Applied Tropical Agriculture
Volume 22, No. 2, 24-35, 2017.
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Haematological and Serum Biochemical Profiles of *Clarias gariepinus* Juveniles Fed Diets Containing Different Inclusion Levels of Mechanically Extracted Sunflower (*Helianthus annuus*) Seed Meal

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ABSTRACT

A fifteen-week experiment was conducted to evaluate changes in the blood indices of Clarias gariepinus juveniles fed with six isonitrogenous and isocaloric diets in which mechanically extracted sunflower seed meal (MESSM) was substituted for soybean meal at 0, 20, 40, 60, 80 and 100%. A total of 360 juveniles of C. gariepinus (mean weight 21.3 ± 0.1 g) were randomly allocated into eighteen rectangular tanks (60 cm x 45 cm x 30 cm) at 20 fish per tank and fed twice daily at 5% body weight. Fish blood samples were collected to determine blood indices. The results showed that fish fed 20% MESSM-based diet had the best weight gain (51.65 g) while those on 100% MESSM-based diet had the least weight gain (21.09 g). There were elevated values of PCV (28.67-38.00%), Hb (9.60-12.70 gm/100ml), RBCs (8.11-10.21 x 1012/mL), WBCs (8.33 - 12.53 x109/mL), platelets (10.00-14.00 x109/mL) and lymphocytes (57.67-75.00%). Fish fed 20% MESSM inclusion had the highest PCV (38.0%), Hb (12.70 gm/100ml), RBC (10.21 x 1012/mL), platelets (14.00 x109/mL), MCV (37.00 µg/ml) and neutrophils (42.33%). Fish fed 40% MESSM had the highest total protein (4.17 g/100 mL), globulin (3.10 g/100 mL), K+ (45.67 mg/dl), Na+ (85.67 mg/dl), creatinine (2.24 mg/dl), alanine aminotransferase (ALT, 37.67 mg/dl) and aspartate aminotransferase (AST, 51.33 mg/dl). Significant difference ($p<0.05$) existed among the values obtained for the various parameters examined. Blood glucose significantly ($p < 0.05$) increased (62.33 - 81.33 mg/dl) in fish fed 20 - 100% MESSM. This study revealed that mechanically extracted sunflower seed meal can replace soybean meal in the diet of C. gariepinus without any adverse effect on its blood parameters.

Key words: Blood samples, *Clarias gariepinus*, Haematology, Soybean meal, Sunflower seed meal

INTRODUCTION

Culture of catfish species offers great potential for their fast growth towards satisfying the national fish demand and thereby reduces fish importation, provides employment opportunities, alleviates poverty and helps to meet the millennium development goals (Williams *et al*., 2007). Since fish are so intimately associated with the aqueous environment, their blood will reveal measurable physiological changes more rapidly than any other physiological assessment parameters (Ezeri *et al.,* 2004). 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).

Furthermore, 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). Haematology has been used as an index of fish health status in many fish species to detect physiological changes following different stress conditions such as exposure to pollutants, diseases, heavy metals, hypoxia, just to mention few. Svobodova *et al*. (1996) stated that study of haematological parameters is carried out on fish to ascertain the normal range of blood parameters, find out the variation with age, sex and season and to determine the effects of disease condition on the fish. Haematological tests and analysis of serum constituents have yielded useful information for detection and diagnosis of metabolic disturbances and disease conditions in fishes (Jamalzadeh *et al*., 2009).

Fish haematological investigation is useful mainly for diagnostic purpose and can also be used to assess the

suitability of new and unconventional feeds, to examine the effect of stress conditions and so on (Svobodova *et al*., 1991). Blood analysis is a valuable means of evaluating the physiological condition of cultured fish while determining the effects of diets and other stress factors on fish health. Changes in fish haematology in response to stress-producing agents are indicators of the stressful stage of fish which give useful clues to arrest any unfavourable condition that may affect fish health (Bello-Olusoji *et al*., 2006). Adeparusi and Ajayi (2004) reported that blood analysis is among important factors that could be considered in fish feed assessment. Soybean meal is currently the most commonly used plant protein source in fish feed (El-Sayed, 1999). However, there are other plant protein sources which are less expensive and can be beneficial in reducing feed cost when made to replace soybean meal (Barros *et al*., 2002). Therefore, this study investigated the haematological and serum biochemical changes that occurred in *C. gariepinus* juveniles fed with diets in which mechanically extracted sunflower seed meal was substituted for soybean meal.

MATERIALS AND METHODS

Preparation of mechanically extracted sunflower seed meal (MESSM)

Two kilograms of dehulled sunflower seeds were dried in an electric oven at 80^0C for 30 minutes (Alegbeleye, 2005) to reduce the moisture content and facilitate grinding. The resulting paste was defatted in an improvised mechanical screw press for 48 hours. The resulting mechanically extracted sunflower seed meal (MESSM) was hand-crumbled and oven-dried at 60^0C for 6 hours in a Gallenkamp oven prior to proximate analysis.

Experimental design and formulation of mechanically extracted sunflower seed meal–incorporated diets in substitution for soybean meal

Six isonitrogenous diets (at 40% crude protein level) were formulated and prepared using Pearson's square method. Mechanically extracted sunflower seed meal (MESSM) was incorporated in the six diets at graded levels of 0%, 20%, 40%, 60%, 80% and 100% to replace soybean meal (SBM) with 0% being the control diet (Table 1). Other ingredients which were measured and mixed together in the diets included fish meal, groundnut cake, vitamin and mineral premix, bone meal, oyster shell, maize, cassava starch, salt and palm oil. 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 broken 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.

Table 1: Gross ingredient composition (g/100g diet) of mechanically extracted sunflower seed meal diets as replacement for soybean meal at graded levels for *C. gariepinus* juveniles

Ingredients	MESSM 1	MESSM 2	MESSM 3	MESSM 4	MESSM 5	MESSM 6
	(Control) 0%	-20%	-40%	-60%	-80%	-100%
Sunflower seed meal		7.58	15.16	22.74	30.32	37.9
Soybean meal	37.9	30.32	22.74	15.16	7.58	
Fishmeal	19.45	19.45	19.45	19.45	19.45	19.45
Groundnut cake	19.45	19.45	19.45	19.45	19.45	19.45
Yellow maize	17.44	17.44	17.44	17.44	17.44	17.44
Vitamin/mineral premix*	2	2	2	2	2	2
Bone meal	1	1	1	1	1	1
Oyster shell	0.5	0.5	0.5	0.5	0.5	0.5
Palm oil	0.75	0.75	0.75	0.75	0.75	0.75
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Cassava starch	1	1	1	1	1	1
Total (%)	100	100	100	100	100	100
Calculated crude protein content (%)	40	40	40	40	40	40

MESSM: mechanically extracted sunflower seed meal diet. 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.

Six graded levels (0%, 20%, 40%, 60%, 80% and 100%) of mechanically extracted sunflower seed meal (MESSM) 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 mechanically extracted sunflower seed meal and soybean meal respectively:
Treatment 1: 0% MESSM + 100% SBM;
Treatment 2: 20% MESSM + 80% SBM;
Treatment 3: 40% MESSM + 60% SBM;
Treatment 4: 60% MESSM + 40% SBM;
Treatment 5: 80% MESSM + 20% SBM;
Treatment 6: 100% MESSM + 0% SBM.

Arrangement of fish feeding experiment

The feeding trial was conducted inside eighteen rectangular 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' interval to maintain relatively uniform physico-chemical parameters and prevent fouling from feed residues. The tanks were well aerated with 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 according to the new body weight gain.

Haematological assessment of experimental fish

Collection of blood samples and analytical procedures

Prior to the commencement of the nutritional experiment, ten juveniles were randomly selected and tranquilised with 150 mg/litre of tricane methane sulphonate (Finquel TMS-222, NAC No. 1026002, Argent Chemical Laboratories Inc., 8702-152nd Avenue, N.E. Redmond, Washington, USA) as described by Wagner *et al.* (1997). Eight ml of blood sample was carefully collected from the caudal artery in the caudal peduncle of fish specimens using sterile disposable 2 ml plastic syringes and needles and mixed with ethylene diamine tetra-acetic acid (EDTA, an anti-coagulant) inside EDTA bottles.

Similarly, at the end of the experiment, six samples of live fish were randomly selected from each treatment and 8 ml of blood sample was collected from them as previously described. The blood samples were analyzed in the laboratory of the Department of Haematology, Faculty of Veterinary Medicine, University of Ibadan where haematological and biochemical parameters were determined using standard methods as described by Schalm *et al.* (1975).

Haematological parameters

Packed cell volume (Haematocrit)

Pre-heparinised capillary tubes were filled up to 2 ml (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 (SP 6–500 UV spectrophotometer) 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 obtained as:

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

Blood volume was calculated as follows:

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

Haemoglobin concentration (Hb)

Determination of Hb concentration involved the cyanomethaemoglobin methods described by Schalm *et al.* (1975) and Kelly (1979). Each 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 allowed to settle for 10 minutes at room temperature to allow all the haemoglobin to react with the reagent to form cyanomethaemoglobin (that is, for proper colour development). The absorbance of the resultant solution was read at 540 nm inside a Unicam spectrophotometer (Spectrumlab 23a Model) against a blank.

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 (Olympus Japan-312545) 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 formula proposed by Dacie and Lewis (2001) as follows:

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

Mean corpuscular haemoglobin concentration (MCHC)

This was calculated from the relationship between haemoglobin concentration and number of red blood cells per 100 ml blood (Dacie and Lewis, 2001).

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

Determination of lymphocytes, neutrophils and monocytes was done using Neubauer-type haemocytometer with Turk's solution as the diluting fluid as described by Rusia and Sood (1992).

Determination of serum biochemical parameters

Serum total protein, albumin and globulin were estimated colorimetrically by Biuret and Bromocresol Green method (Peter *et al*., 1982). Sodium and potassium ion concentrations were measured using potentiometric analysis as described by Langhoff and Steiness (1982). Blood glucose was measured colorimetrically using a spectrophotometric method (WPAS2000-UV/VIS Cambridge, UK) (Trinder, 1969). Serum enzymes [creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] were determined colorimetrically by means of standard enzymatic methods as described by Bush (1991) while blood urea nitrogen (BUN) was measured according to Patton and Crouch (1977).

Statistical analysis of data

All data obtained in this study were presented as mean ± standard deviation. Comparisons were made between the control and experimental groups. One-way ANOVA and Duncan's multiple range tests (Duncan, 1955) were used on SPSS statistical software (Version 17.0 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 less than 0.05 (i.e. $p<0.05$).

RESULTS

Haematological indices of *C. gariepinus* juveniles fed graded levels of mechanically extracted sunflower seed meal-based diets

Haematological indices of *C. gariepinus* juveniles fed graded levels of mechanically extracted sunflower seed meal-based diets in substitution for soybean meal are shown in Table 2. Packed cell volume (PCV) of the experimental fish increased from initial value of 20.67% to final values of 28.67% and 38.0%. Haemoglobin content (Hb) content increased from initial value of 6.80 gm/100ml to final values of 9.60 gm/100ml and 12.70 gm/100ml. Red blood cells (RBCs) increased from an initial value of 5.62 x 10^{12}/ml to the final values of 8.11 x 10^{12}/ml and 10.21 x 10^{12}/ml. Initial WBC count was 11.57 $x10^9$/ml compared to final values of 8.33 $x10^9$/ml and 12.53 $x10^9$/ml. Initial platelet count was 10.0 $x10^9$/ml while final values were 10.0 $x10^9$/ml to 14.0 $x10^9$/ml.

Mean corpuscular volume (MCV) was initially 37.0 µg/ml compared to final values of 34.67µg/ml and 37.0µg/ml. Initial mean corpuscular haemoglobin (MCH) was 12.0 µg/ml compared to final values of 11.0 µg/ml and 12.0 µg/ml. Mean corpuscular haemoglobin concentration (MCHC) rose from 32.0 gm/100ml to final values of 32.67 to 33.0 gm/100ml which, however, were not significantly different ($p>0.05$). The initial lymphocyte count was 59.33% while the final values were 57.67% to 75.0%. Initial neutrophil count was 40.0% compared to the final values of 24.67% to 42.33%. Initial monocyte count was 1.0% while the final values were 0.5% to 2.0%. Significant difference ($p<0.05$) existed among the values obtained for PCV, Hb, RBCs, WBCs, MCV, MCH, MCHC, lymphocytes, neutrophils and monocytes.

Serum biochemical indices of *C. gariepinus* juveniles fed graded levels of mechanically extracted sunflower seed meal based diets

Table 3 presents serum biochemical indices of *C. gariepinus* juveniles fed graded levels of mechanically extracted sunflower seed meal-based diets. Significant differences ($p<0.05$) were observed in the values of indices determined. Initial total protein level was 3.77 g/100ml compared to final values of 2.60 g/100ml and 4.17 g/100ml. Initial albumin level was 1.07 g/100ml while the final values were between 1.0 g/100ml and 1.37 g/100ml. Initial value of globulin was 2.70 g/100ml compared to final values of 1.60 g/100ml and 3.10 g/100ml. Initial potassium ion (K^+) concentration was 41.33 mg/dl while the final values were 30.0 mg/dl and 45.67 mg/dl. Initial sodium ion (Na^+) concentration was 82.0 mg/dl while the final values were 57.67 mg/dl to 85.67 mg/dl.

Table 2: Haematological indices of *C. gariepinus* juveniles fed graded levels of mechanically extracted sunflower seed meal-based diets for 15 weeks

Parameters	Initial values	Experimental Dietary Inclusion Levels					
		MESSM 1 (0%)	MESSM 2 (20%)	MESSM 3 (40%)	MESSM 4 (60%)	MESSM 5 (80%)	MESSM 6 (100%)
PCV (%)	20.67±0.58^{f}	29.67±0.58^{d}	38.00±0.00^{a}	32.67±0.58^{c}	35.00±0.00^{b}	35.67±0.58^{b}	28.67±0.58^{e}
Hb (gm/100ml)	6.80±0.17^{f}	9.87±0.06^{d}	12.70±0.00^{a}	10.87±0.06^{c}	11.80±0.00^{b}	11.87±0.06^{b}	9.60. ±0.35^{e}
RBCs (x10^{12}/ml)	5.62±0.02^{g}	8.23±0.01^{e}	10.21±0.01^{a}	8.87±0.01^{d}	10.08±0.07^{c}	10.13±0.01^{b}	8.11±0.01^{f}
WBCs (x10^{9}/ml)	11.57±0.06^{c}	8.57±0.06^{e}	12.37±0.06^{b}	9.33±0.12^{d}	12.27±0.12^{b}	12.53±0.12^{a}	8.33±0.12^{f}
Platelets (x10^{9}/ml)	10.00±0.00^{c}	12.00±0.00^{b}	14.00±0.00^{a}	12.00±0.00^{b}	14.00±0.00^{a}	14.00±0.00^{a}	10.00±0.00^{c}
MCV (µg/ml)	37.00±0.00^{a}	35.67±0.58^{b}	37.00±0.00^{a}	36.67±0.58^{a}	34.67±0.58^{c}	34.67±0.58^{c}	34.67±0.58^{c}
MCH (µg/ml)	12.00±0.00^{a}	11.67±0.58^{a}	12.00±0.00^{a}	12.00±0.00^{a}	11.00±0.00^{b}	11.00±0.00^{b}	11.67±0.58^{a}
MCHC (gm/100ml)	32.00±0.00^{b}	33.00±0.00^{a}	33.00±0.00^{a}	33.00±0.00^{a}	33.00±0.00^{a}	33.00±0.00^{a}	32.67±0.58^{b}
Lymph (%)	59.33±1.15^{e}	63.33±1.15^{d}	57.67±0.58^{f}	70.00±0.00^{c}	64.33±1.15^{d}	72.67±0.58^{b}	75.00±0.00^{a}
Neutrophils (%)	40.00±1.00^{b}	36.00±0.00^{c}	42.33±0.58^{a}	29.67±0.58^{d}	35.67±0.58^{c}	27.00±0.00^{e}	24.67±0.58^{f}
Mono (%)	1.00±0.00^{b}	2.00±0.00^{a}	0.50±0.00^{c}	1.00±0.00^{b}	1.00±0.00^{b}	1.00±0.00^{b}	1.00±0.00^{b}

a,b,c,d,e,f,g: indicate that mean values with different superscripts along the same row are significantly different (p<0.05).

MESSM: mechanically extracted sunflower seed meal diet; 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.

Table 3: Serum biochemical indices of *C. gariepinus* juveniles fed graded levels of mechanically extracted sunflower seed meal-based diets for 15 weeks

Parameters	Initial values	Dietary Inclusion Levels					
		MESSM 1 (0%)	MESSM 2 (20%)	MESSM 3 (40%)	MESSM 4 (60%)	MESSM 5 (80%)	MESSM 6 (100%)
Total protein (g/100ml)	3.77±0.06^{c}	3.17±0.06^{e}	2.60±0.00^{f}	4.17±0.06^{a}	3.47±0.06^{d}	3.80±0.00^{c}	4.00±0.00^{b}
Albumin (g/100ml)	1.07±0.06^{bc}	1.07±0.06^{bc}	1.00±0.00^{c}	1.07±0.06^{bc}	1.17±0.06^{b}	1.37±0.06^{a}	1.17±0.06^{b}
Globulin (g/100ml)	2.70±0.00^{c}	2.13±0.12^{f}	1.60±0.00^{g}	3.10±0.00^{a}	2.33±0.12^{e}	2.47±0.06^{d}	2.87±0.06^{b}
K^{+} (mg/dl)	41.33±1.15^{c}	35.00±0.00^{f}	30.00±0.00^{g}	45.67±0.58^{a}	36.00±0.00^{e}	40.00±0.00^{d}	43.67±0.58^{b}
Na^{+} (mg/dl)	82.00±2.00^{c}	69.67±0.58^{e}	57.67±0.58^{e}	85.67±0.58^{a}	74.67±0.58^{d}	80.67±0.58^{c}	84.00±0.00^{b}
Creatinine (mg/dl)	1.11±0.01^{f}	2.00±0.00^{d}	1.21±0.01^{e}	2.24±0.01^{a}	1.22±0.01^{e}	2.12±0.00^{c}	2.22±0.01^{b}
ALT (mg/dl)	24.33±0.58^{f}	30.67±0.58^{d}	25.67±0.58^{e}	37.67±0.58^{a}	31.33±1.15^{d}	34.00±0.00^{c}	35.67±0.58^{b}
AST (mg/dl)	47.67±1.53^{b}	40.00±0.00^{d}	21.33±1.15^{f}	51.33±1.15^{a}	37.33±1.15^{e}	45.67±0.58^{c}	48.67±0.58^{b}
BUN (mg/dl)	2.10±0.10^{b}	1.20±0.00^{d}	1.00±0.00^{e}	2.40±0.00^{a}	1.17±0.06^{d}	2.00±0.00^{c}	2.17±0.06^{b}
Glucose (mg/dl)	60.00±2.00^{b}	47.00±1.73^{c}	81.33±1.15^{a}	68.67±1.15^{b}	68.00±13.86^{b}	62.33±4.62^{b}	68.67±1.15^{b}

a,b,c,d,e,f,g: indicate that mean values with different superscripts along the same row are significantly different (p<0.05).

MESSM: mechanically extracted sunflower seed meal diet; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen.

Initial creatinine content was 1.11 mg/dl was lower than the final values of 1.21 mg/dl and 2.24 mg/dl. Initial blood urea nitrogen (BUN) concentration was 2.10 mg/dl while the final values were 1.0 mg/dl and 2.40 mg/dl. Alanine aminotransferase (ALT) concentration increased from 24.33 mg/dl to final values of 25.67 and 37.67 mg/dl. Aspartate aminotransferase (AST) concentration was initially 47.67 mg/dl compared to final values of 21.33 mg/dl and 51.33 mg/dl. Initial glucose concentration was 60.0 mg/dl compared to final values of 47.0 mg/dl to 81.33 mg/dl.

DISCUSSION

Haematological indices of *C. gariepinus* juveniles fed graded levels of MESSM diets

Blood could be used as a means through which general condition of the animal body could be assessed. The PCV values in this study agreed with 27.58 - 35.50% obtained by Musa and Omoregie (1999) and 36.0% reported by Adeyemo (2007). The observed values were higher than 25.65 ± 5.89% found in *Synodontis membranacea* (Owolabi, 2011) and considerably higher than 0.37 ± 0.01 g/l in *C. gariepinus* (Ayoola, 2011). Ajiboye (2009) reported PCV values ranging between 29.15% and 37.61% in *Synodontis nigrita* fed different dietary crude protein levels while Lawali *et al.* (2015) obtained 31.14±8.55%) for *Channa striatus*. Agbabiaka *et al*. (2013) recorded 38.0 to 44.7% for *C. gariepinus* fingerlings fed graded levels of tigernut-based diet. However, Sotolu and Faturoti (2011) reported lower PCV values which were attributed to anaemia resulting from shrunken red blood cells, a situation that probably resulted in fish asphyxiation and death as confirmed by Adeyemo (2005).

Values of haemoglobin were high and within the range of 5.6 to 15.8 g/100 ml) reported for *Esox lucius* (Mulcahy, 1970) and compared well with 8.70 g/100 ml for *C. gariepinus* (Sowunmi, 2003) as well as 7.90 to 8.90 g/100 ml reported by Dienye and Olumuji (2014). These values, however, were higher than 4.46 g/100 ml reported for *Heterotis niloticus* (Fagbenro *et al*., 2000) and Ajiboye (2009) who recorded between 4.70 and 7.84 gm/100ml in *Synodontis nigrita* fed different dietary crude protein levels. The present values were within the range of baseline values established by Adedeji *et al*. (2000) for Nigerian freshwater fishes. The RBC values were greater than 2.11 x 10^{12}/ml to 2.93 x 10^{12}/ml reported by Ajiboye (2009) and 3.01±0.56×10^{12}/l recorded for *Channa striatus* (Lawali *et al*., 2015). Furthermore, the values were also higher than 3.81±1.49×10^{12}/l for *Synodontis membrancea* (Owolabi, 2011), 1.9×10^{12}/l found in *C. gariepinus* juveniles (Ayoola, 2011) and 1.67 × 10^{12}/l in *Parachanna obscura* (Kori Siakpere *et al*., 2005). The increased RBC count 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).

WBC counts were lower than 22.33 ± 2.52 × 10^9 /ml recorded for *Clarias batrachus* (Maheswaran *et al*., 2008) but higher than 19.07 ± 1.47 x 10^3 /mm^3 obtained for *Cyprinion macrostomus* (Orun *et al*., 2003), 6.62±1.23×10^9 /ml for *Channa striatus* (Lawali *et al*., 2015) and 1.61 ± 0.01×10^6/mm^3 for *Ictalurus punctatus* (Klinger *et al*., 1996). 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. Douglas and Jane (2010) stated that their amount has implication in immune responses and the ability of the animal to fight infection. Platelet counts were much higher than 175.92 x 10^3/µL recorded for adult *Sarotherodon melanotheron* (Akinrotimi *et al*., 2007) and 132.0 x 10^3/µL for juvenile *C. gariepinus* (Sunomonu and Oyelola, 2008). The values were also greater than 19.25 x 10^3/µL, 17.33 x 10^3/µL, 16.67 x 10^3/µL and 10. 67 x 10^3/µL recorded for *Clarias anguillaris*, *Clariabranchus*, *Heteroclarias* and *Heterobranchus bidorsalis* respectively (Diyaware *et al*., 2013).

The mean MCV values obtained in this study were lower than 240.18 fl recorded for juvenile hybrid African catfish (*Heteroclarias*) reported by Kori-Siakpere and Ubogu (2008), 200.93 fl for *C. gariepinus* fingerlings (Gbore *et al*., 2006), 113.07 to 138.07 fl for juvenile intergeneric hybrid catfishes (Diyaware *et al*., 2013) and 96.62 fl for *C. gariepinus* fingerlings (Ochang *et al*., 2007). MCV as an estimate of the volume of RBCs indicates the status or size of the RBCs and reflects normal or abnormal cell division during RBC production (erythropoiesis). Larsson *et al.* (1985) attributed increase in MCV to swelling of the RBCs due to hypoxic condition (low oxygen condition), impaired water quality, somatic 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 Bhagwant and Bhikajee (2000), Adesina (2008) and Alwan *et al.* (2009).

The lack of statistical variation between MCH values suggested that MCH was not affected by dietary treatments. However, these MCH values were lower than values obtained in earlier reports such as 24.24 pg for *C. gariepinus* juveniles (Omitoyin, 2006), 33.10 pg for *C. gariepinus* (Ochang *et al*., 2007) and 51.50 pg for juvenile *Heteroclarias* (Gbore *et al*., 2006). The results of the present study also disagreed with Olasunkanmi (2011) who

reported a significant increase in the final MCH values in *C. gariepinus* fed raw mucuna seed meal-based diets. Higher MCH indicates a good volume of haemoglobin which indicates effective oxygen transportation in the bloodstream for healthy wellbeing of the fish (Diyaware *et al*., 2013).

MCHC values were within the range recommended by Bhaskar and Rao (1989) for healthy fish and they closely agreed with 35.47 g/dL recorded for *Heteroclarias* (Kori-Siakpere and Ubogu, 2008) and 33.67 to 39.03 g/dL for juvenile inter-generic hybrid catfishes (Diyaware *et al*., 2013). The variation between the present study and previous reports might be due to species differences and age of the fishes that greatly influence the values of haematological indices (Docan *et al*., 2010). Lymphocyte counts compared closely with 76.49% in *Synodontis membranacea* (Owolabi, 2011) but were higher than 15.38% recorded for *Channa striatus* (Lawali *et al*., 2015) and 33.00% for *C. gariepinus* juveniles (Adeyemo, 2007). However, the present values were lower than 82.8% reported for juvenile *C. gariepinus* (Yaji and Auta, 2007) and 99.20% in *Cyprinus carpio* (Orun *et al*., 2003). Monocyte counts were lower than 16.14 ± 8.25% found in *Synodontis membranacea* (Owolabi, 2011) and 23.76 ± 2.84 % in *Channa striatus* (Lawali *et al*., 2015).

Serum biochemical indices of *C. gariepinus* juveniles fed graded levels of MESSM diets

In fish, proteins are among the main energy sources which play an important role in the maintenance of blood glucose (Shwetha *et al*., 2012). The observed decrease in the total protein content of fish at 20% and 60% MESSM 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*. 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. 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 a reduction in total protein as a means by which fish generates energy to cope with detrimental conditions imposed by a toxicant.

A similar trend was observed for albumin and globulin. Protein depletion in experimental animals (including fish) has been reported to be a physiological strategy in the animals to adapt to alterations in their metabolic systems. This leads to degradative processes such as proteolysis and utilization of degraded products for increased metabolism (Yadav *et al.*, 2003; Adeyemo, 2005). In the present study, the slight decrease in the serum total protein, albumin and globulin levels might be due to their degradation and utilization for metabolic purposes. 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 be dependent 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 decrease in total protein and albumin levels may be due to impaired synthesis of protein or enhanced loss of protein through excretion and is also suggestive of some problem in the kidney (Jee *et al*., 2005).

Values of potassium ion (K^+) concentration were greater than 13.36±4.55 mmol/l concentrations reported by Owolabi (2011) as well as 13.24±2.4 mmol/l (Lawali *et al*., 2015) but were within the range (22.34 to 56.40 mmol/l) recorded for juvenile *C. gariepinus* fed coconut (*Cocos nucifera*) water (Ayotunde *et al*., 2015). Values of sodium ion (Na^+) concentration were above 1.10 - 74.54 mmol/l recorded for juvenile *C. gariepinus* fed coconut (*Cocos nucifera*) water (Ayotunde *et al*., 2015). Although, creatinine values were comparatively above 0.35 – 0.97 µmol/L recorded for *C. gariepinus* juveniles exposed to paraquat dichloride (Ogamba *et al.*, 2011), the low values suggested that creatinine was effectively used up by fish muscle in response to the presence of anti-metabolites in the diets. Blood urea nitrogen (BUN) concentrations observed in the present study (1.00 – 2.40 mg/dl) compared favourably well with 2.33 - 3.23 µmol/L earlier reported for *C. gariepinus* juveniles exposed to paraquat dichloride (Ogamba *et al.*, 2011). The liver is the main source of urea, thus the lower BUN levels have been suggested to be an indication of the impaired functioning of the liver (Hlophe and Moyo, 2014).

The higher values of alanine aminotransferase (ALT) suggested that the blood serum enzyme in the experimental fish efficiently utilized amino acids for metabolic purposes, confirming the observation of Adesina (2008). Transaminases are important enzymes for monitoring the health status of fish (Racicot *et al*., 1975) and leak out into the bloodstream from dying or damaged liver cells. Increased levels of transaminases in the blood serum of fish are usually associated with dying or damaged liver cells while a decrease could suggest leakage of enzymes into the serum (Yilmaz *et al*., 2006; Ozovehe, 2013).

The increased values of aspartate aminotransferase (AST) enzyme at higher MESSM inclusion levels could be linked to stress due to increased levels of anti-nutrients at higher MESSM inclusions. Aminotransferase levels in fish increase in response to stress (Tiwari and Singh, 2004). This observation agreed with that of Dienye and Olumuji (2014) who reported elevated AST, ALT and ALP activities in fish fed 30% *M. oleifera* leaf meal diet and above which suggested hepatic cellular damage leading to their leakage into the bloodstream (Mousa *et al*., 2008). The lower AST values recorded in fish fed 20% and 60% MESSM-based diets corroborated the report of Mousa and Khattab (2003) that there was 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 associated the reduction in enzyme activity with 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 their inhibition or a reduction in the rate of their synthesis in the liver.

The values of blood glucose were relatively higher than values available in some literature reports (Akintayo *et al*., 2008). These values closely agreed with 66.03 to 85.98 mg/dl found in *C. gariepinus* fingerlings (Anene *et al*., 2014) and 62.075 mg/dl in *Heteropneustes fossilis* (Srivastava and Sanjeev (2011). However, Tavares-Dias (2000) and Yilmaz *et al*. (2006) reported higher blood sugar levels ranging from 153 to 208 mg/dl in *C. gariepinus* which are considerably higher than the present findings. Values of blood serum glucose ranging from 25 to 350 mg/dl have also been reported by authors who associated such differences with variations in the chemical compositions of the diets administered (Shaakori *et al*., 1996; Ezenwaji *et al*., 2012). The rise in the blood glucose level observed in the fish fed 20% to 100% MESSM-included diets could be due to the fish generating energy from all available sources to combat stress (Colombo *et al.*, 1990). 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 as a stored form of energy can be easily mobilized for energy production. Furthermore, a rapid secretion of glucocorticoids (Fryer, 1975) and catecholamines (Nakano and Tomlinson, 1967) from the adrenal tissue after the exposure of fish to anti-metabolites enhances the process of glycolysis which causes increase in serum glucose.

CONCLUSION

The results showed that fish fed 20% MESSM-based diet had the best weight gain (51.65 g) while those on 100% MESSM-based diet had the least weight gain (21.09 g). The result of this study has indicated that mechanically extracted sunflower seed meal can replace soybean meal in the diets of African catfish, *C. gariepinus*, without any adverse effect on its physiology and health status.

ACKNOWLEDGMENTS

The author is thankful to Mrs. A. S. Adetiba and her team (Haematology laboratory, Faculty of Veterinary Medicine, University of Ibadan) for technical assistance with blood analysis and Mr. S. O. Adediran for statistical analysis of raw data.

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