EFFECTS OF FERMENTED *Hibiscus sabdariffa* SEED BASED DIETS ON HAEMATOLOGICAL PARAMETERS OF *Clarias gariepinus* (BURCHELL, 1822) FINGERLINGS

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ABSTRACT
A Twenty-one week experiment was conducted to evaluate changes in the blood indices of Clarias gariepinus fingerlings fed with five Iso-nitrogenous diets with 40 % crude proteins were formulated by incorporating 0, 25, 50, 75, and 100% of fermented Hibiscus sabdariffa seed meal as a replacement for soya bean meal. A total of 150 fingerlings of C. gariepinus (weight 4.0 - 4.4g) were randomly allocated into fifteen rectangular net hapa (1 m x 1 m x 1.2 m) at 10 fish per hapa installed in a polythene lined pond (7 x 5 x 1.2 m) and fed twice daily at 5% body weight. Fish blood samples were collected to determine blood indices. The results showed that fish fed 75% FHSSM based diet had the best PCV (37.67%) while those on 25% FHSSM -based diet had the least PVC (27.00%). There were elevated values of Hb (8.93 to 13.60g/dl), RBC (2.28-2.57 $x10^6/mm^3$*), WBC (4.15-4.38* $x10^3/mm^3$*), MCV (11.72 – 14.71fl), MCH (52.81-39.60) and MCHC (34.33-47.83) for the entire treatments. Fish fed 75% FHSSM inclusion had the highest Hb (13.60g/dl), RBC (2.57* $x10^6/mm^3$*), WBC (4.38* $x10^3/mm^3$*), MCV (14.71), MCH (47.83) Lymphocytes (76.00%) and neutrophils (42.33%). The haematological parameters indicate that there was no significant difference (P>0.05) among the treatments in most of the parameters. All the indices fell within recommended ranged for healthy fish.*

Key words: Blood sample, *Clarias gariepinus*, Haematology, Fermented *Hibiscus Sabdariffa* seed

INTRODUCTION
Cultures of catfish species offers great potential for their fast growth towards satisfying the national fish demand and thereby reduces fish importation, provide 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, the 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).

There is need to understand the physiological concept of fish health in respect to blood and therefore the quality of dietary protein fed. Any changes within the value of a component of a blood sample, compared to the conventional values, may accustomed the metabolic state and health status of animal. 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, and 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). 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 effects of replacing soybean meal (SBM) with Fermented *Hibiscus Sabdariffa* (HSSM) seed meal as protein sources in the diet of *Clarias gariepinus* fingerlings.

MATERIALS AND METHODS

Collection and Processing of *Hibiscus sabdariffa* seed

Hibiscus sabdariffa seeds were procured from Gamboru market Maiduguri. They were winnowed to remove debris while stones were remove manually and kept in an Air tied bag until required.

Processing Methods of *Hibiscus Sabdariffa* Seed Meal

The seed of *Hibiscus sabdariffa* were subjected to fermentation. Five hundred (500) grams of the *Hibiscus Sabdariffa* seeds were boiled at 1:3 seeds to water for 30 minutes at 100^0C, the water was drained and allowed to cool. The boiled seed were kept in an air tight sack for 72hours to ferment (solid fermentation). The processed seeds of *H. sabdariffa* were sun-dried for three days, ground and labeled as fermented seed.

Table 1: Composition and calculated analysis of the Experimental Diets Ingredients (%)

Ingredient (%)	Levels of SBM replaced with HSSM (%)				
	0	**25**	**50**	**75**	**100**
FHSSM	0.00	10.37	20.75	31.12	41.49
Soya Bean Meal	41.49	31.12	20.75	10.37	0.00
Fish Meal	41.49	41.49	41.49	41.49	41.49
Maize	13.03	13.03	13.03	13.03	13.03
Premix	0.50	0.50	0.50	0.50	0.50
Methionine	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
Ascorbic Acid	0.50	0.50	0.50	0.50	0.50
Vegetable Oil	0.50	0.50	0.50	0.50	0.50
Binder (starch)	1.00	1.00	1.00	1.00	1.00
Salt (NaCl)	0.20	0.20	0.20	0.20	0.20
Di-calcium Phosphate	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
Crude protein	42.91	41.41	39.93	38.42	37.24
Crude fiber	3.16	3.34	3.55	3.76	3.99
Ether Extract	3.87	3.80	3.77	3.71	3.69
Ash	8.15	8.21	8.30	8.35	8.42
NFE	37.75	37.58	37.13	37.29	37.12
ME (Kcal/kg)	3349	3284	3319	3356	3356

*****Min-vitamins premix supplies the following per kg of feed:
Vitamin A = 3400000IU; vitamin D3 = 600 000IU; vitamin E = 4 000 mg; vitamin K3 = 600 mg; vitamin B1 = 640 mg; vitamin B2 = 1 600 mg; pantothenic = 2000 mg; vitamin B6 = 600 mg; vitamin B12 = 4 mg; folic acid = 200 mg; biotin H2 = 300 mg; choline chloride = 70 000 mg; cobalt = 80 mg; copper = 1200 mg; iodine = 400mg; iron = 8000mg; manganese = 16 000 mg; selenium = 80 mg; zinc = 12000 mg and anti-oxidant = 500 mg.
Keys: FHSSM = Fermented Hibiscus sabdariffa seed meal; ME = Metabolisable energy; NFE = Nitrogen-free extract; SBM = soya bean meal. ME (Kcal/kg) is calculated according to the formula of pauzenga (1985) as: 37 x %CP + 35.5 X %NFE

Proximate composition of *Hibiscus sabdariffa* Seed Meal

Proximate composition of raw and Fermented *Hibiscus sabdariffa* seed were carried out according to Near Infrared Reflectance (NIR) methods. The moisture, crude protein, crude fibre, ether extract, ash nitrogen-free extract of each sample was determined in triplicates using Near Infrared Reflectance (NIR) Multi check Analyser.

Experimental Diets

Five iso-nitrogenous diets with 40 % crude proteins were formulated by incorporating 0, 25, 50, 75, and 100% of fermented *Hibiscus sabdariffa* seed meal as a replacement for soya bean meal. These were balanced with other major co-ingredients, using Pearson's Square method along the least cost formulae and labelled as 1 (Control), 2, 3, 4 and 5, respectively. The ingredients were ground, and measured using weighing scale balance as commonly practiced for fish feed preparation. The ingredients were mixed thoroughly and pelleted using (Pelletizing machine), dried, labeled accordingly and stored at room temperature in the laboratory until its ready for use.

Table 2: Mean Proximate Composition of Fermented *Hibiscus sabdariffa* seed Meal

Parameters (%)	Levels of SBM replaced with FHSSM (%)					SEM
	0	**25**	**50**	**75**	**100**	
Dry Matter	93.55[a]	93.55[a]	93.56[a]	93.40[a]	93.80[a]	0.12[NS]
Crude Protein	30.05[e]	33.71[d]	36.34[c]	38.95[b]	40.93[a]	0.01*
Crude Fibre	4.90[a]	4.21[b]	3.85[c]	3.75[d]	3.47[e]	7.07*
Ether Extract	8.00[e]	8.33[d]	8.55[c]	9.21[b]	9.78[a]	7.74*
Ash	6.45[c]	8.40[b]	8.70[b]	3.75[d]	9.85[a]	0.13*
NFE	44.30[a]	38.39[b]	36.11[c]	32.94[d]	29.77[e]	0.02*
Moisture content	6.45a	6.45a	6.44a	6.60a	6.20a	0.06[NS]

Means within the same row with different superscripts differ significantly (P< 0.05).
Keys: SEM = Standard error of Means, FHSSM = Fermented *Hibiscus sabdariffa* seed meal, NFE = Nitrogen free extract, *= Significant (P<0.05), NS= Not significant

Experimental Design

Different replacement levels of the soya bean with fermented *Hibiscus sabdariffa* seed meal; (0 control, 25, 50, 75 and 100 %) were allotted to five groups of *C. gariepinus* fingerlings (4.0 - 4.4g) randomly stocked at 10 fish per unit in 1 m x 1 m x 1.2 m hapa installed in a polythene lined pond (7 x 5 x 1.2 m) in completely randomized design. The treatments were in triplicates. The *Clarias gariepinus* were weighed every four weeks to adjust the feed rate accordingly. The experiment lasted for 150 days.

Blood sample collection

Blood samples were collected from three (3) *C. gariepinus*in each treatment through caudal peduncle puncture as described by AQUALEX (2004) as cited by Diyaware *et al.,* (2013). Approximately 0.1- 0.5 ml of heparin was drawn from ethylene-diamine-tetra-acetic acid (EDTA) bottle using 2 ml plastic syringe with 22-gauge hypodermic needle as described by Diyaware *et al.* (2013) before drawing blood from the fish. The blood sample was collected from each fish and deposited into labeled bottle containing EDTA. The samples were transported in a cold pack to a haematology laboratory, faculty of Veterinary Medicine University of Maiduguri for haematological analysis.

Haematological Analysis

The haematological parameters were determined using AOAC 2000 method, White blood cell (WBC) count was performed with WBC detector block using Direct Current (DC) detection method. Red blood cell (RBC), Haemoglobin (Hb), Packed Cell Volume (PCV) and Platelets (PL) were determined using Haematocrit and micro haematocrit centrifuge. The following blood indices for each of the treatment were estimated using the following formulae: Mean corpuscular volume (MCV fl) $= \frac{PCV}{RBC} X\ 10\ (Miale, 1982)$

Mean corpuscular haemoglobin (MCH pg) $= \frac{Hb}{RBC} X\ 10\ (Miale, 1982)$

Mean corpuscular haemoglobin concentration (MCHC %) $= \frac{Hb}{pcv} X\ 100\ (Miale, 1982$

Statistical analysis of data

Data obtained from the study were subjected to One-way Analysis of Variance (ANOVA). Differences between the means were determined using Least Significant Difference (LSD) at 95% confidence level (P= 0.05) with the aid of Statistix 10.0, a statistical package.

RESULTS

Haematological indices of *Clarias gariepinus* fed replacement of SBM with FHSSM are presented in the Table 3 packed cell volume (PCV) were 29.33%, 27.00%, 31.33%, 37.67% and 31,33% in the five respective diets. There was no significant (P>0.05) difference in PCV values among the treatments. The highest (37.67%) PCV was recorded in fish fed 75% replacement of SBM with FHSSM while, the lowest (27.00%) PCV was recorded in fish fed 25% replacement of SBM with FHSSM diets. The haemoglobin (Hb) values ranged between 8.93 and 13.60g/dl. There was variation in the Hb values of *Clarias gariepinus* fed varying replacement levels of FHSSM. However, Hb in fish fed 25% and 50% replacement of SBM with FHSSM are similar to the Hb of fish fed 0% (control) replacement of SBM with FHSSM, while the highest (13.60g/dl) Hb was recorded in fish fed 75% replacement of SBM with FHSSM, followed by (12.85g/dl) Hb in fish fed 100% replacement of SBM with FHSSM. The lowest (8.98 g/dl) was observed in fish fed 25% replacement of SBM with FHSSM. There was no variation (P<0.05) in fish fed 75% and 100% replacement of SBM with FHSSM. Red blood cell (RBC) values of fish fed varying levels of FHSSM as a replacement for SBM ranged from 2.28 to 2.57 $x10^6/mm^3$. The highest (2.57 $x10^6/mm^3$) RBC was recorded in fish fed 50% and 75% replacement of SBM with FHSSM, while the lowest (2.28 $x10^6/mm^3$) RBC was recorded in fish fed 25% replacement of SBM with FHSSM. There were no significant (P<0.05) differences among the entire treatments group.

Table 3: Haematological Indices of *Clarias gariepinus* Fed Fermented *Hibiscus sabdariffa* Seed Meal Replacing Soya Bean Meal

Parameters	Levels of SBM replaced with HSSM (%)					SEM
	0	25	50	75	100	
Packed Cell Volume (%)	29.33^{ab}	27.00^{b}	31.33^{ab}	37.67^{a}	31.33^{ab}	3.97*
Haemoglobin Conc. (g/dl)	10.96^{ab}	8.93^{b}	10.80^{ab}	13.60^{a}	12.86^{a}	1.62*
Red Blood Cell ($x10^6/mm^3$)	2.52^{a}	2.28^{a}	2.57^{a}	2.57^{a}	2.55^{a}	0.15^{NS}
White Blood Cell ($x10^3/mm^3$)	4.23^{a}	4.15^{a}	4.38^{a}	4.37^{a}	4.32^{a}	0.11^{NS}
MCV (fl)	11.73^{a}	11.73^{a}	12.24^{a}	14.71^{a}	12.40^{a}	1.97^{NS}
MCH (pg)	43.93^{a}	39.60^{a}	41.97^{a}	52.81^{a}	50.32^{a}	6.60^{NS}
MCHC (%)	37.37^{ab}	37.17^{ab}	34.33^{b}	36.60^{ab}	47.83^{a}	5.36*
Differential Count						
Neutrophils (%)	14.33^{a}	14.67^{a}	14.00^{a}	14.33^{a}	16.00^{a}	2.78^{NS}
Lymphocytes (%)	76.00^{a}	74.33^{a}	70.00^{a}	70.33^{a}	72.66^{a}	3.81^{NS}
Basophils (%)	N.D	N.D	N.D	N.D	N.D	0.00^{NS}
Monocytes (%)	N.D	N.D	N.D	N.D	N.D	0.00^{NS}
Esinophils (%)	N.D	N.D	N.D	N.D	N.D	0.00^{NS}

Means with the same superscripts in the same row are not significantly different (P>0.05)
Key: SEM = Standard Error of Mean, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, HSSM = *Hibiscus sabdariffa* seed meal, SBM = Soya bean Meal, * = Significant (P<0.05), NS= Not Significant, ND-Not detected

White blood cell (WBC) value of fish fed varying level of FHSSM ranged from (4.15 to 4.38 $x10^3/mm^3$). The highest (4.38 $x10^3/mm^3$) WBC was record in fish 50% replacement of SBM with FHSSM, followed by (4.32 $x10^3/mm^3$) WBC in fish fed 100% replacement of SBM with FHSSM. The lowest (4.15 $x10^3/mm^3$) was observed in fish fed 25% replacement of SBM with FHSSM diet. There were no significant (P<0.05) difference among the entire treatment.

MCV value of fish fed varying levels of FHSSM ranged from (11.72 to 14.71fl) with the highest (14.71) in fish fed 75% replacement of SBM with FHSSM, followed by (12.40fl) in fish fed 100% replacement of SBM with FHSSM and the least (11.72fl) MCV was recorded in fish fed 0% (control) and 25% replacement of SBM with FHSSM diet. There were no significant (P<0.05) difference among the entire treatments.

MCH value was highest (52.81 pg) in fish fed 75% replacement of SBM with FHSSM diets, followed by (50.32 pg) in fish fed 100% replacement of SBM with FHSSM while the lowest (39.60 pg) MCH was recorded in fish fed 25% replacement of SBM with FHSSM diets. There were no significant (P<0.05) difference among the treatment groups.

MCHC value of fish fed replacement of SBM with FHSSM ranged from 34.33 to 47.83 %. The highest (47.83 %) MCHC was recorded in fish fed 100% replacement of SBM with FHSSM diets, followed by (37.37 %) in fish fed 0% (control). The lowest (34.33 %) MCHC value was recorded in fish fed 50% replacement of SBM with FHSSM. There was no significant difference (P<0.05) in fish fed 0, 25, 50 and 75% replacement of SBM with FHSSM diets. However, fish fed 100% replacement of SBM with FHSSM differed (P>0.05) with fish fed 50% replacement of SBM with FHSSM diets.

The leucocyte differential count (LDC) parameters (neutrophils, eosinophils, lymphocytes, monocytes and basophils) recorded in fish fed replacement of SBM with FHSSM showed no significant variation (P>0.05) between the fish group fed 0% (control) replacement of SBM with FHSSM and those groups fed diets containing 25, 50, 75 and 100% replacement of SBM with FHSSM. Monocytes, basophils and eosinophils however were not detected in the blood samples collected from the various groups fed diet with graded levels of FHSSM as a replacement for SBM.

DISCUSSIONS

The packed cell volume (PCV) values recorded in this study were higher than the value (20.01 – 20.16%) reported by Gabriel *et al.* (2011). Similar values of PCV (26.33-36.33%) were reported by Aderolu *et al.* (2018). The results (25.23-33.23%) obtained by Fagbohun *et al.* (2019) were also close to the results (27.00 – 37.67%) recorded in this study. However, all the values (20.00 to 37.67s) are within the range for healthy fish as reported by Akinrotomi *et al.* (2011). The haemoglobin concentration (Hb) value in this study were within the range (8.6-10.58 g/dl) reported by Fagbohun *et al.* (2019). Similar value of (Hb) (7.98-10.87 g/dl) were reported by Onimisi *et al.* (2015). Fagbohun *et al.* (2019) observed Hb value (8.6 – 10.58 g/dl) for *C. gariepinus* fed vary level of roselle leaf meal, While Aderolu *et al.* (2018) reported values of (8.76-10.13 g/dl) which are close to the values reported in this study. High value (Hb) 15.31 g/dl were documented by Kori-soakpere and Ubogu,

(2008) for juvenile hybrid as well as 13.00 g/dl recorded for *C. gariepinus* by Ogueji *et al.* (2017) which are similar to 13.60 g/dl (Hb) recorded in this study. This variation may be due to differences in the species, environment and the diets.

Similarly, the red blood cell (RBC) values (2.28-2.57x10^6/mm^3) in this study are similar to the value of *Heteroclarias* (2.46x10^6/mm^3) as reported by Diyaware *et al.* (2010). Higher value of (RBC) 3.53 – 4.02 were reported by Fagbohun *et al.*, (2019) for fish fed roselle leaf meal. Aderolu *et al.* (2018) reported higher value (3.48 – 3.75 x10^6/mm^3) (RBC) in *C. gariepinus* fed 35% CP diet. Lower value of (1.90 x10^6/mm^3) (RBC) was reported by Onimisi *et al.* (2015) for *C. gariepinus* fed 100% fermented *Senna obtusifolia* seed meal. White blood cell value 4.15 – 4.38 reported in this study are close to the WBC value 4.25 – 4.87 (Onimisi *et al.*, 2015). However, lower value (2.09x10^3/mm^3) in *C. gariepinus* fed *Lagenaria vulgaris* as reported by Mamman *et al.* (2013). Similar report by Omitiyin (2006) who observed WBC decreased from 9.2 to 5.0 x 10^3/mm^3 in *C. gariepinus* fed poultry litters. WBC are higher in adult fish than juvenile as observed by Adeyemo *et al.* (2012). Higher value (98.23x10^3/mm^3) WBC was reported by Diyaware *et al.* (2010) for *C. anguillaris* juveniles. MCV values recorded in this study ranged from (117.3 - 147.1 fl) which are similar to the values (117.68 – 138.07 fl) reported by Diyaware *et al.* (2010) for *C. anguillaris* juvenile and *H. bidorsalis* respectively. Similar values 126.0 – 147 fl were reported by Onisimi *et al.* (2015) for *C. gariepinus* fed fermented 0 and 75% *Senna obtusifolia* seed meal. Lower value of (74.96 – 82.10 fl) MCV were reported by Aderolu *et al.* (2018) for *C. gariepinus* fed 35% CP diet. MCH value in this study were similar to the value (41.30 pg) reported for *Clariabranchus* by Diyaware *et al.* (2010). Lower values 24.93- 27.30 were reported by Aderolu *et al.* (2018) for *C. gariepinus* fed 35% CP diet. The lymphocytes values reported in this study ranged from 70.33 – 76.00%. higher value by various authors 90.00 – 98.00% Onimisi *et al.*, (2015), 98.23%, 98.03% and 98.30% values were reported by Diyaware *et al.*, (2010) for *C. anguillaris, Clariabranchus* and *Heteroclarias*, respectively.

CONCLUSION

The study revealed that fermented *Hibiscus sabdariffa* seed meal had no deleterious effects on the fish as evidenced by the haematological parameters which were within the normal range for healthy fish.

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