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HAEMATOLOGICAL PROFILE OF BLOOD OF AFRICAN CATFISH (*Clarias gariepinus*, Burchell 1822) FED SUNFLOWER AND SESAME MEAL BASED DIETS

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ABSTRACT

The effect of substituting soybean meal with raw sunflower and sesame seed meal was evaluated using haematological indices of Clarias gariepinus fed the diets containing sunflower and sesame seed meal. Seven isonitrogenous and isocaloric diets containing sunflower and sesame seed meal replacing soybean meal at a rate of 15, 30 and 45% were prepared. A diet without replacement of soybean meal served as control. The result of fish fed test diets showed significant ($P < 0.05$) difference over the fish fed control diet with respect to PCV and mean corpuscular volume (MCV). Fish fed control diet had the lowest H_b , PCV, WBC, RBC, MCV, MCHC, ESr. There was no significant difference ($P > 0.05$) in the fish fed control diet and test diets with respect to MCH, MCHC, ESr, Neutrophil, Monocytes, Eosinophils and Lymphocytes. Hence it can be concluded that the haematological parameter of Clarias gariepinus is not significantly ($P > 0.05$) changed with increase dietary inclusion of raw sunflower and sesame seed meal.

INTRODUCTION

Catfish farming and indeed aquaculture offer strong potential for growth to meet the natural fish demand thereby reducing importation, provides employment, alleviates poverty and helps to meet the millennium development goals (William et al., 2007). There are many culturable catfish species which are but not limited to *Clarias gariepinus*, *C. anguillaris*, *Heterobranchus bidorsalis*, *H. longifilis*, *C. isheriensis*, *C. submarginatus*, *Chrysichthys nigrodigitatus*, *Bagrus spp.*, *Synodontis Sp.* But *Clarias gariepinus* is the most cultured fish in Nigeria and indeed Africa (Garibaldi, 1996). Its hardiness, omnivory, ability to withstand adverse condition, high fecundity and mass artificial seed technique also ease its culture (Haylor, 1992, Hecht et al., 1996). Ichthyohaematological investigation serves mainly for diagnostic purpose; apart from this main purpose, it is also used to assess suitability of feeds and feed mixture pellets, to evaluate the effect of stress situation etc. (Svobodova et al., 1991). As with all animal production, disease is considerable constraint in production, development and expansion in the aquaculture industry. There can be a wide variation in the quality of diet received by farmed fish. Factors include the poor availability of suitable constituents, poor formulation and processing, lack of knowledge and understanding of dietary requirement or in appropriate storage (Southgate, 1993). Soybean meal (SBM) is currently the most commonly used plant protein source in fish feed (El-Sayyed, 1999). However, other plant protein sources which are less expensive would be

beneficial in reducing feed cost when made to replace soybean meal (Barros *et al.*, 2002).

Blood analysis is a valuable means of evaluating the physiological condition of cultured fish with respect to determining the effect of diets and other stress factors on fish health. Changes in haematology of fish in response to stressing agents are indicators of the stressful stage of fish, producing useful information to curb any unfavourable condition that may affect the fish health. (Bello-Olusoji *et al.*, 2006). Adeparusi and Ajayi (2004) reported that analysis of blood is an important factors that could be considered in fish feed assessment. Hence, this study investigates the haematological characteristic of *Clarias gariepinus* fed with sesame and sunflower meal based diets replacing soybean meal based diet. The use of haematological values as indices of diagnosing diseases and stress induced condition as well as for feed assessment is well documented (Fagbenro *et al.*, 1993; Adeparusi and Ajayi, 2004; George *et al.*, 2007; Yue and Zhou, 2008; Akintayo *et al.*, 2008).

MATERIALS AND METHODS

Sources and Processing of Ingredients

The dehulled seeds of sunflower and sesame were obtained from a farm in Kebbi State, Nigeria. They were ground in a hammer mill and the oil therein was removed using the pressure generated from locally made screw press (cassava-presser type). The cakes therefore were analyzed for their proximate composition (AOAC 1990). Fish meal, soybean meal and other feedstuffs obtained from commercial sources in Nigeria were separately milled screened to fine particles size and triplicate samples were analyzed for their proximate composition (AOAC, 1990). Based on the nutrient composition of the protein feed stuff (Table 1), a control diet and six test diets (40% crude Protein, 12% crude Lipid, 18 MJ energy) were formulated. The control diets contained soybean meal, providing 50% of the total protein. Three of the test diets contained sunflower meal protein at a replacement levels of 15, 30 and 45% for the soybean meal to serve as test diets RSF₁₅, RSF₃₀, and RSF₄₅ respectively while the other three contained sesame meal protein at the same replacement levels for the soybean meal to serve as test diets RSM₁₅, RSM₃₀, and RSM₄₅ respectively (Table 2). The feedstuffs were blended, moistened, steam pelleted and oven dried for 24hrs.

Table 1: Proximate Composition of Carcass of Protein Feed Ingredients

Parameter	Fishmeal	Soybean Meal	Sunflower Meal	Sesame Meal	Corn Meal
Moisture	7.59	8.92	9.48	8.39	9.21
Crude Protein	69.76	42.81	40.01	42.21	8.89
Crude Lipid	8.82	18.56	20.28	15.92	1.49
Crude Fibre	-	5.63	12.80	5.48	29.78
Ash	13.83	6.01	5.89	7.27	3.81
NFE	-	18.07	11.54	20.73	46.82

Table 2: Gross and Proximate Composition (G/100g Dry Matter) of Experimental Diets fed to *Clarias gariepinus* at Varying Replacement Levels of Raw Samples of Sunflower and Sesame Seedmeal Based Diets.

Ingredients	Experimental Diets						
	CTR	RSF ₁₅	RSF ₃₀	RSF ₄₅	RSM ₁₅	RSM ₃₀	RSM ₄₅
Fishmeal	27.24	27.24	27.24	27.24	27.24	27.24	27.24
Soybean meal	46.71	39.71	32.70	25.70	39.71	32.70	25.70
Raw samples	-	7.48	14.96	22.44	7.08	14.16	21.25
Corn meal	11.25	11.25	11.25	11.25	11.25	11.25	11.25
Fish oil	5.09	5.09	5.09	5.09	5.09	5.09	5.09
Vita/Min	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Premix							
Starch	4.76	4.22	3.76	3.28	4.63	4.26	4.17
Total	100	100	100	100	100	100	100
Proximate Composition							
Moisture	9.17±.15 ^b	9.67 ± .72 ^a	9.36±.46 ^{ab}	9.57±.09 ^{ab}	9.39 ±.19 ^{ab}	9.43 ± .26 ^{ab}	9.37±.15 ^{ab}
Crude protein	40.60±.72	40.47 ± .46	40.28 ± .87	40.18 ± .28	40.48±.61	40.42 ± .36	40.24±.38
Crude lipid	11.74±.04 ^c	12.18±.12 ^{ab}	12.28±.05 ^a	12.32±.05 ^a	11.93±.12 ^{bc}	12.33 ± .06 ^a	12.46±.35 ^a
Crude fibre	5.84±.10 ^b	5.84 ±.08 ^b	6.44±.29 ^a	6.44±.28 ^a	5.53 ±.28 ^b	5.90 ±.04 ^b	5.87±.22 ^b
Ash	9.35±.10 ^a	8.71±.24 ^{bc}	8.80±.11 ^{bc}	9.04±.47 ^{ab}	8.91±.13 ^{abc}	8.81 ±.11 ^{bc}	8.51±.27 ^c
NFE	23.13±.74	23.42 ± .39	23.05 ± .75	22.46 ± .13	23.76 ± .93	22.92 ± .26	23.55 ± .94

Culture Condition

Clarias gariepinus fingerlings were acclimated to experimental condition for 7 days prior to the feeding trial. Groups of 15 catfish fingerlings (3.38 ± 0.015 g) were stocked into aquaria comprising 60 litre-capacity rectangular plastic tanks. Each diet was fed to the catfish in triplicate tanks twice daily (09.00h, 16.00h) at 5% body weight for 70 days. Fish was removed from each tank every 14 days and batch-weighed and the amount of feed was adjusted accordingly. Eight catfish and 6 catfish per treatment were respectively sacrificed at the beginning and end of the feeding trial respectively

HAEMATOLOGICAL STUDIES

The blood analyses were determined according to the method described by Svobodova *et al.*, (1991). The following were done.

Blood Analysis

5–10ml blood samples were collected from cardiac puncture using 2ml disposable heparinised syringe treated with EDTA as anti-coagulant.

Blood Cell Count

Haemocytometer was used in blood cell count. The blood diluting fluid was prepared as described by Svobodova *et al.*, (1991). The blood cells were counted on the counting chamber of haemocytometer with the aid of compound microscope.

RBC = No of cells counted x 3 x 10 x 200
(10⁶mm³)

WBC = no of cells counted x 0 x 25 x 10 x
20 (10⁴mm³)

Haemoglobin Estimation

Haemoblobinometer was used for haemoglobin estimation based on acid haematin method (SAHLI)

Haemoglobin =

$$\frac{\text{Value obtained} \times 17.2 \text{ mg/100ml}}{100}$$

Packed Cell Volume

The packed cell volume was measured after placing sealed microhaematocrit tube in a centrifuge at 10,500rpm using microhaematocrit reader and expressed as percentage.

Erythrocyte Sedimentation Rate (ESR)

ESR was determined the procedures of Svobodava et al (1991). The volume of ESR with the given time interval is the difference between 100% and the percentage part presented by the corpusde volume Mean Corpuscular Volume (MCV) was calculated from the haematocrit value (PCV, % and the Erythrocyte count (Er mm³)

$$\text{MCV } (\mu^3) = \frac{\text{PCV} \times 10}{\text{Er}}$$

Mean Corpuscular Haemoglobin Concentration (MCHC).

This was obtained using the formula

$$\text{MCHC}(\%) = \frac{\text{Hb} \times 100}{\text{PCV}}$$

Mean Corpuscular Haemoglobin (MCH)

This was expressed in picogrammes (pg)

$$\text{MCH} (\text{pg}) = \frac{\text{Hb}}{\text{Er}} \times 10^2$$

Statistical Analysis

Data obtained from the experiment was expressed in mean \pm SD and it was subjected to one way Analysis of Variance (ANOVA) using SPSS 16.0 version. Where the ANOVA reveals significant difference (P<0.05) Duncan multiple range test was used to compare differences among individual treatment means.

RESULTS AND DISCUSSION

The summary of comparison of haematological parameters of *Clarias gariepinus* fed sunflower and sesame meal based diets replacing soybean meal at different levels is presented in Table 4.

Table 4:HAEMATOLOGICAL PROFILE OF BLOOD OF *Clarias gariepinus* FED RAW SUNFLOWER AND SESAME MEAL BASED DIETS.

Parameters	Experimental Diets						
	CTR	RSF ₁₅	RSF ₃₀	RSF ₄₅	RSM ₁₅	RSM ₃₀	RSM ₄₅
Hb	4.7 \pm .57	9.55 \pm 2.05	10.7 \pm .85	10.75 \pm .35	10.85 \pm 4.88	7.65 \pm 4.31	5.4 \pm .99
PCV	14 \pm 1.42 ^b	28.5 \pm 6.36 ^{ab}	32 \pm 2.82 ^{ab}	34.75 \pm 1.34 ^a	32.5 \pm 14.84 ^{ab}	23.00 \pm 12.72 ^{ab}	16.00 \pm 2.83 ^{ab}
WBC	3600 \pm 707.10	6050 \pm 636.40	6000 \pm 1697.06	6075 \pm 388.91	6600 \pm 1697.06	4850 \pm 2050.61	3800 \pm 141.42
RBC	1.85 \pm .21	3.35 \pm .78	3.7 \pm .42	3.95 \pm .35	3.90 \pm 1.84	2.78 \pm 1.31	2.05 \pm .21
MCHC	33.5 \pm .71	33.5 \pm .28	33.4 \pm .28	33.45 \pm .35	33.40 \pm .28	33.10 \pm .42	33.65 \pm .21
MCV	75.70 \pm .99 ^b	85.15 \pm .78 ^{ab}	86.60 \pm 2.26 ^a	83.50 \pm 4.24 ^{ab}	83.65 \pm 1.34 ^{ab}	81.15 \pm 7.57 ^{ab}	77.75 \pm 5.73 ^{ab}
MCH	25.4 \pm .14	28.55 \pm .50	29.00 \pm .99	27.30 \pm 1.56	28.00 \pm .71	26.9 \pm 2.83	26.2 \pm 2.12
ESR	4.50 \pm .42	4.55 \pm 1.76	4.60 \pm .42	4.65 \pm .92	4.55 \pm .92	4.60 \pm .71	4.75 \pm .78
Neutrophils	49.00 \pm 1.41	59.00 \pm 11.31	63.50 \pm 2.12	64.45 \pm 5.16	64.50 \pm 6.36	65.50 \pm 12.02	62.00 \pm 14.14
Lymphocytes	50.00	41 \pm 11.31	35.50 \pm 3.54	32.30 \pm 3.11	35 \pm 7.07	33 \pm 11.31	38 \pm 14.14
Monocytes	1 \pm 1.41	ND	ND	ND	.50 \pm .71	1.5 \pm .71	ND
Eosinophils	ND	ND	1 \pm 1.41	1 \pm .71	ND	ND	ND
HIS	1.76 \pm 0.00	1.81 \pm 0.025	1.81 \pm 0.08	1.74 \pm 0.08	1.82 \pm 0.03	1.80 \pm 0.57	1.77 \pm 0.32

Figures in each row with different superscript are significantly different (P<0.05) from each other.

Figures in rows without superscripts are not significantly different (P>0.05) from each other

The Erythrocyte count (Er) ranges between 10.85 in fish fed RSM₁₅ to 4.7 in fish fed diet CTR. Er increases with increment in levels of sunflower inclusion and decreases with level of sesame seed meal inclusion. A measurable increase in the haematological parameter observed in the fish fed test diets with respect to fish fed control diets was also observed by Akintayo *et al.*, 2008 who fed the toasted sunflower seed meal to *Clarias gariepinus* and Yue and Zhou, 2008 who fed cotton seed meal to juvenile hybrid Tilapia, Barros *et al.*, (2002) for channel catfish and El-Saidy and Gaber (2004) for Nile Tilapia. Although, the result trend deviates from the trend reported by Adeparusi and Ajayi (2004), who fed Lima bean to *Oreochromis niloticus*. Other researchers who have also reported a decrease in haematocrit and haemoglobin with increase level of ingredients are Blom *et al.*, 2001, Dabrowski *et al.*, 2001, Richard *et al.*, 2003. However there was no significant ($P>0.05$) difference in the Er of fish fed control diet and the fish fed test diets. The Leucocytes counts also ranges between $6.6 \times 10^6 \text{mm}^3$ in fish fed diet RSM₁₅ and $3.6 \times 10^6 \text{mm}^3$ in fish fed diet CTR. Same pattern as recorded for Er was observed for WBC. There was no significant ($P>0.05$) difference in fish fed control diet and fish fed test diets with respect to MCH, MCHC, ESr. However significant ($P<0.05$) variation existed in the packed cell volume (PCV) and mean corpuscular volume (MCV) of fish fed the control diets and other test diets. The highest PCV was obtained in the blood of fish fed diet RSF₄₅ while the lowest PCV was obtained in the fish fed diet CTR. However there was no significant difference ($P>0.05$) in the PCV of the blood of fish fed diets RSF₁₅ RSF₃₀, RSF₄₅, RSM₁₅, RSM₃₀, and RSM₄₅. The highest MCV was obtained in the blood of fish fed RSF₃₀ while the lowest was in the fish fed diet CTR. There was no significant ($P>0.05$) variation in the MCV of fish fed other test diets apart from RSM₃₀. There was no significant ($P>0.05$)

difference in the Leucocytes, Eusinophils, Monocytes and Lymphocytes of fish fed control diets and other test diets. Akinwade *et al.*, 2004 opined that a measurable increase in white blood count of fish or any animal is a function of immunity and animals' resistance to some vulnerable illness or disease. This increase might indicate that the fish under study had high immunity or resistance to disease. George *et al.* (2007) observed that when 50% fish meal was replaced by soybean meal in the diet for *Clarias gariepinus*, there was increase in PCV, HB, and RBC of the fish fed the diet. High value of Erythrocyte count recorded in this study was also observed by Fagbenro *et al.*, 1993, and Akintayo *et al.*, 2008 which indicates high oxygen absorption and transportation capacity of the cells of the fish under study. Hecht *et al.*, 1996 reported the hardiness of *Clarias gariepinus* to be due to the presence of aborescent air-breathing organ. The decrease in haematological parameter with increasing level of incorporation of sesame meal might not be unconnected to the presence of tannin and phytate present in the seed meal. Wedemeyer and Yasutake, 1981 reported that blood infection might reduce haematocrit value and erythrocyte count. The result agreed with the observation of Blom *et al.*, 2001, Dabrowski *et al.*, 2001, Richard *et al.*, 2003. But the presence of these anti-nutrients was not to a level to induce pathological changes in the fish. Thus it can be concluded that not much stress is placed on the health of *Clarias gariepinus* fed RSM₄₅ and sesame seed meal replacing soybean meal at RSM₁₅, RSM₃₀ and RSM₄₅.

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