

HAEMATOLOGICAL RESPONSE OF *Clarias gariepinus* (Burchell, 1822) FINGERLINGS FED VARYING LEVELS OF TOASTED *Sesbania sesban* SEED MEAL

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ABSTRACT

This study examined the haematological effect of partial replacement of soybean meal (SBM) with toasted *S. sesban* seed meal (TSSSM) in the diet of *Clarias gariepinus* fingerlings. Four iso-nitrogenous (42% crude protein) diets were formulated to replace SBM with TSSSM at the rates of 0, 10, 20, and 30%. The experimental diets were fed to fingerlings for 10 weeks in triplicates in hapas of dimension (1m x 1m x 1m) outdoor earthen ponds (7x5x1.2m) partitioned into 4. The fish were stocked at 10 fish/1m³. The proximate composition and anti-nutritional compound of the raw and toasted SSSM were determined. The haematological indices of *C. gariepinus* fingerlings were also studied. The results showed that TSSSM had (34.27%) crude protein. Toasting resulted in a decline in the concentration of anti-nutritional compounds in this order 67.5%, 84.4%, and 57.8% for oxalates, alkaloids, and tannins respectively. The haematological parameter indicates that there was no significant difference ($P < 0.05$) between fish fed control (0% TSSSM) diet and fish fed 10% TSSSM diet. All the haematological indices fell within the recommended range for normal healthy fish. This implies that 10% of the soya bean meal in African catfish could be substituted with toasted *S. sesban* seed meal without deleterious effect on fish health status.

Keywords: Physiology, Processed seeds, Catfish Fingerlings

INTRODUCTION

The evaluation of nutrient utilization and biological values of feeds and feedstuffs may seem indeterminate without sufficient consideration of their implications on the animal's physiological and health status (Anyanwu et al., 2011). Haematological parameters of different species of fish and screening test provide a bank from which useful information which could draw valuable and informative conclusions (Nlewadim and Alum, 1999). According to (Bhaskar and Rao 1990), regular monitoring of haematological parameters of farmed fish can be used to prevent harm to fish farming activities. The use of haematological parameters in diagnosing fish's health is gaining ground as a tool in the management of fish farms. It has been observed that changes in haematological parameters due to unfavourable exogenous factors like adverse water quality, overstocking, starvation, etc., are indices of cultured fish's health status (Anyanwu, et al., 2011). Similarly, Anyanwu, et al. (2011) reported a range of 5.48 - 6.25g/100ml, 2.35 - 3.0x10⁶/mm³, 2.335 - 24.75%, and 4.48 - 5.03X10⁴/mm³ for the heamoglobin, red blood cells, packed cell volume, and white blood cells values for *Clarias gariepinus* respectively, fed varied dietary inclusion levels of *Carica papaya* leaf meal. Bhasker and Rao (1990) reported MCV, MCH, and MCHC values of 132.8-308.4fl, 20.90-47.20pg, and 10.90-38.10% respectively, studies about stocking and feeding conditions. The objective of this study was to determine the haematological response of (*Clarias*

gariepinus Teugells, 1982) fingerlings fed graded levels of Toasted *Sesbania sesban*, seed meal.

MATERIALS AND METHODS

Experimental Site

The research was conducted in the ponds of the Department of Fisheries and Aquaculture, Federal University Dutse, Jigawa State, Nigeria. It is located within latitudes 11.00°N and 13.00°N and longitudes 8.00°E and 10.15°E, respectively in the Sudan Savannah agro-ecological zone of Nigeria.

Source and Processing of *Sesbania sesban* seeds

The seeds of *Sesbania sesban* seeds were collected from the Federal University Dutse campus, Jigawa State, Nigeria. Leaves and pods of the plant were taken to the Herbarium of the Department of Forestry and Wildlife, the plant was identified using a plant press key for identification. The seeds were then removed out from the plant pulp and processed by drying and then toasted under gentle heat (80°C) for about 5 minutes. The toasted seeds were milled into a fine powder and analyzed for proximate composition, mineral elements, and anti-nutritional compounds.

Experimental Fish

One hundred and twenty (120) *C. gariepinus* fingerlings with average body weight (2.5g) and standard length (4.30cm) were procured from a commercial fish hatchery in Kano. They were then transported to the fisheries laboratory, Department of Fisheries and Aquaculture, Federal

University Dutse, Jigawa State in 50litres plastic containers and allowed to acclimatize for four days while they were fed 0.8mm floating pelleted commercial diet before the commencement of the experiment.

Experimental Design

Completely Randomized Design (CRD) was employed in this research. Four treatments were used. The inclusion levels were: 0% (control) i.e., without *S. sesban*, 10%, 20%, and 30% of the *Sesbania sesban* and a commercial diet. Each of the four diets had three replicates. Twelve (12) hapas of dimension (1m×1m×1m) were installed in a polyethylene earthen fish pond (7×5×1.2m). Ten (10) *C. gariepinus* fingerlings were stocked into each of the twelve hapas and fed for ten weeks (70 days).

Feed Formulation and Pellet Preparation.

The experimental diets contained fish meal (FM), soybean meal (SBM), yellow maize meal (YMM), toasted *Sesbania sesban* seed meal (TSSSM). All ingredients were milled into a fine powder using a hammer mill and sieved through a 0.25mm sieve. FM, SBM, and YMM were obtained from commercial suppliers. Before feed formulation, proximate compositions of these ingredients were determined (Table 1). Based on the proximate composition of the ingredients. Five isonitrogenous diets (42% CP) and Isocaloric (gross energy 2777 kcal kg⁻¹) were prepared where the toasted *Sesbania sesban* seed meal (TSSSM) was replaced soyabean meal (SBM) at 0%, 10%, 20%, 30%. While 0% (TSSSM) feed served as the control diets (Table 1). Diet samples were subjected to proximate analysis as described by the (A.O.A.C.2000).

Determination of Haematological Parameters

At the end of the 70 days of feeding, three fish from each treatment were randomly selected for haematological analysis. The following parameters were determined: Packed cell volume (PCV) as described by (Kelly, 1979), Red blood cell (RBC) and white blood cell (WBC) counts, carried out by use of the Neubauer improved counting chamber as described by (Kelly, 1979), the haemoglobin concentration using the cyanomethaemoglobin method described by (Schalm *et al.*, 1975 and Kelly, 1979). The mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular hemoglobin concentration (MCV, MCH, and MCHC) were calculated as described by (Siang *et al.*, 2007).

STATISTICAL ANALYSIS

All data collected were subjected to one-way analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS

(SAS, 2004). Duncan Multiple Range Test (DMRT) was used to separate means respectively. Values of P<0.05 were considered significant.

RESULTS

The proximate composition and the anti-nutritional composition of the raw and processed *S. sesban* are shown in Table 2. The crude protein (CP) level was slightly higher (34.66%) in the raw than the toasted seed (34.27%). The moisture content of the toasted seed was significantly (P<0.05) lower (9.00%) than the raw seed (9.71%). This may be as a result of the heat while toasting the seed. The ash, crude fiber (CF), and lipid contents 3.55%, 7.55%, and 7.89% respectively were significantly (P<0.05) higher than the toasted samples. Nitrogen-free extract (NFE) in the raw *S. sesban* seed was 37.79% which was significantly (P<0.05) lower compared to the toasted seed (45.36%).

Generally, there was a reduction in the anti-nutrients due to toasting and they differ significantly (P<0.05) with the raw samples.

Haematological indices

The mean haematological indices of *C. gariepinus* fingerlings fed diets with various inclusion levels of TSSSM are presented in table 3. The range of PCV, RBC, and Hb was 23.47 to 27.57%, 2.01 x 10⁶mm⁻³ to 2.39 x 10⁶mm⁻³ and 6.17 to 7.80g/100ml respectively, these values increased as the level of TSSSM increased in the diet significantly (P<0.05), however, these variations are not significantly different (p>0.05). There is no significant (P>0.05) difference between the fish-fed control diet and those fed the other treatments (10, 20, and 30% TSSSM) respectively to the mean corpuscular volume (MCV). However, there was a significant difference (P<0.05) between the MCV value of fish fed 20% TSSSM diet, compared to that fed 10% replacement level.

There was no significant (P>0.05) variation between the mean corpuscular haemoglobin concentration (MCHC) of fish fed the control diet and those fed the other treatment (10, 20, and 30% TSSSM) except for fish fed diet containing 0% TSSSM. The MCHC value of the fish fed 0% TSSSM, was significantly (P<0.05) lower compared to those fed the other treatments (10, 20, and 30% TSSSM). The leucocyte differential count (LDC) parameters (neutrophils, eosinophils, lymphocytes, monocytes, and basophils) recorded, showed no significant (P>0.05) between the fish group fed the control diet and those of the other Monocytes, basophils, and eosinophils however, were not detected in the blood samples collected from fish fed 20% TSSSM diet. The results obtained for the white blood cell (WBC) showed that there was an increase as TSSSM increased in the diet. The fishes fed the control diet and 10% TSSSM diet recorded the values of 186.83 × 10³/mm³ and 201 × 10³ mm³,

respectively. These values showed a significant ($P < 0.05$) difference from the values obtained in fishes fed a diet containing 20 and 30% TSSSM.

DISCUSSIONS

The hematological characteristics of fishes are an essential part of assessing their health status. However, the diet composition, metabolic adaptation and variation in fish activity are the main factors responsible for the change in hematological parameters of fish (Keri Alhadi *et al.*, 2012). The ranges of hematological values recorded in this study is within recommended values suitable for *C. gariepinus*. The packed cell volume (PCV) and haemoglobin concentration (Hb) values in this study are within the range of values (23.0-28.0% and 6-7g/dl) this show an increase as the inclusion of TSSSM increased which is an indication that the fish is not anaemic. The increase in the red blood cell and haemoglobin concentrations in fish may be attributed to the increase in the size of the fish as a result of growth and metabolic activities (Onimisi, *et al.*, 2015). Similar results were also obtained by Okore *et al.* (2016) who incorporated Housefly Larva *Musca domestica* as Feed Supplement. However, the PCV value recorded in this study was higher than the values (0.29-0.37%) obtained by Simeon, (2010) who included poultry hatchery waste at graded levels (0, 25, 50, 75, and 100%).

The red blood cell (RBC) value of ($2.0 \times 10^6 - 2.4 \times 10^6 \text{ mm}^3$) in this study for fish fed 20 and 30% TSSSM diet shows an increase which might be due to increase in size of the fish and possibly increase in fish activities. The increase in RBC might also be as result of high oxygen absorption and transportation capacity of the cells of the fish as opined by (Fagbenro *et al.*, 1993, and Akintayo *et al.*, 2008). According to (Hetch *et al.*, 1996) the presence of arborescent air-breathing organ is responsible for the hardiness of *Clarias gariepinus*.

White blood cells (WBC) and lymphocytes results recorded in this study showed an increase as the level of TSSSM increased in the diet. The increase in the WBC and lymphocytes as TSSSM increased in the diet could be resulting from likely feed toxicity as a result of residual anti-nutrients in the feed. Hence, it is, therefore, likely that the fish with higher inclusions of the TSSSM might be stressed. But the presence of these anti-nutrients was not to a level to induce pathological changes in the fish. White blood cells (WBC) and lymphocytes are the defense cells of the body. Douglas and Jane (2010) demonstrated that the amount has implications in immune responses and the ability of the animal to fight infection.

The mean corpuscular volume (MCV) range (109.17 to 120.27 fl) recorded in this study was lower than (156.33 to 218.0 fl) reported for *C. gariepinus* juveniles by (Simeon, 2010), meanwhile, the mean corpuscular haemoglobin concentration

(MCHC) range (26.13 to 28.30%) recorded in this study for fish fed TSSSM compared fairly well with (28.78 to 37.02%) reported for *C. gariepinus* (Dienye and Olumuji 2014).

The mean corpuscular haemoglobin (MCH) results showed that the fish fed diet containing 10% TSSSM recorded the highest values for the experiment. The MCH range (29.27 to 32.70pg) obtained in this study was lower than the range (51.92 to 72.44pg) reported for *C. gariepinus* juveniles fed poultry hatchery waste incorporated feed (Simeon 2010).

In conclusion, this investigation revealed that a 10% substitution rate of TSSSM for soya bean meal in (*Clarias gariepinus*) diet did not have any adverse effect on the fish as shown by the haematological indices.

It is, therefore, recommended that 10% of toasted *S. sesban* seed meal (TSSSM) could be included in the diet of *C. gariepinus* without adverse effect on its haematology. Other processing methods to reduce the anti-nutrients in the test ingredients such as fermentation, boiling, soaking could be tried.

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Table 1 Gross composition of the experimental diets

Ingredient	Experimental Diet			
	0%	10%	20%	30%
Fishmeal	30.17	30.17	30.17	30.17
Soya bean meal	30.17	27.15	24.14	21.12
TSSSM	0	3.02	6.034	9.05
Wheat offal	14.84	14.84	14.84	14.84
Yellow maize	14.84	14.84	14.84	14.84
Methionine	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5
Premix	0.5	0.5	0.5	0.5
Common salt	0.5	0.5	0.5	0.5
Ascorbic acid	1	1	1	1
Dicalcium phosphate	1	1	1	1
Vegetable oil	1	1	1	1
Binder	5	5	5	5
Total	100	100	100	100

Table 2: Mean Proximate Composition and Anti-nutritional composition of toasted and raw *S. sesban* seed

Parameter (%)	Processing Methods			SEM
	Raw	Toasted		
Moisture	9.00 ^b	9.71 ^a		±0.01
Ash	3.55 ^a	1.41 ^b		±0.34
Crude fiber	7.55 ^a	3.05 ^b		±0.03
Crude protein	34.64 ^a	34.28 ^b		±0.04
Lipid	7.89 ^a	6.17 ^b		±0.04
N.F.E	37.37 ^b	45.45 ^a		±0.20
Levels of Anti-nutrients in the seed				% Reduction
Oxalates	11.68 ^a	7.83 ^b	±0.04	67.5
Alkaloids	1.67 ^a	1.41 ^b	±0.05	84.4
Tannins	7.13 ^a	4.12 ^b	±0.13	57.8

Note: N.F.E; Nitrogen free extract

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Table 3. Mean Haematological indices of *Clarias gariepinus* fingerlings fed graded levels of TSSSM

Parameter	Levels of SBM replaced with TSSSM (%)				
	0	10	20	30	SEM
Packed cell volume (%)	23.47 ^b	25.50 ^{ab}	26.13 ^{ab}	27.57 ^a	±1.55
Haemoglobin conc. (g/dl)	6.17 ^a	7.00 ^a	7.00 ^a	7.80 ^a	±0.76 ^{NS}
Red blood cell count (× 10 ⁶ /mm ³)	2.01 ^a	2.15 ^a	2.39 ^a	2.39 ^a	±0.23 ^{NS}
White blood cell (× 10 ³ /mm ³)	186.83 ^b	201.07 ^{ab}	211.93 ^a	215.83 ^a	±10.26
Mean corpuscular volume (fl)	119.03 ^a	120.27 ^a	118.17 ^a	115.33 ^a	±11.35 ^{NS}
MCH (pg)	30.53 ^b	32.70 ^a	29.27 ^b	32.60 ^a	±0.72
MCHC (%)	26.13 ^a	27.57 ^a	26.80 ^a	28.30 ^a	±2.57 ^{NS}
Platelets	48.33 ^b	33.67 ^b	64.00 ^{ab}	100.00 ^a	±16.34
Differential counts (%)					
Neutrophils	1.67 ^a	1.67 ^a	2.00 ^a	2.00 ^a	±0.33 ^{NS}
Eosinophils	0.33 ^a	0.33 ^a	0.34 ^a	0.33 ^a	±0.41 ^{NS}
Lymphocytes	97.85 ^a	97.83 ^a	98.13 ^a	97.97 ^a	±0.28 ^{NS}
Monocytes	0.33 ^a	0.33 ^a	0.35 ^a	0.33 ^a	±0.41 ^{NS}
Basophils	0.33 ^a	0.33 ^a	0.34 ^a	0.33 ^a	±0.41 ^{NS}

a, b, c, d = Mean within the same row with different superscript differ significantly (P<0.05)

Keys: NS = Not Significant (P>0.05), SBM = soya bean meal, TSSSM = Toasted *Sesbania sesban* seed meal, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration