higher ($p< 0.05$) than was recorded for fish fed 100% GCLM.

***Mean corpuscular haemoglobin* (MCH%)**

Values obtained for the MCH were 15.07±0.65% for T_1, 18.52±0.96% for T_2, 14.91±0.93% for T_3, 13.89±0.96% for T_4 and 12.51±0.60% for T_5. There were significant differences ($p<0.05$) among the various treatment with respect to MCH. Fish fed with diet containing 25% GCLM had the highest value (18.52±0.96) while those on diets containing 100% GCLM had the lowest value of 12.51±0.60.

Mean Corpuscular Haemoglobin Concentration (%)

Mean Corpuscular Haemoglobin Concentration in fish fed with diets containing varying levels of GCLM were 32.65% (T_1), 31.99% (T_2), 31.80% (T_3), 31.74% (T_4), and 32.26% (T_5). There were no significant differences ($p>0.05$) among the treatment means with respect to MCHC values.

Table 4. Serum biochemistry of *Clarias gariepinus* fingerlings fed on varying levels of *Gomphrena celosiodes* leaf meal

PARAMETERS	$T_1$0% (GCLM)	$T_2$25% (GCLM)	$T_3$50% (GCLM)	$T_4$75% (GCLM)	$T_5$75% (GCLM)
Blood sugar (mg/dl)	$72.73^a \pm 0.64$	$71.47^a \pm 1.41$	$70.97^a \pm 1.17$	$66.40^b \pm 1.30$	$60.40^c \pm 1.36$
Cholesterol (mg/dl)	$83.40^a \pm 0.62$	$86.10^a \pm 0.12$	$78.30^b \pm 1.13$	$78.27^b \pm 1.13$	$66.53^c \pm 0.92$
Urea (mg/dl)	$12.53^c \pm 0.12$	$15.23^a \pm 0.29$	$13.53^b \pm 0.84$	$12.80^c \pm 0.15$	$12.80^c \pm 0.42$
Creatinine (mg/dl)	$0.12^a \pm 0.01$	$0.11^a \pm 0.01$	$0.12^a \pm 0.02$	$0.13^a \pm 0.03$	0.12 ± 0.42
ALT(U/I)	$50.30^d \pm 0.91$	$65.47^a \pm 6.86$	$46.53^e \pm 6.65$	$55.50^c \pm 9.72$	$62.43^b \pm 3.32$
AST(U/I)	$25.31^c \pm 0.66$	$28.40^b \pm 2.14$	$24.93^c \pm 1.54$	$28.53^b \pm 1.44$	$30.4^a \pm 0.18$
Alkaline phosphate (ALP) (U/I)	$56.27^b \pm 0.98$	$54.33^b \pm 6.86$	$46.53^c \pm 6.65$	$55.5^b \pm 9.72$	$62.43^a \pm 3.32$
Total Protein (mg/dl)	$7.27^c \pm 0.15$	$7.17^c \pm 0.05$	$7.23^c \pm 0.22$	$8.23^b \pm 0.38$	$9.33^a \pm 0.09$

Means and Standard Deviation (SD) were obtained from triplicate samples. Means in the same row with different superscripts are significant difference ($p<0.05$) while similar super scripts are not ($p>0.05$).

Serum biochemistry

The results of the effect of feeding *G. celesiodes* leaf meal on serum biochemistry of *C. gariepinus* fingerlings fish are represented in Table 4.

Blood Sugar (mg/dl)

Blood sugar concentration in the serum of *C. gariepinus* fingerlings ranged from 72.73± 0.64 mg/dl in fish fed diets with 0% GCLM to 60.40±1.36 mg/dl in fish fed 100% GCLM. There were no significant differences ($p>0.05$) in blood sugar of fishes fed on T_1, T_2 and T_3. However, blood sugar levels in fish fed on diets containing 75-100% GCLM was significantly lower ($p>0.05$) than fishes fed on 0-50% GCLM.

Cholesterol (mg/dl)

The cholesterol concentrations was 83.40±0.62mg/dl in fishes fed on 0% GCLM and was not significantly different ($p>0.05$) from 86.10±0.12mg/dl for those fed 25% GCLM. These cholesterol levels were significantly higher ($p>0.05$) than was recorded for fish fed diets containing 50-100% GCLM. The concentration of cholesterol in blood of fishes fed experimental diets decreased significantly ($p>0.05$) with increase in the quantity of GCLM in the diet.

Urea (mg/dl)

The urea results were 12.53mg/dl for fishes fed diet T_1, 15.23mg/dl, for those fed diet T_2, 13.53mg/dl, for fishes fed on diet T_3 and 12.80mg/dl for those diet T_4 and 12.80mg/dl for T_5. Urea concentrations in fishes fed diets T_1, T_3, T_4 and T_5 were not significantly different ($p>0.05$) from each other but were significantly lower than that in fishes fed on diet T_2. Means and Standard Deviation (SD) were obtained from triplicate samples. Means in the same row with different superscripts are significant difference ($p<0.05$) while similar super scripts are not ($p>0.05$).

***Creatinine* (mg/dl)**

The values for creatinine in experimental fish are presented in Table 4. The results were 0.12 mg/dl for T_1, 0.11 mg/dl for T_2, 0.12 mg/dl for T_3, 0.13 mg/dl for T_4, and 0.12 mg/dl for T_5 and there were no significant differences ($p>0.05$) in creatinine concentration amongst the various treatments.

***Alanine Transaminase* (ALT** U/I**)**

The ALT values of the fish were 25.37, 28.00, 24.73, 28.53 and 30.4U/I in fishes fed diets T_1, T_2, T_3, T_4 and T_5 respectively. There were significant differences ($p>0.05$) among the treatment groups in ALT values of the experimental fish.

***Aspertate Transaminase* (AST** U/I**)**

AST concentrations in the blood of experimental fish fed on the following experimental diets T_1, T_2, T_3, T_4 and T_5 were significantly different ($p>0.05$) and ranged from 24.93 U/I in fishes fed diet T_3 to 30.4±0.18 U/I in fishes on diet T_5. There were significant differences ($p>0.05$) among the treatment groups.

***Alkaline Phosphate*(ALP** U/I**)**

Blood serum of *C. gariepinus* fed on the following experimental diets T_1, T_2, T_3, T_4 and T_5 contained the following concentrations of alkaline phosphate 56.27, 54.33, 46.53, 46.53, 55.50 and 62.43U/I respectively. The alkaline phosphate concentration in blood of fish fed with a diet containing 100% GCLM was significantly higher ($p>0.05$) than the concentrations in fish fed on other diets.

Total Protein

Total Protein concentrations in blood of experimental fish fed on the control diet T_1 was 7.27mg/dl, T_2 7.17mg/dl, $T_3$7.23mg/dl, T_4 7.23mg/dl, and T_5 7.33mg/dl. There were no significant difference ($p>0.05$) among the treatment groups in total protein values of the fish.

DISCUSSION

The protein level as recorded for *Gomphrena celosiodes* in this study is comparable to a protein level of 18.4% in baobab seeds (Anene *et al.*, 2012). It is also comparable with the level 23.57% in sweet potato (*Ipomoea batatas*) and this was found to satisfy dietary protein requirements in *Tilapia zilli* fingerlings (Adewolu, 2008).Aduku (1993) asserted that any feed ingredients that contain crude protein at above

13.77% can be a good protein source for fish fingerlings. Therefore the proximate quality of the amounts of protein in *G. celesiodes* and the experimental diets in this study conform to the recommended optimum dietary protein level of fish Luquet (1991). The RBC and Hb values in this study indicate that experimental fish were not anaemic and compare favorably with literature on RBC and Hb in *C. gariepinus* (Adeyemo *et al*., 2003; Adewoye, 2010; Anyanwu *et al*., 2011; Ozovehe, 2013). The trend in RBC in this study shows that inclusion of GCLM in the diet of experimental fish reduced the number of RBC in blood of fish but a further increase (25, 50, 75 and 100%) did not similarly reduce the red blood cell count. Results of this study show that there is a decrease in PCV and Hb concentration in blood of experimental fish with increase in the inclusion rate of GCLM in the experimental diet. Similar response patterns in PCV have been reported for *C. gariepinus* fed on sesame leaf meal, jack bean seed meal, (Osuigwe *et al*., 2005; Omitoyin, 2006, Fagbenro *et al*., 2013; Ozovehe, 2013) and this decrease may be attributed to the presence of anti-metabolites in the diets. The principal function of red blood cells is in the distribution of oxygen and nutrients via the blood flow in the circulatory systems to all tissues and organs in animals including fish. This function is enhanced by the presence of haemoglobin an iron containing bio-molecule thus the fortunes of RBC in experimental fish is linked to that of Hb. Since the RBC was not reduced by the addition further inclusion of GCLM the observed reduction in Hb in the same circumstances may be due to some other factor which is unrelated to the physiological functions of the RBC in fish tissues.

Even though there were no significant differences in WBC of experimental fish fed on diets containing 25% and 50% GCLM, the general trend is that WBC increased in number with increase in the inclusion level of GCLM. White blood cells are important in the disease fighting capacity of fish. Low WBC is a decrease in disease-fighting cells (leukocytes) circulating in the blood. Thus the increase in WBC with increase in the level of GCLM in the diet may be due to an increase in the defense mechanism of the fish to tolerate the presence of sub-lethal concentrations of anti-nutritional factors in the leaf meal. It has been observed (Ates *et al*., 2008) that increase in WBC during acute and sub-lethal treatment may be due to stimulated lymphomyeloid tissue as a defense mechanism of the fish to tolerate the toxicity. Eosinophils and Basophils were not significantly different ($P<0.05$) amongst all the diets.

The blood sugar levels in *C. gariepinus* in this study ranged from 60.40 to 72.73 mg/dl and were comparable to some literature reports (Shakoori *et al.,* 1996; Akintayo, 2008). However other reports (Tavares-Dias, 2000; Yilmaz *et al*., 2006) on blood sugar level in *C. gariepinus* were higher than is being reported in his study. These differences in blood sugar levels may be due to differences in the chemical composition of the diets. The blood sugar levels in experimental fish fed diets T_3 and T_4 which contained higher quantities of GCML were significantly lower than those fed diets T_1 and T_2. Thus increase in GCML may have accelerated metabolism of glucose in the experimental fish fed diets T_3 and T_4 and may also be an index of the absence of sub-lethal stress at those levels of inclusion of GCML (Connors *et al*., 1978). The concentration of cholesterol in blood of fishes fed experimental diets were relatively low when compared with literature (Yilmaz *et al*., 2006; Ezenwaji, *et al*., 2012) and decreased significantly ($p>0.05$) with increase in the quantity of GCLM in the diet. This observation was not in tandem with observations made by Yilmaz *et al*., (2006), Ezenwaji, *et al*., (2012). These researchers had variously reported increases in cholesterol level with increase in a dietary components. *G. celesiodes* contains more fiber than both energetic diets and bioactive yeast and this factor may have accounted for the depressed cholesterol level that was recorded in this study. Intakes of 9 to 16.5 g/day of a variety of soluble fibers have been shown to produce net reductions in serum cholesterol levels in humans (Rubin, 2011) and may also be applicable in fishes. Without evidence from literature we can further suggest that decreasing levels of cholesterol as recorded in this study may be due to the fact that experimental diets were low in saturated fat.

Transaminases (aspertate transaminase, alkaline phosphate and alanine transaminase) are important enzymes for monitoring the health status of fish (Racicot *et al*., 1975). Yilmaz *et al.* (2006) reported that dietary energy level influenced all enzyme activities in *C. gariepinus* fed on energetic diets. Ozovehe (2013) also reported that there was an increase in the activities of serum enzymes in *C. gariepinus* fed on Morienga leaf meal. Our results is in tandem with these observations and illustrates that the plasma activities of ALT, AST and ALP increased significantly ($p>0.05$) among experimental fishes fed increasing quantities of GCML. Decreases in transaminases may suggest leakage of enzymes from the liver into the serum, inefficient liver function, excessive fat digestion and possibly a diet low in nitrogen. The experimental diets used in this study were not deficient in protein and nitrogen hence it is possible that that inefficient liver function may have accounted for the increase in transaminases recorded in this study.

The serum total protein value in *C. gariepinus* as reported in (Adeyemo *et al*., 2003, Ezenwaji *et al*., 2012) was lower than values of 7.17-7.33mg/dl obtained in the present study which was comparable to values in some literature (Tavares-Dias, 2000, Adeyemo *et al*., 2003). Literature that report values higher than what is being reported in this study is also available (Ayoola, 2011). Environmental conditions of rearing facilities and handling have been shown to influence blood protein levels and may account for the variances in total plasma protein records (Ayoola, 2011). Increase in GCML level in the diets did not result in increase in the total protein in blood thus it can be inferred that the ability of the liver in deamination of protein in experimental diets was not compromised.

Conclusion

The amount of protein recorded in leaves of *G. celesiodes* is considered to be substantial and qualifies as commendable protein supplement in fish feed formulations. This study also highlights the fact that all the experimental diets containing various quantities of GCML were acceptable to *C. gariepinus*. Consequently, *G. celosiodes* could be substituted with soy bean in *C. gariepinus* diet without any negative effect on the haematological and blood indices that were studied.

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