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EVALUATION OF HAEMATOLOGICAL AND SERUM BIOCHEMICAL CHANGES IN *CLARIAS GARIEPINUS* JUVENILES FED GRADED DIETARY LEVELS OF BOILED SUNFLOWER (*HELIANTHUS ANNUUS*) SEED MEAL REPLACING SOYBEAN MEAL

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ABSTRACT

The effects of boiled sunflower seed meal (BSSM) on the haematological parameters and serum biochemical indices of *Clarias gariepinus* juveniles were investigated for fifteen weeks. Boiled sunflower seed meal was substituted for soybean meal (SBM) at 0%, 20%, 40%, 60%, 80% and 100% in formulating six isonitrogenous and isocaloric diets. The formulated dietary treatments comprised 0% BSSM: 100% SBM, 20% BSSM: 80% SBM, 40% BSSM: 60% SBM, 60% BSSM: 40% SBM, 80% BSSM: 20% SBM and100% BSSM: 0% SBM respectively. Diets were fed twice daily, 07.00 - 08.00 and 17.00 – 18.00 hours, at 5% body weight to 360 *C. gariepinus* juveniles in eighteen rectangular tanks at the rate of 20 juveniles per tank in triplicate treatments. Blood samples collected from fish were analysed for haematological and serum biochemical parameters. Fish fed 40% BSSM inclusion had the highest PCV (31.67%), Hb (10.40 gm/100ml), RBC (8.83 x 10^{12}/mL) and WBC (9.33 x10^{9}/mL) while those fed 60 to 100% BSSM had depressed values of PCV (21.00 to 22.00%), Hb (6.90 to 7.20 gm/100ml), RBC (5.65 x 10^{12}/mL) and WBC (6.33 to 6.57 x10^{9}/mL). Fish fed 40% BSSM had the highest total protein (3.60 ± 0.0 g/100 mL) and globulin (2.57 g/100 mL) while those fed 100% BSSM had reduced protein (2.3 ± 0.0 g/100 mL) and globulin (1.40 g/100 mL). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) varied between 27.67- 46.67 mg/dl and 31.67–72.00 mg/dl respectively and were statistically different ($p < 0.05$). Blood glucose varied between 51.33 and 84.00 mg/dl and fish fed above 20% BSSM inclusion had significant increase in blood glucose level except those fed at 100% BSSM inclusion. This study indicated that boiled sunflower seed meal could replace soybean meal up to 20% BSSM inclusion level in the diet of *Clarias gariepinus* without any deleterious effect on its blood parameters.

Keywords: *Clarias gariepinus*, sunflower seed meal, soybean meal, haematology, serum biochemistry

INTRODUCTION

Fish is the cheapest source of animal protein consumed by the average Nigerian and constitutes about 40% of the total protein intake (Atanda, 2007). Fish accounts for about one-fifth of the world's total supply of animal protein and this has risen five-folds within the last 40 years from 20-98 million metric tonnes in 1993 and was projected to exceed 150 million metric tonnes by the year 2010 (Olagunju *et al*., 2007). Aquaculture is one of the fastest growing food production sectors in the world accounting for approximately 50% of fisheries products (FAO, 2010). According to FAO (2006), aquaculture has grown into a multi-billion dollar industry. Rapid growth in the aquaculture industry has helped to alleviate human over-dependence on depleted natural fish stocks. To sustain and boost such high rates of increase in production, a corresponding increase in fish feed production is highly imperative. The high cost, fluctuating quality and uncertain availability of fishmeal have necessitated the need to identify alternative protein sources for fish feed formulation. Therefore, in order to achieve more economically sustainable, environmentally friendly and viable fish feed production, research efforts have been directed towards the evaluation and utilization of non-conventional sources of plant protein such as sunflower seeds.

Fish live in very intimate contact with their environment and are therefore very susceptible to many physical and chemical changes which may be detected in their blood components (Ayoola *et al*., 2014). Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health status of fish (Hrubec *et al*., 2000). In fish, exposure to chemical compounds can induce either increase or decrease in the levels of haematological indices. Since

blood tissues clearly reflect physical and chemical changes occurring in organisms, detailed information can be obtained on general metabolism and physiological status of fish in different groups of age and habitat. The study of physiological and haematological characteristics of cultured fish species is an important tool in the development of aquaculture system, particularly with respect to its use in distinguishing healthy from diseased or stressed fish (O'Neal and Weirich, 2001). Early diagnosis is also possible, when evaluating haematological data, particularly blood parameters (Luskova, 1997).

However, there is a need to understand the physiological concept of fish health in relation to blood and the quality of dietary protein fed. Any changes in the value of a component of a blood sample, when compared to the normal values, could be used to interpret the metabolic state and health status of animal (Babatunde *et al.,* 1992). Low haematological indices are indications of anaemic conditions (Haruna and Adikwu, 2001). In fish, exposure to chemical pollutants can induce either increases or decreases in the levels of haematological indices. Such changes depend on fish species, age, cycle of sexual maturity of spawners and disease (Luskova, 1997). Previous haematological studies on the effect of nutrition (Rehulka, 2000), infectious diseases and pollutants (Rehulka, 2002) revealed that erythrocytes are the major and reliable indicators of various sources of stress (O'Neal and Weirich, 2001).

Cells naturally contain enzymes for their functions such that any damage to cellular membrane leads to their escape into the bloodstream where their presence or activities can be measured as an index of cell integrity (Coppo *et al*., 2002). Analysis of certain serum enzymes could be used to identify tissue damage (Patti and Kulkarni, 1993). Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are enzymes normally found within the cells of the liver, heart, gills, kidneys, muscles and organs (Shalaby, 2009) but their increase in the plasma indicates tissue injury or organ dysfunction (Wells *et al*., 1986). Changes in enzyme profiles are important toxicity indices (bio-markers) and have been used to assess the biochemical and physiological health of vital tissues and organs in fishes (Gabriel and George, 2005).

African mud catfish, *Clarias gariepinus*, is among the most preferred species among fish farmers and consumers in Nigeria because it commands high commercial value in Nigeria and all over Africa. It can tolerate a large variety of feedstuffs and is very resistant to changing and sub-optimal water conditions. It can tolerate excessive crowding, rigours of high transport stress (Inyang, 2008) and can be cultured in high densities reaching production levels of 6 to 16 metric tonnes/hectare annually when raised in monocultures and fed with high quality feed (Faturoti, 1989). The aim of this experiment was to investigate the effects of graded dietary levels of boiled sunflower seed meal on the haematological parameters and serum biochemical indices of *C. gariepinus* juveniles.

MATERIALS AND METHODS

Experimental design and formulation of boiled sunflower seed meal–based diets in replacement for soybean meal

Six isonitrogenous diets (at 40% crude protein level) were formulated and prepared using Pearson's square method. Boiled sunflower seed meal (BSSM) was incorporated in the six diets at graded levels of 0%, 20%, 40%, 60%, 80% and 100% in replacement for soybean meal (SBM) with 0% being the control diet (Table 1). Boiled sunflower seed meal was prepared at 100 ^{0}C for 15minutes in a pressure cooker (Qlink Model No. 9000), oven-dried in a Gallenkamp oven at 60 ^{0}C for 6 hours. Other ingredients included fish meal, groundnut cake, vitamin and mineral premix, bone meal, oyster shell, maize, cassava starch, salt and palm oil. The ingredients were measured and mixed together to formulate a 40% crude protein diet. Each diet mixture was extruded through a 3 mm die pelleting machine (Hobart A-200T GmbH, Rhen-Bosch, Offenbug, Germany) to form noodle-like strands, which were manually crumbled into a suitable size for the *C. gariepinus* juveniles. The pellets were sun-dried, packed in labeled polythene bags and stored in a cool dry place to prevent fungal growth. The gross composition of the experimental diets is shown in table 1 and diet 1 served as the control with no sunflower seed meal supplementation. Six graded

levels (0%, 20%, 40%, 60%, 80% and 100%) of boiled sunflower seed meal (BSSM) were substituted for soybean meal (SBM) in dietary treatments 1, 2, 3, 4, 5 and 6. The six dietary treatments contained the following percentage composition of boiled sunflower seed meal and soybean meal respectively:

Treatment 1: 0% BSSM : 100% SBM
Treatment 2: 20% BSSM : 80% SBM
Treatment 3: 40% BSSM : 60% SBM
Treatment 4: 60% BSSM : 40% SBM
Treatment 5: 80% BSSM : 20% SBM
Treatment 6: 100% BSSM : 0% SBM.

Fish feeding trial

The experiment was carried out using fifteen plastic tanks (60 cm × 45 cm × 30 cm) for 15 weeks in the research laboratory of the Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria. Each tank was supplied with well water up to 70% capacity which was replaced every three days to maintain relatively uniform physico-chemical parameters and prevent fouling from feed residues. The tanks were well aerated using air stones and aerator pumps. There were six dietary treatments and each had three replicates with 20 fish per replicate. The fish were weighed, distributed into experimental tanks and allowed to acclimatize for 14 days before the experiment. The experiment lasted for 15 weeks during which the fish were fed at 5% body weight (in two equal portions of 2.5%) twice daily. Weight changes were recorded weekly and feeding rates adjusted to the new body weight.

Table 1: Gross ingredient composition (g/100 g diet) of boiled sunflower seed meal diets in substitution for soybean meal at graded levels for *Clarias gariepinus* juveniles

Ingredients	BSSM 1 0%(Control)	BSSM 2 (20%)	BSSM 3 (40%)	BSSM 4 (60%)	BSSM 5 (80%)	BSSM 6 (100%)
Sunflower seed meal	____	7.88	15.75	23.63	31.51	39.38
Soybean meal	39.38	31.51	23.63	15.75	7.88	____
Fish meal	20.19	20.19	20.19	20.19	20.19	20.19
Groundnut cake	20.19	20.19	20.19	20.19	20.19	20.19
Yellow maize	14.50	14.50	14.50	14.50	14.50	14.50
Vitamin/mineral premix	2.00	2.00	2.00	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00	1.00	1.00	1.00
Oyster shell	0.50	0.50	0.50	0.50	0.50	0.50
Palm oil	0.75	0.75	0.75	0.75	0.75	0.75
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Cassava starch	1.00	1.00	1.00	1.00	1.00	1.00
Total (%)	100.00	100.00	100.00	100.00	100.00	100.00
Calculated crude protein content (%)	40	40	40	40	40	40

BSSM: Boiled sunflower seed meal

Vitamin/mineral premix:
Vit. A: 1,000,000 IU; Vit. B_1: 250 mg; Vit. B_2: 1750 mg; Vit. B_6: 875 mg; Vit. B_{12}: 2500 mg; Vit. C: 12,500 mg; Vit. D_3: 600,000 IU; Vit. E: 12,000 IU; Vit. K_3: 15 mg; Calcium D-pantothenate: 5000 mg; Nicotinic acid: 3750 mg; Folic acid: 250 mg; Cobalt: 24,999 mg; Copper: 1999 mg; Iron: 11,249 mg; Selenium (Na_2SeO_3. $5H_2O$): 75 mg; Iodine (Potassium iodide): 106 mg; Anti-oxidant: 250 mg.

Blood collection from experimental fish

Ten juveniles and six juveniles per treatment were randomly selected at the beginning and end of the experiment respectively. The fish were anaesthetized with 150 mg/litre of tricane methane sulphonate as described by Wagner *et al.* (1997). A small cut was made in the caudal peduncle using a sharp dissecting blade according to the method of Stoskopf (1992). 8 ml of blood sample was carefully collected from the caudal artery in the caudal peduncle of each fish using sterile disposable 2 ml plastic syringes and needles and mixed with ethylene diamine tetra-acetic acid (EDTA, an anti-coagulant) inside EDTA bottles. The blood samples collected were finally taken to the analytical research laboratory of the

Department of Haematology, Faculty of Veterinary Medicine, University of Ibadan for various haematological and biochemical analyses. Haematological parameters were determined by standard haematological methods as described by Schalm *et al.* (1975).

Haematological assessment of experimental fish

Packed cell volume (Haematocrit): Pre-heparinised capillary tubes were filled up to 2ml (75%) with blood samples from experimental fish by suction pressure and one end of each tube was immediately sealed with plasticine. The tubes were arranged on a tray and centrifuged for 5 minutes in a micro-haematocrit centrifuge at 12,000 r.p.m. Packed cell volume (PCV) was read by means of a haematocrit reader (UV–VIS Spectrophotometer 108). The results were expressed in percentages (Kelly, 1979). According to Duke (1975), PCV was calculated as:

$$PCV = 100 \frac{(\text{Blood volume} - \text{plasma volume})}{\text{Blood volume}}$$

$$\text{Blood volume} = \frac{\text{Plasma volume} \times 100}{100 - PCV}$$

Haemoglobin (Hb) concentration: Determination of Hb concentration involved the cyanomethaemoglobin methods described by Schalm *et al.* (1975) and Kelly (1979). 0.02 ml of sufficiently mixed blood was added to 4 ml of Drabkin's solution (which is a mixture of 250 mg potassium ferricyanide, 200 mg potassium cyanide and 50 mg potassium dihydrogen phosphate). The resultant mixture (Drabkin's solution) was diluted up to 1 litre mark with distilled water (as the solvent) and pH adjusted to neutral (i.e. pH = 7.0). The entire mixture was left undisturbed for 20 minutes and the haemoglobin concentration was read inside a spectrophotometer (Spectrumlab 23a Model) at 540 nm wavelength.

Red blood cell (RBC) and white blood cell (WBC) counts: Counting of the blood cells was carried out by means of Neubauer haemocytometer as described by Kelly (1979). The number of red blood cells was determined by diluting (at ratio 1:200) each blood sample collected with Dacies fluid (a mixture of 99 ml of 3% aqueous solution of sodium citrate and 1 ml of 40% formaldehyde) which maintained the normal shape of the red blood cells. The number of white blood cells was determined by diluting (at 1:200, i.e. at the same ratio as for red blood cells) the blood sample with 3% aqueous solution of acetic acid and then gentian violet was added. 1 ml of the mixture was dropped on a microscope slide and labeled according to the dietary treatments. A binocular light microscope was used for counting red and white blood cells from $x10^6$ d/litre and $x10^3$ d/litre respectively.

Mean corpuscular volume (MCV): This refers to the mean volume of red blood cells in a blood sample and was determined according to the method used by Oyelese *et al.* (1999) as follows:

$$MCV\ (\mu g/ml) = \frac{\text{Volume of red blood cells in ml per 100 ml blood} \times 100}{\text{Number of red blood cells per 100 ml blood}}$$

Mean corpuscular haemoglobin concentration (MCHC): This was calculated from the relationship between haemoglobin concentration and number of red blood cells per 100 ml blood as follows:

$$MCHC\ (g/100ml) = \frac{\text{Haemoglobin concentration} \times 100}{\text{Number of red blood cells per 100 ml blood}}$$

Serum biochemical parameters: These were determined using standard analytical methods: serum protein (total protein, albumin and globulin), serum sodium, serum potassium, blood glucose and serum enzymes [creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST)].

Data analysis

All data obtained in this study are presented as mean ± standard deviation. Comparisons were made between the control and experimental groups. One-way ANOVA and Duncan's multiple range test (Duncan, 1955) were used on SPSS statistical software (Version 16.0 for Windows; SPSS Inc., Chicago, USA) to detect the significant differences among the control and experimental groups. Differences were considered to be statistically significant at probability levels below 0.05 (i.e. $p<0.05$).

RESULTS AND DISCUSSION

Haematological indices of *Clarias gariepinus* juveniles fed graded levels of boiled sunflower seed meal-based diets

Table 2 shows the haematological indices of *C. gariepinus* juveniles fed graded levels of boiled sunflower seed meal-based diets replacing soybean meal for 15 weeks (Figure 1). Packed cell volume (PCV) or haematocrit value increased from a mean initial value of 20.67% to final values ranging from 21.0% (in the fish fed 60% and 100% BSSM diets) to 31.67% (in the fish fed 40% BSSM diet). The result closely agrees with Korzhuev (1964) who stated that fish haematocrit values ranged between 20% and 35% and rarely attain values greater than 50%. These values are fairly comparable with 27.58 - 35.50% documented by Musa and Omoregie (1999) and 36.0% reported by Adeyemo (2007). Ajiboye (2009) reported PCV values ranging between 29.15% and 37.61% in *Synodontis nigrita* fed different dietary crude protein levels. Dienye and Olumuji (2014) also obtained 21.00 to 32.00% which fell within the range of 20 and 50% reported by Pietse *et al.* (1981) and PCV values above 50% are rarely reported (Etim *et al.*, 1999).

Haematocrit values represent the abundance of red blood cells and Blaxhall and Daisley (1973) reported the essence of using haematocrit values to detect or diagnose anaemic condition in fishes. Piotr *et al.* (2014) stated that an increase of PCV can result from increased number of RBC (due to acute stress and spleen evacuation), erythrocyte swelling (due to lower blood pH, respiratory acidosis) or decreased volume of water in the circulating blood (due to muscle tissue acidification following stress exposure or severe exercise). However, Sotolu and Faturoti (2011) reported lower PCV values which was attributed to anaemia resulting from shrunken red blood cells, a situation that probably resulted in fish asphyxiation and death as confirmed by Adeyemo (2005). Blaxhall (1972) associated reduced PCV values to loss of appetite or disease while Adamu and Audu (2008) reported that a significant decrease in PCV may be attributed to gill damage or impaired osmoregulation causing anaemia and haemodilution.

Haemoglobin (Hb) content increased from a mean initial value of 6.80 g/100 ml to final values which ranged from 6.90 g/100 ml in the fish fed 60% and 100% BSSM diets to 10.40 g/100 ml in the fish fed 40% BSSM diet respectively. Hb significantly differed ($p<0.05$) between the fish fed lower inclusion levels ($\leqslant$ 20%) and those fed higher levels ($\geqslant$40%). These values were high and fell within the range of 5.6 to 15.8 g/100 ml reported for *Esox lucius* (Mulcahy, 1970), 8.70 g/100 ml for *C. gariepinus* (Sowunmi, 2003) and also compared favourably with 7.90 to 8.90 g/100 ml reported for *C. gariepinus* fed dietary levels of *Moringa oleifera* leaf meal (Dienye and Olumuji, 2014). The values also fairly corroborate 9.60 g/100 ml recorded by Omitoyin (2006) for African catfish juveniles fed poultry litter and 10.62 g/100 ml reported by Osuigwe *et al.* (2005) who fed *C. gariepinus* with jackbean meal-based diets. Ajiboye (2009) reported Hb values ranging from 4.70 g/100 ml to 7.84 g/100 ml in *Synodontis nigrita* fed different dietary crude protein levels. The present values, however, are higher than 4.46 g/100 ml reported for *Heterotis niloticus* (Fagbenro *et al.*, 2000).

Clark *et al.* (1978) observed that certain physiological stresses elevate haemoglobin levels in fish and that anaemia can be diagnosed by means of very low Hb values. Physiologically, haemoglobin is crucial to fish survival because of its important role in the oxygen-binding and carrying capacity of blood (Etim *et al.*, 1999). The values of haemoglobin concentrations recorded in this study were quite high and can be related to large anaerobic metabolic capacity of *C. gariepinus*. Abidi and Srivastava (1988) reported that an increase in the Hb% may be due to the catalysing actions of pesticides on the incorporation of body iron stored in the haemoglobin. According to Lenfant and Johansen (1972), haemoglobin concentration is higher in fishes capable of aerial respiration. Therefore, the high Hb values indicate the air-breathing characteristic and high activity of *C. gariepinus*.

Red blood cells (RBCs) increased from a mean initial value of 5.62×10^{12}/ml to final values which ranged between 5.65×10^{12}/ml in the fish fed 60%, 80% and 100% BSSM diets and 8.83×10^{12}/ml in

the fish fed 40% BSSM diet. RBCs significantly differed ($p<0.05$) between fish fed lower inclusion levels ($\leqslant$ 20%) and those fed higher inclusion levels ($\geqslant$ 40%). These values are higher than 1.24 x 10^{12}/ml - 1.88 x 10^{12}/ml reported by Smith and Kaplan (1952), 2.11 x 10^{12}/ml - 2.93 x 10^{12}/ml obtained by Ajiboye (2009), $3.01 \pm 0.56 \times 10^{12}$/l for Malaysian snakehead, *Channa striatus* (Lawali *et al.*, 2015) and $3.81 \pm 1.49 \times 10^{12}$/l reported by Owolabi (2011) on *Synodontis membranacea*. The values were also superior to 1.67×10^{12}/l in *Parachanna obscura* (Kori Siakpere *et al.*, 2005) and 1.9×10^{12}/l reported for *Clarias gariepinus* juveniles (Ayoola, 2011). The increased RBC count recorded during the study might be due to the release of new RBCs from the erythropoietic tissue to improve the oxygen-carrying capacity of fish blood with resultant higher values of erythrocyte count as observed by Rottmann *et al.* (1992) and Alkahem *et al.* (1998).

Effects of feeding BSSM-based diets on haematological parameters in *C. gariepinus* included elevated levels of erythrocyte number (RBCs), haemoglobin concentration and haematocrit values (PVC) compared to their respective initial values. These observations might indicate compensatory erythropoiesis which resulted in the production of more RBCs to replace the older ones which were probably rapidly destroyed due to a decrease in the oxygen-carrying capacity of the blood. Increased haematocrit is an indication of a stress response causing RBC swelling or haemo-concentration due to plasma volume reduction (Wilson and Taylor, 1993). Erythrocyte count greater than 1.00×10^{6} mm^{-3} is considered high and indicative of high oxygen-carrying capacity of the blood, which is characteristic of fishes capable of aerial respiration and with high metabolic activity (Jimoh *et al.*, 2012).

Final white blood cell (WBC) counts of *C. gariepinus* juveniles fed BSSM-included diets ranged between 6.33 x 10^{9}/ml (in fish fed 80% BSSM-based diet) and 9.33 x 10^{9}/ml (in fish fed 40% BSSM-based diet) and were generally lower and significantly different from the initial WBC count (11.57 x 10^{9}/ml). Significant differences ($p<0.05$) in WBCs existed between fish fed lower inclusion levels ($\leqslant$ 20%) and those fed higher levels ($\geqslant$ 40%). The WBC values in this study are lower than $22.33 \pm 2.52 \times 10^{9}$ /ml to $31.65 \pm 95.37 \times 10^{9}$ /ml recorded for *Clarias batrachus* (Maheswaran *et al.*, 2008) but were superior to 19.07 ± 1.47 x 10^{3} /mm^{3} obtained for *Cyprinion macrostomus* (Orun *et al.*, 2003), 16.13×10^{3} - 16.39×10^{3} mm^{-3} for *C. gariepinus* (Sotolu and Faturoti, 2011), $6.40 \pm 0.01 \times 10^{3}$ /mm^{3} for *Chalcalbumus mossulensis* (Orun *et al.*, 2003), $1.53 \pm 0.01 \times 10^{6}$ /mm^{3} for *Alburnoides bipunctatus* (Orun *et al.*, 2003) and $1.61 \pm 0.01 \times 10^{6}$/$mm^{3}$ for *Ictalurus punctatus* (Klinger *et al.*, 1996). White blood cells are known to play an important role in the immune system and responses of living organisms. Low WBC count in the fish fed 60%, 80% and 100% BSSM-included diets could be attributed to a reduction in the number of lymphocytes. Alkahem (1994) also reported reduced WBC counts in *Oreochromis niloticus* and associated them with the effects of toxicants and also to a generalised stress response resulting from increased pituitary-interrenal activity. Alkahem (1994) also linked reduced WBC count with a reduction in the number of circulating thrombocytes and lymphocytes due to a diminution in the delivery of lymphocytes to the circulatory system through a reduced lymphocyte production and a rapid destruction of cells which leads to an increased rate of peripheral removal of lymphocytes.

However, Ajani (2006) and Kori-Siakpere *et al.* (2009) stated that high WBC count means a release of more cells to maintain homeostasis while low WBC count is a common stress response. Therefore, increasing or decreasing numbers of WBCs are normal physiological reactions to toxicants and these show the response of the immune system under toxic conditions. Many researchers have reported low values of leukocyte counts in the blood of fish exposed to different pollutants and attributed them to the reduction in the number of circulating lymphocytes and thrombocytes (Alkahem, 1994; Alkahem *et al.*, 1998; Koprucu *et al.*, 2006). This gives a considerable support to the present investigation. Douglas and Jane (2010) demonstrated that their amount has implication in immune responses and the ability of the animal to fight infection. High WBC count is usually associated with microbial infection of the circulatory system (Oyawoye and

Ogunkunle, 1998).

Platelets were highest ($10.0x10^9$/ml) in fish fed 0% to 40% BSSM-based diets and lowest ($8.0x10^9$/ml) in those fed 60% to 100% BSSM-based diets. There were significant differences ($p<0.05$) between fish fed lower inclusion levels ($\leqslant$ 40%) and those fed higher levels ($\geqslant$ 60%). The lack of variation ($p>0.05$) in platelet count of from the initial value to 40% BSSM treatment and from 60% to 100% BSSM treatments suggests that platelets were not affected by the dietary treatments. The reduced platelet count for fish fed 60% to 100% BSSM-included diets may be due to fish reaction to the effect of the presence of anti-metabolites in the diets and this agrees with the findings of Fagbenro *et al.* (2007) and Ozovehe (2013).

Mean corpuscular volume (MCV) ranged between 35.0 µg/ml and 38.67 µg/ml in the fish fed 40% and 80% BSSM diets respectively and these values were very close to the initial value of 37.0 µg/ml. MCV values obtained at higher BSSM inclusion levels (60% - 100%) were significantly higher than those at lower BSSM inclusion levels($\leqslant$ 20%). These values are lower when compared to 79.20 - 105.32 µg/ml reported for *Heteroclarias* (Anyanwu *et al.*, 2011). MCV indicates the status or size of the RBCs and reflects a normal or an abnormal cell division during the production of red blood cells (erythropoiesis). Increase in MCV may be due to the swelling of the erythrocytes resulting in macrocytic anaemia. Larsson *et al.* (1985) attributed the increase in MCV to the swelling of the RBCs as a result of hypoxic condition (low oxygen condition), impaired water balance (osmotic stress) or macrocytic anaemia (swelling of RBCs) in fishes exposed to metal pollution. Reduced MCV could be linked with shrinkage of RBCs either due to hypoxia or microcytic anaemia (shrinkage of RBCs) as earlier reported by Adesina (2008) and Alwan *et al.* (2009).

Mean corpuscular haemoglobin (MCH) values were consistently uniform (12.0 µg/ml) except at 40% BSSM inclusion level (11.0 µg/ml) and were not statistically different. The lack of variation ($p>0.05$) in MCH from the initial value to the control and the other treatments indicated that MCH was not affected by the dietary treatments. These values are lower than 20.82 to 26.60 µg/ml reported by Anyanwu *et al.* (2011) for *Heteroclarias* fed *Carica papaya* leaf meal-incorporated diet and also disagree with Olasunkanmi (2011) who reported a significant increase in the final MCH values in *C. gariepinus* fed raw mucuna seed meal-based diets.

The final values of the mean corpuscular haemoglobin concentration (MCHC) ranged between 32.0 gm/100ml and 33.0 gm/100ml. MCHC value of 33.0 gm/100ml recorded for both 0% and 20% BSSM inclusions were slightly higher and significantly different from 32.0 gm/100ml recorded at the initial stage and at higher inclusion levels (40% - 100%). MCHC followed the same trend as MCH. These values compare fairly well with 33.97% recorded by Adeyemo (2007), 30.70% reported for *C. gariepinus* from Asejire dam (Adedeji and Adegbile 2011) and values ranging between 28.75 and 37.62% recorded for fish fed *M. oleifera* leaf meal-based diet (Dienye and Olumuji, 2014). The results obtained, however, disagree with Olasunkanmi (2011) who reported that the final MCHC values in *C. gariepinus* fed raw mucuna seed meal-based diets dropped below the initial value but were not statistically different among the treatments. The MCHC is a good indicator of red blood cell swelling (Wepener *et al.*, 1992) and a quantitative measurement of mean amount of haemoglobin per erythrocyte in biological organisms (Moses, 2007).

The final values of lymphocytes ranged from 66.0% (in fish fed 0% BSSM) to 82.0% (in those fed 60% BSSM) and were higher and significantly different from the initial value (59.33%). However, the results obtained disagree with the report of Olasunkanmi (2011) who observed no significant difference between the initial and final lymphocyte values in *C. gariepinus* fed diets containing 10%, 20% and 30% raw mucuna meals. The values of lymphocytes in this study compare favourably with 76.49 $\pm$ 10.81% in *Synodontis membranacea* (Owolabi, 2011) and 99.20 $\pm$ 0.83% in *Cyprinus carpio* (Orun *et al.*, 2003) but are higher than 33.00% recorded for juvenile *C. gariepinus* (Adeyemo, 2007). White blood cells (WBC) and lymphocytes are the defense cells of

the body. Douglas and Jane (2010) demonstrated that their amount has implication in immune responses and the ability of the animal to fight infection.

Neutrophils had the highest (33.67%) value in the fish fed 0% BSSM-based diet and least (17.33%) in those fed 60% BSSM-based diet. These values were generally lower and significantly different from the initial value (40.0%). However, the reduced neutrophil counts probably indicated absence of bacterial or any pathogenic infection. Increase in the total neutrophil count has been reported to be a sign of bacterial infection or as a result of stress. The final values of monocytes ranged between 0.5% (in fish fed 40% BSSM) and 2.0% (in fish fed 20% and 60% BSSM-based diets) and were statistically different ($p<0.05$). These values are lower when compared with 23.76 ± 2.84 % reported for Malaysian snakehead (*Channa striatus*) by Lawali *et al.* (2015) and 16.14 ± 8.25% in *Synodontis membranacea* (Owolabi, 2011).

Table 2: Haematological indices of *Clarias gariepinus* juveniles fed graded levels of boiled sunflower seed meal-based diets for 15 weeks

Parameters	Initial values	BSSM 1 0%(control)	Experimental BSSM 2 20%	Dietary BSSM 3 40%	Inclusions BSSM 4 60%	BSSM 5 80%	BSSM 6 100%
PCV (%)	20.67±0.58^{d}	30.00±0.00^{b}	30.00±0.00^{b}	31.67±0.58^{a}	21.00±0.00^{d}	22.00±0.00^{c}	21.00±0.00^{d}
Hb (gm/100ml)	6.80±0.17^{d}	9.90±0.00^{b}	9.90±0.00^{b}	10.40±0.17^{a}	6.90±0.00^{d}	7.20±0.00^{c}	6.90±0.00^{d}
RBCs ($x10^{12}$/ml)	5.62±0.02^{d}	8.24±0.01^{b}	8.23±0.01^{b}	8.83±0.02^{a}	5.65±0.01^{cd}	5.65±0.02^{cd}	5.65±0.01^{cd}
WBCs ($x10^{9}$/ml)	11.57±0.06^{a}	9.13±0.12^{c}	9.00±0.00^{c}	9.33±0.12^{b}	6.37±0.06^{e}	6.33±0.12^{e}	6.57±0.06^{d}
Platelets ($x10^{9}$/ml)	10.00±0.00^{a}	10.00±0.00^{a}	10.00±0.00^{a}	10.00±0.00^{a}	8.00±0.00^{b}	8.00±0.00^{b}	8.00±0.00^{b}
MCV (µg/ml)	37.00±0.00^{b}	36.00±0.00^{c}	36.00±0.00^{c}	35.00±0.00^{d}	37.00±0.00^{b}	38.67±0.58^{a}	37.00±0.00^{b}
MCH (µg/ml)	12.00±0.00^{a}	12.00±0.00^{a}	12.00±0.00^{a}	11.00±0.00^{a}	12.00±0.00^{a}	12.00±0.00^{a}	12.00±0.00^{a}
MCHC (gm/100ml)	32.00±0.00^{b}	33.00±0.00^{a}	33.00±0.00^{a}	32.00±0.00^{b}	32.00±0.00^{b}	32.00±0.00^{b}	32.00±0.00^{b}
Lymph (%)	59.33±1.15^{f}	66.00±0.00^{e}	70.00±0.00^{d}	81.33±1.15^{ab}	82.00±0.00^{a}	75.67±0.58^{c}	80.67±0.58^{b}
Neut (%)	40.00±1.00^{a}	33.67±0.58^{b}	29.33±1.15^{c}	19.33±1.15^{e}	17.33±1.15^{f}	24.33±1.15^{d}	19.33±1.15^{e}
Mono (%)	1.00±0.00^{b}	1.00±0.00^{b}	2.00±0.00^{a}	0.50±0.00^{c}	2.00±0.00^{a}	1.00±0.00^{b}	1.00±0.00^{b}

BSSM – Boiled sunflower seed meal

The above values are means of triplicate data. Mean values in each row with similar superscripts are not significantly different ($p>0.05$).

PCV – Packed cell volume; Hb – Haemoglobin; RBCs – Red blood cells;
WBCs – White blood cells; MCV – Mean corpuscular volume; MCH – Mean corpuscular haemoglobin;
MCHC – Mean corpuscular haemoglobin concentration; Lymph – lymphocytes; Neut – Neutrophils;
Mono- Monocytes.

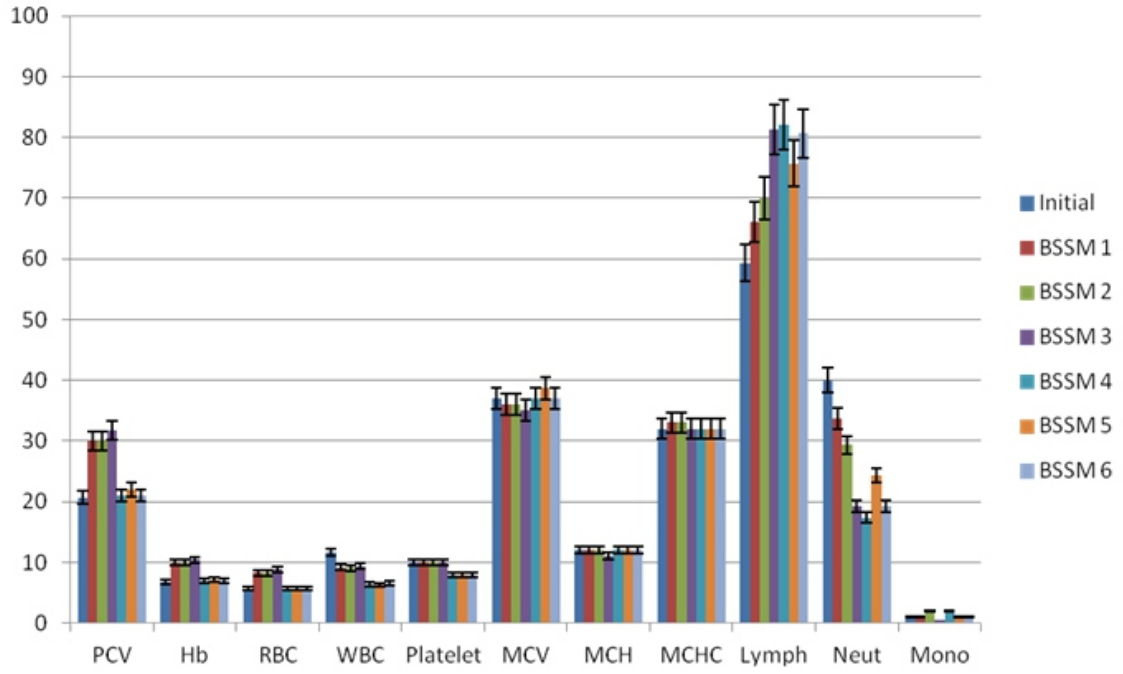

Figure 1: Haematological values (means) of *Clarias gariepinus* juveniles fed graded levels of boiled sunflower seed meal-based diets for 15 weeks

Serum biochemical indices of *Clarias gariepinus* juveniles fed graded levels of boiled sunflower seed meal-based diets

The result of serum biochemical indices of C*larias gariepinus* juveniles fed graded levels of boiled sunflower seed meal-based diets is presented in table 3 and figure 2. Total protein content of fish blood reduced from a mean initial value of 3.77 g/100ml to final values ranging between 2.30 g/100ml (in the fish fed 100% BSSM-based diet) and 3.60 g/100ml (in the fish fed 40% BSSM-based diet) and all the values differed significantly. The observed decrease in the total protein content at 60%, 80% and 100% BSSM inclusion levels could be linked with the level of anti-nutrients present in the diets. Yadav *et al.* (2003) also reported a decrease in serum total protein content in *Channa punctatus* induced with stem-bark extract of *Croton tiglium* while Ajani (2006) attributed such significant decrease in total blood protein level to impaired water quality. Hussein *et al.* (1996) reported a similar decrease in total protein level with increase in atrazine level and exposure time in *Oreochromis niloticus* and *Chrysichthys auratus*. Alkahem *et al.* (1998) attributed the reduction in total protein as a means of meeting an increased energy demand by fish to cope with detrimental conditions imposed by a toxicant.

According to Das and Mukherjee (2000), exposure of fish for a long time to most toxicants including pesticides interferes with protein metabolism. Decrease in total protein in fish exposed to toxic levels of toxicants could be attributed to either a state of hydration and change in water equilibrium in the fish or a disturbance in protein synthesis within the liver or both (Gluth and Hanke, 1984). Similarly, Das and Mukharjee (2000) observed that exposure of fish for a long time to most toxicants including pesticides interferes with protein metabolism, depletion of total protein in the plasma and serum of fish. On the contrary, the total protein values (2.30 ± 0.00 – 3.60 ± 0.00 g/100ml) obtained in the current study were much lower compared with 40.19 ± 7.45 g/100ml recorded by Ayoola (2011) while Olasunkanmi (2011) reported higher total protein levels in *C. gariepinus* fed diets containing raw mucuna meal.

The final values of albumin content ranged between 0.87 g/100ml (in the fish fed 100% BSSM-included diet) and 1.07 g/100ml (in the fish fed 40% BSSM-included diet) and were slightly

lower than the initial value (1.07 g/100ml) except in the fish fed 40% BSSM-included diet which maintained a similar value of 1.07 g/100ml. Globulin content of blood exhibited a similar pattern with total protein and albumin as it also reduced from a mean initial value of 2.70 g/100ml to the final values which varied from 1.40 g/100ml (in the fish fed 100% BSSM-included diet) to 2.57 g/100ml (in the fish fed 40% BSSM-included diet). Significant differences ($p<0.05$) were observed in globulin values between fish fed lower inclusion levels ($\leqslant$ 20%) and those fed higher inclusion levels ($\geqslant$40%). In the present study, the slight decrease in the serum total protein, albumin and globulin levels in fish blood samples in some treatments might be due to their degradation and utilisation for metabolic purposes.

The decrease in total protein, albumin and globulin levels may be due to impaired synthesis of protein or enhanced loss of protein through excretion and may also indicate some problem in the kidney (Jee *et al*., 2005). Protein depletion in experimental animals (including fish) has been reported to be a physiological strategy in the animals to adapt to changed metabolic systems. This leads to degradative processes such as proteolysis and utilisation of degraded products for increased metabolism (Yadav *et al*., 2003; Adeyemo, 2005). Bradbury *et al*. (1987) and Yadav *et al*. (2003) reported that decreased total protein content and albumin level might be due to destruction or necrosis of cells and consequently impairment in protein synthesis mechanism. The quantity of protein has been shown to depend on the rate of protein synthesis or its degradation (Yadav *et al.,* 2003). Protein quality may also be affected by impaired incorporation of amino acids into polypeptide chains (Yadav *et al*., 2003).

The concentrations of serum potassium ions (K^+) obtained in the present study varied significantly between 32.67 mg/dl (in fish fed 0% BSSM-based diet) and 51.33 mg/dl (in fish fed 80% BSSM-based diet) compared to the initial value of 41.33 mg/dl. However, these values are higher than 13.36±4.55 mmol/l concentrations reported by Owolabi (2011) and 13.24±24 mmol/l (Lawali *et al*., 2013). The concentrations of serum sodium ions (Na^+), which statistically varied from 55.33 mg/dl (in fish fed 100% BSSM-based diet) to 73.33 mg/dl (in fish fed 80% BSSM-based diet) compared to the initial value of 82.00 mg/dl, are lower than 139.48±23.19 mmol/l and 137.34±16.36 mmol/l concentrations reported by Owolabi (2011) and Lawali *et al*. (2015) respectively. The observed variations could be due to differences in species, geographical locations, dietary treatments and size or age. Creatinine content was highest (2.14 mg/dl) in fish fed 80% BSSM-based diet and least (1.07 mg/dl) in fish fed 100% BSSM-based diet compared to the initial value of 1.11 mg/dl. Significant differences ($p<0.05$) existed among final creatinine values in the dietary treatments. However, these low levels of creatinine (1.11 - 2.14 mg/dl) are higher than 0.35 – 0.97 µmol/L recorded for *C. gariepinus* juveniles exposed to paraquat dichloride (Ogamba *et al*., 2011) and they suggest that creatinine was effectively used up by fish muscle in response to the presence of anti-metabolites in the diets.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are blood serum enzymes which function as a link between carbohydrate and protein metabolism by catalysing the interconversion of strategic organic compounds such as α–ketoglutarate to pyruvic acid and alanine to glutamic acid (Tiwari and Singh, 2004). In the present study, the final ALT values ranged between 27.67 mg/dl (in fish fed 0% BSSM-based control diet) and 46.67 mg/dl (in fish fed 80% BSSM-based diet) and were significantly higher than the initial ALT value (24.33 mg/dl). Similarly, the initial AST value (47.67 mg/dl) statistically differed from the final values which ranged between 31.67 mg/dl and 72.0 mg/dl in the fish fed 0% and 80% BSSM diets respectively. The observed higher ALT and AST values suggest that the blood serum enzymes in the experimental fish were efficiently utilising amino acids for metabolic purposes, confirming the observation of Adesina (2008).

Furthermore, the elevated ALT and AST values at higher BSSM inclusion levels could be linked to stress due to increased levels of anti-nutrients at higher BSSM inclusions. Stress is generally known to elevate aminotransferase levels in fish (Tiwari and Singh, 2004). The result also conforms to the nature of the function of aminotransferases as

they respond to any stress or altered physiological condition (Knox and Greengad, 1965). The present observations agree with those of Dienye and Olumuji (2014) who reported a significant increase in the activities of serum enzymes - aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) - as the level of *M. oleifera* leaf meal increased considerably by 20% in the diet. Elevated AST, ALT and ALP activities in fish are suggestive of hepatic cellular damage leading to their leakage into the bloodstream (Mousa *et al*., 2008). Transaminases are well known to be very active in the liver, hence they are marker enzymes and their activities can be detected in very small amounts.

On the contrary, the results obtained in the present study disagree with the findings of Mousa and Khattab (2003) which revealed inhibition of AST and ALT activities in the liver of catfish after intoxication with dietary ochratoxin. Abdel Tawwab *et al*. (2001) also observed a similar result in liver AST and ALT of Nile tilapia after exposure to mercury. These workers ascribed the reduction in enzyme activity to liver necrosis caused by the toxicants and a possible damage to the hepatocytes. The decrease in the activities of these enzymes could be attributed to inhibition of the enzymes or a reduction in the rate of synthesis of the enzymes in the liver. Assessment of protein and enzyme activities can be considered as a diagnostic tool to determine the physiological status of cells or tissues (Manoj, 1999). Alterations of ALT and AST activities of fish resulting from toxicant or contaminant effect in various organs of fish have been reported (Gill *et al*., 1991; Begum, 2004). Such biochemical changes in fish are aimed at maintaining equilibrium in the presence of these toxicants, which are known to disrupt physiological and biochemical processes (Wedemeyer and McLeay, 1981).

Blood glucose was initially 60 mg/dl while its final levels varied from 51.33 mg/dl to 84.0 mg/dl in *C. gariepinus* fed BSSM-included diets. This range of values is consistent with 62.075 mg/dL median value of plasma glucose recorded for *Heteropneustes fossilis* by Srivastava and Sanjeev (2011). The increase in the blood glucose level observed in the fish fed 40% to 80% BSSM-included diets might be due to the fish mobilising energy from all available sources to combat stress (Colombo *et al*., 1990). The decreased glucose levels recorded for fish fed diets containing 20% and 100% BSSM inclusions could be due to the severity of stress factor caused by such diets as reported by Mahajan and Dheer (1983). Increase in the serum glucose level and reduction in the liver and muscle glycogen after exposure to anti-metabolites in diets may be due to mobilization of glycogen reserves (Singh and Srivastava, 1981). Glycogen is a stored form of energy which can be easily mobilized for energy production. Furthermore, the rapid secretion of catecholamines (Nakano and Tomlinson, 1967) and glucocorticoids (Fryer, 1975) from the adrenal tissue after the exposure of fish to anti-metabolites enhances the process of glycolysis which results in elevation of serum glucose.

Table 3: Serum biochemical indices of *Clarias gariepinus* juveniles fed graded levels of boiled sunflower seed meal-based diets for 15 weeks

Biochemical parameters	Initial values	BSSM 1 0% (control)	Dietary BSSM 2 20%	inclusion BSSM 3 40%	levels BSSM 4 60%	BSSM 5 80%	BSSM 6 100%
Total protein (g/100ml)	3.77±0.06^{a}	3.00±0.00^{d}	3.17±0.06^{c}	3.60±0.00^{b}	2.37±0.06^{f}	2.50±0.00^{e}	2.30±0.00^{g}
Albumin (g/100ml)	1.07±0.06^{a}	0.93±0.12^{ab}	0.97±0.06^{ab}	1.07±0.06^{a}	0.93±0.12^{ab}	1.00±0.00^{ab}	0.87±0.06^{b}
Globulin (g/100ml)	2.70±0.00^{a}	2.13±0.12^{c}	2.20±0.00^{c}	2.57±0.06^{b}	1.43±0.12^{d}	1.50±0.00^{d}	1.40±0.00^{d}
K^{+} (mg/dl)	41.33±1.15^{cd}	32.67±0.58^{f}	34.67±0.58^{e}	42.00±0.00^{bc}	43.00±0.00^{b}	51.33±1.15^{a}	40.67±0.58^{d}
Na^{+}(mg/dl)	82.00±2.00^{a}	65.00±0.00^{d}	70.00±0.00^{c}	58.00±0.00^{f}	60.00±0.00^{e}	73.33±1.15^{b}	55.33±1.15^{g}
Creatinine (mg/dl)	1.11±0.01^{d}	2.07±0.06^{ab}	2.02±0.01^{b}	1.11±0.01^{d}	1.22±0.01^{c}	2.14±0.12^{a}	1.07±0.05^{d}
ALT (mg/dl)	24.33±0.58^{g}	27.67±0.58^{f}	30.00±0.00^{e}	34.68±0.58^{d}	40.00±0.00^{c}	46.67±0.58^{a}	42.00±0.00^{b}
AST(mg/dl)	47.67±1.53^{e}	31.67±0.58^{g}	40.00±0.00^{f}	57.33±1.15^{c}	62.67±2.31^{b}	72.00±0.00^{a}	51.33±1.15^{d}
Glucose (mg/dl)	60.00±2.00^{c}	51.33±1.15^{e}	55.67±0.58^{d}	61.33±1.15^{c}	67.00±1.73^{b}	84.00±1.73^{a}	57.33±1.15^{d}

The above values are means of triplicate data. Mean values in each row with similar superscripts are not significantly different ($p>0.05$).
BSSM – Boiled sunflower seed meal

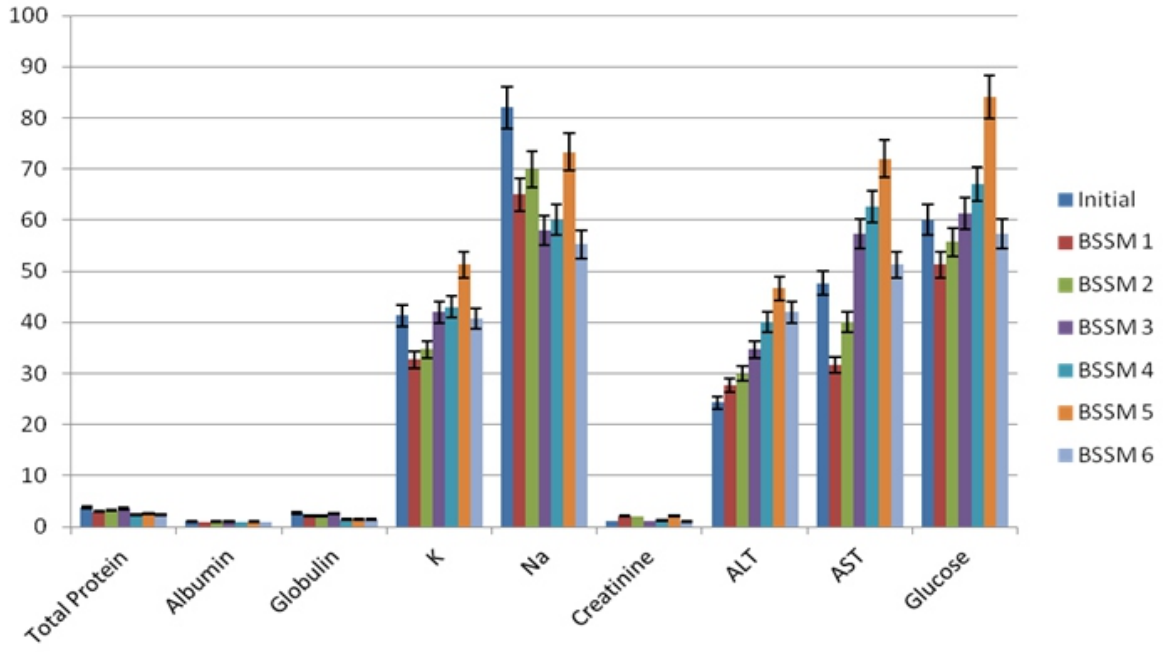

Figure 2: Serum biochemical values (means) of *Clarias gariepinus* juveniles fed graded levels of boiled sunflower seed meal-based diets for 15 weeks

CONCLUSION

The need to develop effective management mechanisms in order to support the ever-growing aquaculture industry constitutes the rationale behind the study of the haematology and biochemistry of fishes. The present study has provided relevant base-line information on the haematology and serum biochemistry of *C. gariepinus* that could be referred to in determining changes in the normal physiology of the species. This will eventually help in the early detection of stressful and other disease conditions that may interfere with the performance of the species in some vital culture operations such as nutritional assessment, artificial breeding and toxicological studies. All the haematological parameters

measured in this study were within the recommended physiological ranges reported for *C. gariepinus*. Improvement in the haematological parameters observed in this study indicated positive contribution of boiled sunflower seed meal (BSSM) protein content in blood formation and improving immunity in the fish. Consequently, the result of this study has indicated that boiled sunflower seed meal could be substituted for soybean meal in the diet of *C. gariepinus* without any negative effect on the haematological and biochemical parameters that were studied.

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