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Some Haematological Responses of Fingerlings of *Clarias gariepinus* Fed Different Dietary Levels of Boiled *Mucuna cochichinensis* Seed Meal (BMSM)

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Abstract: The aims of this study, include to assess the haematological responses of *Clarias gariepinus* fed diets containing Boiled Mucuna Seed Meal (BMSM). The seed meal was incorporated in diets at 0, 25, 50, 75 and 100% of experimental diets. All diets were iso-nitrogenous (39.66-40.29% crude protein). Fingerlings of *Clarias gariepinus* with initial mean weight of 10.15±1.54 g were fed *ad lib.* on allotted diets twice per day at 08.00 and 18.00 h for 60 days. The experiment was a complete randomized design. Fishes fed 25% BMSM had highest white blood cell count values of $15.93\pm0.23\times10^6$ mL^{-1} while the lowest value of $6.00\pm0.28\times10^6$ mL^{-1} was recorded in fishes that were fed with the diet containing 100% BMSM. Haemoglobin (Hb) levels in experimental fish fed experimental diets containing 25 and 50% BMSM was 6.53±0.38 and 6.40±0.67%, respectively and significantly decreased to 4.25±0.05 in fishes fed diets with 100% BMSM. The results for the packed cell volume were 36.3±0.39% for fish fed on control diet, 26.23±0.78% for T_2, 26.55±0.71% for T_3, 23.88±0.41% for T_4 and 17.05±0.09% for T_5. Values obtained for Mean Cell Haemoglobin (MCH) were 17.71±1.09% for T_1, 19.32±0.97% for T_2, 20.78±1.72% for T_3, 18.62±1.08% for T_4 and 23.88±0.36% for T_5. Protein concentrations in blood of experimental fish fed the control diet T_1 was 4.03±0.87 and 3.53±0.54 mg dL^{-1} in T_2. In T_3 and T_4 total protein concentrations were 2.90±0.04 and 3 mg dL^{-1} and 2.58±0.35, respectively. Urea results were 20.13±2.36 mg dL^{-1} for fishes fed diet T_1, 19.43±2.17 mg dL^{-1}, for those fed diet T_2, 20.50±1.16 mg dL^{-1} for fishes fed diet T_3 and 21.35±2.07 mg dL^{-1} for those diet T_4 and 21.90±1.67 mg dL^{-1} for T_5. Alanine transaminase values of the fish were 8.33±1.23, 8.20±1.44, 7.3±1.65, 8.05±0.88 and 10.15±1.13 U I^{-1} in fishes fed diets $T_{1\text{-}5}$, respectively. Aspertate transaminase concentrations in the blood of experimental fish fed the following experimental diets T_1-T_5, respectively. Blood serum of *C. gariepinus* fed on the following experimental diets T_1-T_5 contained the following concentrations of alkaline phosphate 25.43±2.33, 26.20±1.58, 25.83±1.76, 24.55±1.87 and 24.30±1.99, respectively.

Key words: Phosphate, white blood cells, red blood cells, concentration, blood serum

INTRODUCTION

In view of the rising cost of fish feed constituents, development of fish feed technology has shifted towards the use of non-conventional protein feed stuff. Many constituents have been successfully used as protein sources in fish feed feeds. These include leaves of *Gomphrena celosiodes* and broad bean (Magouz *et al*., 2008; Anene *et al*., 2014), termites, garden snail meal, maggot, un-skinned, dried tadpole meal (Sogbesan and Ugwumba, 2008), jack bean *Canavalia ensiformis* (Jimoh *et al*., 2010).

Legumes are one of the most widely used non-conventional protein sources for animal/fish feeds. The wild legume, Mucuna consisting of over 100 varieties has shown to have good nutritional qualities that qualify it, as a good protein source in animal feed. Many leguminous plants including Mucuna are toxic to animals because of the presence of anti-nutritional factors which include protease inhibitors, cyanogenic glycosides, tannins phytates, canavanine, saponines, etc. (Kakade *et al*., 1969). Hydrothermal treatments, fermentation and germination have been shown to be most effective in reducing the anti-nutrients of Mucuna seeds. Several anti-nutritional compounds of Mucuna seeds serve in health care and considerable interest has been drawn towards their antioxidant properties and potential health benefits.

Mucuna seeds are also known to consist of high protein, high carbohydrates, high fiber, low lipids and

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adequate minerals and meet the requirement of essential amino acids. The seeds also possess good functional properties and *in vitro* protein digestibility. Mucuna could be characterized, as being medium in protein and low in ether extract high in nitrogen-free extract, low to medium in crude fiber and low in ash (Ukachukwu and Obioha, 1997). Ukachukwu *et al.* (1999) reported gross metabolizable energy value of 4.60 and 1.05 kcal g^{-1}, respectively. It is agreed that the nutritive value of Mucuna beans is similar to that of soya beans. Also, the seed is deficient in the sugar containing amino acids.

Mucuna compares well with other little known legume crops such as jack bean (*Canavalia ensiformis*) and yam bean (*Sphenostylis sternocarpa*). It also compares well with other little known legume of West Africa, such as kidney bean (*Phaseolus vulgenis*), lima bean (*Phaseolus linatis*), pigeon pea (*Cajanus cajan*) and Bambara nut (*Voandzein subterranean*) (Ologhobo, 1992; Ukachukwu and Obioha, 1997). It is reported that Mucuna seeds contain level, as low as 13.9 and 1.88 ppm of vitamins. It also contains low amount of calcium, phosphorus, magnesium and sodium (Ukachukwu and Obioha, 1997).

This study was carried out to assess the potentials of Boiled Mucuna Seed Meal (BMSM) 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 Boiled Mucuna Seed Meal (BMSM) based diets replacing soybean meal, as a protein source. Indices that were used for assessment include haematological, serum biochemistry indices including Haemaglobin (Hb), Red Blood Cell Count (RBC), White Blood Cell Count (WBC) and Packed Cell Volume (PCV), aspertate transaminase, alanine transminase, alkaline phosphate, serum protein, creatinine, blood sugar. The effect of the BMSM on the proximate composition of fillets of fish fed experimental diets was also assessed.

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. About 300 fingerlings of *C. gariepinus* fish were obtained from a private fish farm in Umudike, Abia State, Nigeria. They were transported in 350 L 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%.

Processing of boiled Mucuna seed meal: Raw seeds of *Mucuna cochichinensis* were collected from a local farm in Ikwuano, in Abia State, Nigeria. The seed were soaked for 24 h in water and boiled for 1 h at 105°C without change of water. The Mucuna seeds were oven dried at 60°C before milling. Milling was done using hammer miller. Each of the diet was compounded and mixed separately, as shown in Table 1. The mixture was made into pellets using a machine, sun dried and stored in sack bays for further use. Triplicate determination was made for each treatment to produce Boiled Mucuna Seed Meal (BMSM). The leaf meal was used to formulate 5 iso-nitrogenous (41-42% crude protein) *C. gariepinus* diets replacing soyabean 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). The leaf meal was used to formulate 5 iso-nitrogenous (41-42% crude protein) *C. gariepinus* diets replacing soyabean at 0, 25, 50, 75 and 100% inclusion levels. All experimental diets were analysed for crude protein, crude fiber, moisture and ash according to methods described by AOAC (2000).

Table 1: Proximate analysis of boiled Mucuna seed constituents

Composition	Seed (%)
Moisture	5.34±1.85
Crude protein	30.86±2.45
Ether extract	13.20±2.56
Crude fiber	7.50±1.07
Ash	3.93±0.92
Carbohydrate	39.10±3.44

Stocking/feeding regime: All fish were starved for 24 h prior to the commencement of the experiment. The feeding trial was carried out in 15 transparent plastic tanks of 40 L capacity with water depth of 0.40 m. The tanks were placed on wooden table with a height of 1.4 m. 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 5 iso-nitrogenous (41-42% crude protein) diets formulated with graded levels of inclusion of (0, 25, 50, 75 and 100%) Boiled Mucuna Seed Meal (BMSM). Each diet was fed to apparent satiation to triplicate groups of 10 fingerlings of *C. gariepinus* (mean = 10.15±1.54 g wet body weight) 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 500 L 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 (1 h after feeding) after which water level was topped to maintain a constant 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, 3 fishes from each of the replicates were selected for blood samples. About 2 mL of blood were collected into labeled sterile universal bottle containing a drop of Ethylene Diamine Teracetate (EDTA). A second sample of 3 mL 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 Haemoglotan (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):

$$\text{MCHC}(\%)=(\text{Hb}/\text{PCV})\times 10$$

$$\text{MCH}(\text{pg})=(\text{Hb}/\text{RBC})\times 10$$

$$\text{MCV}(\text{fl})=(\text{PCV}/\text{RBC})\times 100$$

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 Steel and Torrie (1980) and differences between paired means were tested using Duncan New Multiple range test as outlined by Obi (1990). Differences were considered significant at 5% probability levels ($p<0.05$).

RESULTS

The result of the proximate composition of boiled Mucuna seeds are presented in Table 1. The moisture content of Mucuna seeds was 5.34±1.85%, crude protein content was 30.86±2.45%, cruder fiber content of 7.50±1.07%, either extracts level of 13.20±2.56% and ash content of 3.93±0.92%.

Table 2 shows the percentage composition of the various ingredients used in the feed formulation of the experimental diets. Treatment T_1 contained 0% BMSM and treatments T_2-T_5 contained 4, 14.25, 24.5 and 28.5% BMSM, respectively. These represented 0, 25, 50, 75 and 100% of the total protein source in the experimental diets, respectively. Other sources of protein in the test diets were soya bean and fish meal.

The proximate composition of experimental diets is presented in Table 2. The result revealed diets were generally iso-nitrogenous. The protein content ranged from 39.69±2.70 to 40.27±0.99% 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.

Haemaological responses: The haemaological responses of fingerlings of *Clarias gariepinus* fed varying levels of BMSM are presented in Table 3.

White blood cell ($\times 10^6$ mL^{-1}): Results for the white blood cell counts of fingerlings of *Clarias gariepinus* fed varying levels of BMSM showed that there were significant differences ($p<0.05$) in the white blood cell counts. This result shows that fishes fed 25% BMSM had highest WBC values of 15.93±0.23$\times 10^6$ mL^{-1} while the lowest value of 6.00±0.28$\times 10^6$ mL^{-1} was recorded in fishes that were fed with the diet containing 100% BMSM. The general trend is that WBC decreased in number with increase in the inclusion level of BMSM. However, there was no significant difference in WBC in fishes fed 50 and 75% BMSM.

Lymphocytes in WBC of experimental fish decreased significantly from 1.95±0.57% in diet containing 100% BMSM to 3.68±0.35% in diet containing 75% BMSM. The percentage neutrophils in the WBC of *C. gariepinus* fed varying levels of BMSM is also presented in Table 3. Peak neutrophils were recorded in fish fed on diet containing 25% BMSM while the lowest neutrophil level was 1.95±0.57% in fish fed on 100% BMSM and these were significantly different ($p>0.05$). Monocytes in fish fed diets containing 25, 50 and 75 BMSM were not significantly different ($p<0.05$) but was significantly higher ($p<0.05$) than in fishes fed on diets containing 0 and 100% BMSM.

Haemoglobin (%): The effect of feeding BMSM on the haemoglobin concentration of fingerlings of *C. gariepinus* showed that fishes fed control diet were 7.28±0.45%. Similarly, Hb in fish fed diets containing 25 and 50% BMSM had the following values of 6.53±0.38,

Table 2: Percentage (%) composition and proximate composition of the experimental diets

Ingredients	T_1 0% (BMSM)	T_2 25% (BMSM)	T_3 50% (BMSM)	T_4 75% (BMSM)	T_5 100% (BMSM)
Maize	49.8	49.8	49.80	49.8	49.8
Soya bean	28.5	24.5	14.24	4.00	0.00
BMSM	0.00	4.00	14.24	24.5	28.5
Wheat offer	11.0	11.0	11.00	11.0	11.0
Palm kernel cake	5.10	5.10	5.10	5.10	5.10
Fish meal	3.00	3.00	3.00	3.00	3.00
Bone meal	1.00	1.00	1.00	1.00	1.00
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10	0.10
Palm oil	1.00	1.00	1.00	1.00	1.00
Chemical composition of diets					
Moisture	10.36±1.31	9.59±0.78	10.11±1.05	10.72±1.09	9.79v1.16
Crude fiber	4.89±1.17	5.77±0.98	4.78±1.19	5.17±0.81	4.39±0.56
Crude protein	39.69 ± 2.70^{a}	40.19 ± 3.98^{a}	39.66 ± 2.17^{a}	39.89 ± 3.44^{a}	40.27 ± 0.99^{a}
Ash	4.67±0.88	5.6±0.56	5.67±0.96	4.89±0.99	4.4±1.10
Nitrogen free extract	40.39 ± 2.34^{a}	38.85 ± 2.69^{a}	39.78 ± 1.56^{a}	39.33±1.79	41.15±2.10

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 fingerlings of *C. gariepinus* fed varying levels of BMSM

Parameters	T_1 0% (BMSM)	T_2 25% (BMSM)	T_3 50% (BMSM)	T_4 75% (BMSM)	T_5 100% (BMSM)
WBC ($\times10^6$ mL^{-1})	$15.93^{a}\pm0.23$	$9.23^{c}\pm0.85$	$13.05^{b}\pm0.11$	$13.15^{b}\pm0.63$	$6.00^{d}\pm0.28$
Lymph (%)	$2.15^{c}\pm0.11$	$3.12^{ab}\pm0.09$	$2.93^{b}\pm0.11$	$3.68^{a}\pm0.35$	$1.95^{c}\pm0.57$
Neutro (%)	$4.13^{c}\pm0.45$	$7.58^{a}\pm0.66$	$4.65^{b}\pm0.51$	$3.75^{b}\pm0.21$	$1.55^{c}\pm0.33$
Mono (%)	$2.95^{c}\pm0.34$	$5.23^{a}\pm0.28$	$5.48^{a}\pm0.24$	$5.48^{a}\pm0.19$	$2.05^{b}\pm0.79$
Hb (%)	$7.28^{a}\pm0.45$	$6.53^{ab}\pm0.38$	$6.40^{ab}\pm0.67$	$5.68^{b}\pm0.31$	$4.25^{c}\pm0.05$
PCV (%)	$36.3^{a}\pm0.39$	$26.23^{b}\pm0.78$	$26.55^{b}\pm0.71$	$23.88^{c}\pm0.41$	$17.05^{d}\pm0.09$
RBC ($\times10^3$ mL^{-1})	$4.11^{a}\pm0.05$	$3.38^{b}\pm0.28$	$3.08^{c}\pm0.55$	$3.05^{c}\pm0.91$	$1.78^{d}\pm0.84$
MCV (%)	$88.32^{b}\pm2.45$	$77.60^{d}\pm4.11$	$86.20^{c}\pm2.28$	$78.29^{d}\pm2.21$	$95.78^{a}\pm0.66$
MCH (%)	17.71 ± 1.09^{d}	$19.32^{b}\pm0.97$	$20.78^{b}\pm1.72$	$18.62^{c}\pm1.08$	$23.88^{a}\pm0.36$
MCHC (%)	$2.01^{b}\pm0.36$	$2.49^{a}\pm0.08$	$2.41^{a}\pm0.77$	$2.38^{a}\pm0.32$	$2.49^{a}\pm0.28$

Means in the same row with different superscripts are significantly different ($p<0.05$); HBC = 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

and 6.40±0.67% Hb and these values were not significantly different ($p<0.05$). However, Hb level significantly dropped to 4.25±0.05 in fishes fed with 100% BMSM. Generally, these data indicate a decreasing trend in Hb value of blood with increase in the quantity of BMSM in the diet.

Packed Cell Volume (PCV%): The results for the packed cell volume were 36.3±0.39% for fish fed on control diet, 26.23±0.78% for T_2, 26.55±0.71% for T_3, 23.88±0.41% for T_4 and 17.05±0.09% for T_5. There was a significant difference ($p<0.05$) among the treatment means. Treatments containing 0% BMSM (T_1 control diet) inclusion had the highest value of PCV while treatment 5 containing (100% BMSM) inclusion had the lowest value of PCV, thus showing a significant decrease ($p<0.05$) in PCV with increase the BMSM in the experimental diet.

Red blood cell ($\times10^6$ mL^{-1}): The highest value of red blood cell counts were $4.11\pm0.05\times10^6$ mL^{-1} for fishes fed with diet containing 0% BMSM and was significantly higher ($p<0.05$) than the red blood cell count in fishes fed with diets containing 25, 50, 75 and 100% BMSM. There were no significant differences ($p<0.05$) in red blood cell counts of fishes fed on diets containing 50 and 75% BMSM.

Mean Corpuscular Haemoglobin (MCH%): Values obtained for the MCH were 17.71±1.09% for T_1, 19.32±0.97% for T_2, 20.78±1.72% for T_3, 18.62±1.08% for T_4 and 23.88±0.36% for T_5. There were significant differences ($p<0.05$) among the various treatment with respect to MCH. Fish fed with diet containing 100% BMSM had the highest value (23.88±0.36) while those on diets containing 0% BMSM had the lowest value MCH value.

Mean Corpuscular Volume (MCV%): The MCV value of 95.78±0.66% recorded in fish fed diets containing 100% BMSM was significantly higher ($p<0.05$) than in the other treatments.

Serum biochemistry: The results of the effect of feeding BMSM on serum biochemistry of *C. gariepinus* fingerlings fish are represented in Table 4.

Total protein: Total protein concentrations in blood of experimental fish fed control diet T_1 was 4.03±0.87 and

Table 4: Serum biochemistry of *Clarias gariepinus* fingerlings fed on varying levels of BMSM

Parameters	T_1 0% (BMSM)	T_2 25% (BMSM)	T_3 50% (BMSM)	T_4 75% (BMSM)	T_5 100% (BMSM)
Total protein (mg L^{-1})	$4.03^{a}\pm0.87$	$3.53^{b}\pm0.54$	$2.90^{c}\pm0.04$	$2.58^{c}\pm0.35$	$2.55^{c}\pm0.08$
Blood sugar (mg dL^{-1})	$85.98^{a}\pm3.25$	$79.10^{b}\pm1.19$	$76.18^{c}\pm2.41$	$72.68^{d}\pm2.11$	$66.03^{e}\pm1.94$
Urea (mg L^{-1})	$20.13^{b}\pm2.36$	$19.43^{c}\pm2.17$	$20.50^{b}\pm1.16$	$21.3^{ab}\pm2.07$	$21.90^{a}\pm1.67$
Creatinine (gL^{-1})	$0.11^{a}\pm0.07$	$0.14^{a}\pm0.02$	$0.10^{a}\pm0.01$	$0.14^{a}\pm0.01$	$0.15^{a}\pm0.01$
GPT (ALT) (uL^{-1})	$8.33^{b}\pm1.23$	$8.20^{b}\pm1.44$	$7.3^{c}\pm1.65$	$8.05^{b}\pm0.88$	$10.15^{a}\pm1.13$
AST (Got) (uI^{-1})	$11.90^{c}\pm1.45$	$14.50^{a}\pm2.31$	$14.63^{a}\pm2.08$	$11.90^{c}\pm1.11$	$13.30^{b}\pm0.95$
Alkaline phosphate (uI^{-1})	$25.43^{b}\pm2.33$	$26.20^{a}\pm1.58$	$25.83^{ab}\pm1.76$	$24.55^{c}\pm1.87$	$24.30^{c}\pm1.99$

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 ($p>0.05$)

3.53 ± 0.54 mg dL^{-1} in T_2. In T_3 and T_4 total protein concentrations was 2.90 ± 0.04 and 3 mg dL^{-1} and 2.58 ± 0.35, respectively. The value for this parameter in T_5 was 2.55 ± 0.08 mg dL^{-1}. There were significant differences ($p>0.05$) among the treatment groups in total protein values in fish blood.

Blood sugar (mg dL^{-1}): Blood sugar concentration in the serum of *C. gariepinus* fingerlings fed various amounts of BMSM ranged from 66.03 ± 1.94 mg dL^{-1} in fish fed diets with 100% BMSM to 85.98 ± 3.25 mg dL^{-1} in fish fed on 0% BMSM. Blood sugar levels of fish fed on the various diets decreased with increase in the quantity of BMSM in the diet.

Urea (mg dL^{-1}): The urea results were 20.13 ± 2.36 mg dL^{-1} for fishes fed diet T_1, 19.43 ± 2.17 mg dL^{-1}, for those fed on diet T_2, 20.50 ± 1.16 mg dL^{-1}, for fishes fed on diet T_3 and 21.35 ± 2.07 mg dL^{-1} for those on diet T_4 and 21.90 ± 1.67 mg dL^{-1} for T_5. Urea concentrations in fishes fed on diets T_4 and T_5 were not significantly different ($p>0.05$) from each other but were significantly higher than that in fishes fed on diet T_1-T_3.

Creatinine (mg dL^{-1}): The values for creatinine in experimental fish are also presented in Table 4. The results were 0.11 ± 0.07 mg dL^{-1} for T_1, 0.14 ± 0.02 mg dL^{-1} for T_2, $0.10\pm0.$ mg dL^{-1} for T_3, 0.14 ± 0.01 mg dL^{-1} for T_4 and 0.15 ± 0.01 mg dL^{-1} for T_5 and there were no significant differences ($p>0.05$) in concentration of creatinine amongst the various treatments.

Alanine Transaminase (ALT UI^{-1}): The ALT values in experimental fish were 8.33 ± 1.23, 8.20 ± 1.44, 7.3 ± 1.65, 8.05 ± 0.88 and 10.15 ± 1.13 UI^{-1} in fishes fed diets T_1-T_5, respectively. There were significant differences ($p>0.05$) among the treatment groups.

Aspertate Transaminase (AST UI^{-1}): The AST concentrations in the blood of experimental fish fed the following experimental diets T_1-T_5 were significantly different ($p>0.05$) and ranged from $11.90^{c}\pm1.45$ UI^{-1} in fishes fed diet T_1 and T_4 to 14.63 ± 2.08 UI^{-1} in fishes on diet T_5. There were significant differences ($p>0.05$) among the treatment groups.

Alkaline Phosphate (ALP UI^{-1}): Blood serum of *C. gariepinus* fed on the following experimental diets T_1-T_5 contained the following concentrations of alkaline phosphate 25.43 ± 2.33, 26.20 ± 1.58, 25.83 ± 1.76, 24.55 ± 1.87 and 24.30 ± 1.99, respectively. The alkaline phosphate concentration in blood of fish fed a diet containing 100% GCLM was significantly lower ($p>0.05$) than the concentrations in fish fed on other diets.

DISCUSSION

The protein level of boiled seeds of *Mucuna cochinchinensis* used in this study was $30.86\pm2.45\%$. Protein content of species of Mucuna have been severally reported and values reported and the content are as varied. Superior levels of protein ranging from 33-38% has been reported for 6 different species of Mucuna seeds (Adebowale *et al*., 2005). In a study of seed characteristics and nutrient and anti-nutrient composition of 12 Mucuna accessions from Nigeria (Ezeagu *et al*., 2003) recorded protein values ranging from 24.50-29.79%. Similarly Fathima *et al*. (2010) recorded little variation and contain higher crude protein ranging from 28.80 g/100 g to 32.48 g/100 g which are in tandem with protein level being reported in the present dispensation. Other researchers (Ezeagu *et al*., 2003; Adebooye and Phillips, 2006; Tuleun *et al*., 2008) have also recorded protein levels lower in different species of Mucuna seed than in the present study. Many factors account for the wide variation in the protein content of Mucuna, chief amongst them are species variation and processing methods. Heat processing has been shown to affect seed nutrients contents of Mucuna seeds either by enhancing its crude protein content (Dahouda *et al*., 2009) or by decreasing it (Iorgyer *et al*., 2009). This study has further confirmed the potentials of boiled *Mucuna cochinchinensis*, as a suitable source of protein for animal feeds.

The trend in RBC, PVC and Hb levels decreased significantly ($p<0.05$) with increasing dietary BMSM such that experimental fish fed diets containing 0% BMSM had the highest value of 7.28±0.45 that was significantly higher ($p<0.05$) than the value obtained from fish fed other diets. In the same vein, PVC was highest in blood of fish fed on the control diet (T_1) with a value of 36.3±0.39% and was significantly higher ($p<0.05$) than values for other experimental fish fed other diets. Osuigwe *et al*. (2005), Omitoyin (2006), Fagbenro *et al*. (2007) and Ozovehe (2013) similarly reported decreasing trends in RBC, PVC and Hb levels and attributed this decrease to the presence of anti-metabolites in the diets. Tuleun *et al*. (2008) reported the presence of anti-nutrient factor including L-3, 4 dihydroxyphenylalanine (L-Dopa), tannin, phytate, oxalate and saponin. Except for l-DOPA, all anti-nutritional factors are reported to be heat-labile and can be destroyed by boiling as was applied in this experiment. Consequently, l-DOPA in the test diet may have accounted for the depression in haematological factors in *C. garieinus* observed in this study.

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.

The serum total protein in experimental fish fed on graded quantities of BMSM in this study are slightly lower than was reported by Adeyemo *et al*. (2003), Yilmaz *et al*. (2006) and Ezenwaji *et al*. (2012). Protein in the serum of fish here studied varied significantly, decreasing with increase in BMSM in the diet. It can be inferred that the ability of the liver in deamination of protein in experimental diets was not compromised by increase in BMSM level in the diets, since such increases did not result in increase in the total protein in blood.

The blood sugar levels in *C. gariepinus* in this study ranged from 85.98±3.25 to 66.03±1.94 mg dL^{-1} and were relatively higher than what is available in some literature reports (Shakoori *et al*., 1996; Akintayo *et al*., 2008). However, Tavares-Dias (2000) and Yilmaz *et al*. (2006) reported blood sugar levels ranging from 153-208 mg dL^{-1} in *C. gariepinus* which are considerably higher than the findings. Some other researchers have reported that blood serum glucose ranges 25-350 mg dL^{-1} (Connors *et al*., 1978; Shakoori *et al*., 1996; Ezenwaji *et al*., 2012) and attributed such differences to differences in the chemical composition of the diets. Depletion in glucose with increase in dietary level of BMSM may be due to the direct utilization of the sugar in energy generation. Thus, increase in BMSM may have accelerated metabolism of glucose in the experimental fish (Connors *et al*., 1978).

Transaminases (aspertate transaminase, alkaline phosphate and alanine transaminase) are important enzymes for monitoring the health status of fish (Racicot *et al*., 1975). Transaminases are generally enzymes produced by dying or damaged hepatocytes, the major cell type in the liver. As cells are damaged, these enzymes leak out into the bloodstream. ALT value of 10.15±1.13 UI I^{-1} in serum of fish fed on diets with 100% BMSM was significantly higher than values for fish fed on diets containing lower quantities of BMSM. However, ALT value of 8.33±1.23 to 14.50^a±2.31 (control group) was not significantly affected when 25, 50 or 75% of dietary protein was replaced by BMSM. AST value of in serum of 14.63±2.08 UI I^{-1} fish fed on diet T_3 was not significantly higher than a value of 14.50±2.31 UI I^{-1} in serum of fish fed diet T_2. The Alkaline Phosphate (ALP) results show that a value of 26.20±1.58 UI I^{-1} recorded in fish fed diet T_2 was significantly higher than those fed diets T_1, T_4 and T_5. An elevated activity of transaminases in serum of fish is usually associated with dying or damaged hepatocytes, the major cell type in the liver while decreases could suggest leakage of enzymes into the serum (Yilmaz *et al*., 2006; Ezenwaji *et al*., 2012; Ozovehe, 2013). It is important to note that ALT, AST and alkaline phosphate values in *C. gariepinus* fed various amounts of BMSM are lower than has been reported in literature (Yilmaz *et al*., 2006; Ezenwaji *et al*., 2012). Given the comparatively low values of transaminases in experimental fish in this study and it appears that leakage of enzymes into the serum rather than liver damage is a prevailing physiological condition in the *C. gariepinus* fed on various quantities of BMSM.

CONCLUSION

The amount of protein recorded in of BMSM 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 BMSM were acceptable to *C. gariepinus*. Consequently, boiled seeds of *Mucuna cochichinensis* 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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