Research Journal of Food Science and Quality Control (RJFSQC) E-ISSN 2756-5483
P-ISSN 2695-2459 Vol 9. No. 3 2023 www.iiardjournals.org

Haematology of fingerlings of all Males Nile Tilapia *Oreochromis niloticus* fed with differently processed lima bean (*Phaseolus lunatus*) seed meal

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DOI: 10.56201/rjfsqc.v9.no3.2023.pg78.91

ABSTRACT
This study investigates the haematological effects of fingerlings of Oreochromis niloticus fed with differently processing methods of (Phaseolus lunatus) lima bean seed meal in the fisheries research centre of the Moshood Abiola Polytechnic Abeokuta. 10 fingerlings each of Oreochromis niloticus were placed in plastic tanks and fed for 13 weeks with feed containing different processed lima beans control, raw, autoclave, fermented and germinated P. lunatus lima beans. The substitutions were at the rate of 10%, 20% and 30%, in triplicate and the control diet. The mean temperature was (24.10±0.03_27.50±0.150C). pH (6.90±0.20-7.35±0.20) and dissolved oxygen was 4.50±0.20-4.79±0.50mh/l. The proximate analysis of P. lunatus showed crude protein highest in Fermented Lima Bean Seed Meal (FLBSM) 35.00±0.25%. This had a positive effect on the growth of O. niloticus. The determination of haematological parameters followed standard procedures. The Packed Cell Volume (PCV) (50.00±$1^0$28%) and Haemoglobin (Hb) (16.70±$1^0$89g/dl) value were highest in fish fed with 30% Autoclaved Lima Bean Seed Meal (ALBSM), while the least Packed Cell Volume (PCV) (29.00±1.52%, and Hb (9.82±0.25g/dl) were obtained in fish fed 10% Raw Lima Bean Seed Meal (RLBSM). The Raw Lima Bean Seed Meal (RLBSM) increases in the Red Blood Cell (RBC) and White Blood Cell (WBC) as inclusion level increases while Autoclaved Lima Bean Seed Meal (ALBSM). Feed Lima Bean Seed Meal (FLBSM) and Germinated Lima Bean Seed Meal (GLBSM) showed a significant decrease (p>0.05) among the Red Blood Cell (RBC), and White Blood Cell (WBC). Conclusively, 30% level of substitution in Autoclaved Lima Bean Seed Meal (ALBSM) yielded an appreciable haematological parameter.

Keywords: *Red blood cell, Oreochromis lunatus, Phaseolus lunatus.*

INTRODUCTION

The availability of good and quality feeds in needed by fish farmers. The use of soybean and a means of protein sources is not sufficient as there is competition between man and livestock leading to high cost of soybean. Hence there is urgent need for a ready alternative for other plant protein rich plant that can supplement or substitute the conventual protein feed ingredient (Sotolu and Faturoti, 2009). Lima beans *Phaseolus lunatus* is a warm season plant (NRC, 1993) propagated through its seed. National Academy of Science (NAS) 1979 assessed the nutrient value of some lima beans varieties and gave protein content 21.4 to 36/1%; fat 1.00 to 2.2% as 0.3.4 to 17.9%; fibre 01.7 to 04.9% carbohydrate 55.6% to 73.2% Adeniji et al., (2021)

obtained Ash 1.50 to 5.50%; moisture 2.50 to 12.10% crude lipid 2.61 to 4.30%; crude fibre 4.51% to 5.31%; and crude protein 27:42 to 35:00 and carbohydrate 43.0% to 47.89%. From proximate lima beans analysis the presence of anti-nutritional factors such as saponin, phytic acid and oxalic acid has been a major setback in the use of the benefit of its nutritional status.

The haematological parameter of fish is important as it provide the nutritional as well as health status of the fish. (Wagner et al., 1997), (Idris et al., 2005). Haematological characteristic of most fish have been studied with the aim of establishing normal value range. And deviation from it may indicate a disturbance in the physiological process (Shah and Altin dag, 2004), (Ogunji et al., 2005).

Different factors such as nutrition, size, age, genetic properties, insufficient food supply, diet, population density and stressors have been reported to affect the haematological status of fish (Binu Kamar et al., 2011), (Akinmuimi et al., 2002; Okoh et al., 1998). The effect of some nonconventional rich plant ingredients such as *Vigna Subterannae*, Jack Bean, Meal, Moriga Leaf, Pigeon Pea; sesame meal as well as Carica Pipaya Leaf Meal on haematological parameters have been well documented Osuigwe et al., 2007; Sotolu and Faturoti, 2009; Fagbenro et al., 2013; Diaz et al., 2006; Agbo et al., 2017.

Lima bean *Phaseolus lunatus* has a relative high protein content, it is available in Nigeria and other sub sahara region. This study evaluated the haematological response of all males *Oreochromis niloticus* fingerlings to the different processing methods of *P. lunatus* seed meal.

MATERIALS AND METHODS

The experiment was conducted at the Fisheries Research Centre at the biological garden of Moshood Abiola Polytechnic Abeokuta which is within latitude $6^0.21^1$ and $7^0.10$N; and longitude 2^031^1 and 3.33^0 E in the south western zone of Nigeria.

COLLECTION AND PROCESSING OF *Phaseolus lunatus*

1000g *Phaseolus lunatus* were obtained from Itoku Market in Abeokuta South Local Government of Ogun State. The lima beans were handpicked and thereafter assigned into 5 different portions of treatment: raw, autoclaved, fermented, germinated and control.

Raw: A batch of 200g raw or unprocessed lima beans was grounded and kept in an air-tight container for chemical analysis and later incorporated into the diet.

Autoclaved Lima Beans: This method of processing was described by Johnson et al., (1991). 200g of the lima seeds were autoclaved at 121^0C at 15ppm: grounded and kept in an air tight container for further chemical analysis.

Fermented Lima Beans: 200g of the lima beans were taken for fermentation according to (Ruskin and Djen, 1974) where distilled water (5ml) was added to pure culture of *Rhizopus* oligosporus on a taoge sucrose agar for 7days @ 37^0C. Suspended spores were scrapped off and mixed with cooked beans. The inoculated beans were spread to a loose layer of aluminium tray. Lima beans were incubated at 37^0C in a standard incubator without air. Dried inoculated

beans were oven dried @ 50^0C. After which it was grounded into flour and kept in polythene for further chemical analysis.

Germination: About 200g of lima beans was used as described by Esonu et al., (1988). A portion of lima beans were covered with moisten cloth using distilled water for three days at room temperature. After sprout for 72 hours, harvested sprint is dried at 72^0C. Later processed into flour and stayed in air tight container for chemical analysis.

Control Treatment: Lima beans were absent in this treatment.

Formulation of Experimental Diet

Imported fish feed Eco float (68% crude protein, vitamin premix, groundnut cake, maize, soybean cake, methionine was purchased from a reputable feed company Animal Arena (Nig) Ltd in Abeokuta while salt and vegetable oil were purchased from a local market in Abeokuta.

Each feed ingredient was weighed according to the formulation (person's square method). They were grounded into fine powder; these ingredients were later mixed together pelleted into 2 min using an ad locally fabricated pellatizing machine. This is according to method described by Gabriel et al., (2007).

The pellets were sundried. Three experimental diets containing 10%, 20% and 30% *Phaseolus lunatus* were formulated. While fish meal based diet served as control. The different samples are Raw Lima Beans Seed meal (RLBSM), Autoclaved Lima Beans Seed Meal (ALBSM) Fermented Lima Beans Seed Meal (FLBSM), Germinated Lima Beans Seed Meal (GLBSM). The different samples of each diet were kept for proximate analysis, AOAC, (1990). While the remaining sample was packed into polytene bag. The gross composition of the experimental diets is show Table 1.

Ingredients	CONTROL 0%	RLBSM 1 10%	RLBSM 2 20%	RLBSM 3 30%	ALBSM 1 10%	ALBSM 2 20%	ALBSM 3 30%	FLBSM 1 10%	FLBSM 2 20%	FLBSM 3 30%	GLBSM 1 10%	GLBSM 2 20%	GLBSM 3 30%
Fish meal (68%)	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Soy bean meal (40%	41.90	39.31	36.49	33.40	39.23	36.33	33.19	38.33	34.64	30.82	38.70	35.33	31.77
Groundnut cate (40%)	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Maize (4%)	21.60	20.82	18.89	16.79	20.91	19.08	17.09	21.91	21.20	20.47	21.50	20.34	19.11
P. lunatus (Raw)	-	4.37	9.12	14.31	-	-	-	-	-	-	-	-	-
P. lunatus (Auto)	-	-	-	-	4.36	9.08	14.22	-	-	-	-	-	-
P. Lunatus (Fermented)	-	-	-	-	-	-	-	4.26	8.66	13.21	-	-	-
	-	-	-	-	-	-	-	-	-	-	4.30	20.34	13.62
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

P. lunatus (Germinated)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lysine	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Methonine	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Premix	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Salt													
Vegetable Oil													
TOTAL %													

Table 1: Gross Compositions of experimental diets

Experimental Fish and Procedure

Three hundred and Seventy fingerlings of all males Tilapia *Orechromis niloticus* were purchased from premium an Aqua Limited Igboora in Oyo State and transported in 50 litres "Jerry cans". The fingerlings were acclimatized in a rectangular plastic tank in the Fisheries Research Centre of Moshood Abiola Polytechnic Abeokuta for 14days and fed with coppers (an imported feed from Belgium) every morning to remove left over feed and faces were flushed out every morning. Dead fish were removed and recorded.

The control consists of commercial feed. Each treatment had three triplicates. The fishes were fed at 3% body weight twice daily (Lovell et al., 1989) for 13 weeks. Water quality parameters (Table) were taken twice a week. The parameters include water temperature using mercury in glass thermometers calibrated in degree a company from U.K. The fingerlings were than starved for 24 hours prior to the commencement of the experiment. The fingerlings were randomly sorted and stocked at 10 fingerlings per tank.

A total of 39 white plastic 50 litres capacity (49.0cm x 33.5cm) were used for the trial experiment. Water was supplied from a bole hole on the campus, filled with overhead plastic tank of 5,000 litres capacity. The tank was cleaned. Dissolved oxygen was determined using oxygen meter (Hanne model D, 1946) while the pH was determined with pH meter (pH - 099) model.

Table 2: Proximate composition of differently processed lima bean seed meal.

PROCESSING METHODS PARAMETERS (%)	RLBSM	ALBSM	FLBSM	GLBSM
Ash content	5.50±0.75^{a}	3.51±0.24^{b}	5.00±0.01^{a}	1.50±0.41^{c}
Moisture content	11.51±0.02^{b}	10.00±3.10^{c}	2.50±0.31^{d}	12.10±0.20^{a}
Crude lipid	2.61±0.21^{b}	4.01±0.22^{a}	3.30±0.01^{b}	4.30±0.35^{a}
Crude fibre	5.21±0.11^{a}	5.13±0.01^{a}	5.31±0.03^{a}	4.81±0.05^{a}
Crude protein	27.42±0.07^{c}	28.00±0.41^{c}	35.00±0.25^{a}	32.08±0.01^{b}
Nitrogen free extract	1.22±0.05^{c}	3.18±0.02^{a}	1.00±0.25^{c}	2.15±0.03^{b}
Carbohydrate	46.54±0.05^{a}	46.17±0.10^{a}	47.89±0.00^{a}	43.06±0.40^{b}

KEY:
RLBSM – Raw Lima Bean Seed Meal
ALBSM – Autoclaved Lima Beans Seed Meal
FLBSM – Fermented Lima Beans Seed Meal
GLBSM – Germinated Lima Bean Seed Meal

Haematological Parameters

At the end of the feeding trial, two fishes per tank were randomly taken from the 13 treatments. Blood samples were taken from the fishes according to methods described by Wu et al., 2004. The fish was placed on its back in a triangle and blood was collected with a razor to cut the

operculum. The blood was emptied into EDTA (Ethylene Diamine Tetra Acetate), pre treated bottles to prevent coagulation. After which the blood was analyzed according to the haematological procedures as described by (Klontz and Smith et al., 1986). Haematocrit was determined by centrifugation of blood in heparinised capillary tube (with one end sealed) using haematocrit centrifuge method Haemoglibin was determined calorimetrically by cyamethemoglobin method. Red blood cell and leukocyte counts were determined according to (Hyduke et al., 1975).

Table 3: Haematological profile of *Orechromis niloticus* fingerlings fed on differently processed lima bean seed meal

TREATMENTS	CONTROL	RLBSM 1	RLBSM 2	RLBSM 3	ALBSM 1	ALBSM 2	ALBSM 3	FLBSM 1	FLBSM 2	FLBSM 3	GLBSM 1	GLBSM 2	GLBSM 3
	%	10%	20%	30%	10%	20%	30%	10%	20%	30%	10%	20%	30%
Parameters													
PCV (%)	34.00±1.04^{c}	29.00±1.52^{c}	30.00±1.31^{c}	32.00±1.51^{c}	37.00±1.19^{b}	39.00±1.18^{b}	50.00±1.28^{a}	42.06±1.53^{b}	42.50±0.56^{b}	38.12±0.18^{b}	35.00±0.44^{c}	33.56±0.44	31.95±0.41^{c}
HB (g/dL)	11.40±0.31^{b}	9.82±0.25^{c}	10.10±1.35^{c}	10.80±1.33^{c}	12.46±0.51^{b}	13.10±0.22^{b}	16.70±1.89^{a}	14.10±0.35^{b}	13.40±0.15^{b}	12.80±0.61^{b}	11.65±0.53^{b}	11.60±0.53^{b}	10.40±0.45^{c}
RBC (x106/L)	3.80±0.11^{b}	3.63±0.14^{b}	4.80±0.12^{a}	4.61±0.42^{a}	4.20±0.35^{a}	5.62±0.48^{b}	3.20±0.43^{b}	4.80±0.15^{a}	4.66±0.25^{a}	4.20±1.15^{a}	3.81±0.24^{b}	3.60±0.41^{b}	3.40±0.43^{b}
WBC (x103)	5.00±0.51^{a}	5.80±0.89^{a}	6.00±0.64^{a}	5.85±0.55^{a}	5.20±0.74^{a}	5.42±0.51^{a}	5.60±0.58^{a}	5.20±0.11^{a}	6.25±0.67^{a}	5.66±0.42^{a}	6.00±0.11^{a}	5.69±0.23^{a}	5.83±0.66^{a}
NEUT (%)	65.00±1.21^{b}	64.15±0.42^{c}	67.35±0.74^{b}	66.52±0.32^{b}	66.21±0.31^{b}	68.45±0.14^{a}	67.00±0.34	69.02±0.80^{a}	66.25±0.36^{b}	65.42±0.95^{b}	69.00±0.47^{a}	66.45±0.41^{b}	65.00±0.35^{b}
LYM (%)	29.00±0.11^{a}	29.10±0.44^{a}	28.05±0.80^{b}	26.13±0.11^{b}	27.54±0.42^{b}	26.51±0.69^{b}	27.12±0.42^{b}	26.65±0.77^{b}	30.59±0.91^{a}	29.05±0.62^{a}	27.16±0.23^{b}	29.55±0.30^{a}	30.65±0.33^{a}
EOS (%)	4.00±0.47^{b}	5.32±0.41^{a}	6.00±0.33^{a}	4.25±0.21^{b}	6.02±0.21^{a}	4.03±0.53^{b}	5.43±0.09^{a}	4.00±0.33^{b}	5.02±0.21^{a}	4.25±0.15^{b}	3.00±0.34^{c}	3.26±0.73^{c}	4.11±0.41^{b}
MON (%)	1.00±0.31^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.60±0.00^{a}	0.67±0.33^{a}	0.78±0.41^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
BAS (%)	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Means along the same row with same letter are not significantly different (p>0.05)
KEY:
PCV: Packed Cell Volume; Hb: Haemaglobin; RBC: Red Blood Cell; WBC: White Blood Cell; NEUT: Neutrophil; LYM: Lymphocyte; EOS: Eosinophil; MON: Means along the same row with same letter are not significantly different (p>0.05)
KEY: PCV: Packed Cell Volume; HB: Haemalobin; RBC: Red Blood Cell; WBC: White Blood Cell;
NEUT: Neutrophil; LYM: Lymphocyte; EOS: Eosinophil: MON: Monocyte; BAS; Basophil

Statistical Analysis

All data collected were subjected to one-way analysis of variance (ANOVA) to test for significant differences among the treatment using SPSS version 15.0 followed by Duncan's multiple range les (DMRT). This was used to separate significantly different means. The level of significance set for all the treatment was $P<0.05$.

RESULTS

The initial and final carcass composition of the fish fed with different processed lima bean seed meal are found in Table 2. The carcass moisture composition showed the highest at 7.40±0.17% $GLBSM_3$ at 30% inclusion level, and lowest with 7.00±0.12% FLBSM, at 10% inclusion level. When compared to the initial carcass 7.71±0.10%; the treatment in the different processed lima bean seed meal had a lower moisture content.

The carcass protein value ranged from 63.20±0.04% RLBSM, at 10% inclusion level to 66.50±0.22% $FLBSM_3$ at 30% inclusion level; when compared to 54.41±0.52% initial value. All carcass lipid value was highest in fish fed with $GLBSM_3$ at 30% inclusion level 12.41±0.40%, and lowest in fish fed ALBSM, at 10% inclusion level (10.70±0.50%). Carcass ash was highest in fish fed with FLBSM, (7.80±0.21%) and least with (1.30±0.26%) fish fed with $GLBSM_3$ when compared to initial value of 11.54±0.06%. All the values of the processed lima bean seed meal are lower than the initial value. Carcass fibre value had no significant value $GLBSM_3$ had the highest Nitrogen Free Extract (NFE) 13.71±0.55 $GLBSM_3$ and the lowest value was 6.99±0.33% FLBSM; when compared with the initial value of (18.82±0.05%). The values of the processed lima bean seed meal are lower than the initial values.

DISCUSSION

Blood is a good indicator in determining the health of an organism (Yoshi et al., 2002). The haematological parameters of fish are reported to be affected by a wide range of factor, which include species size, age, physiological status, environmental conditions and dietary regime such as the quantity and quality of food, dietary ingredients, protein sources, vitamins, probiotics (Hrubec and Smith, 2000; Kim et al., 2000; Osuigwe et al., 2007).

Result in this study showed that there was a slight decrease in the values of haematological parameters of *O. niloticus* fed with differently process *P. lunatus*. The four different processing methods of *P. lunatus* had no significant effect on the packed cell volume PCV, of the fish fed with the diets except fish fed 30% ALBSM which had higher PCV values when compared to fish fed in RLBSM. This might be an indication of the well being of the fish. The result obtained in this study are similar to Navaro et al., (2017). However, the mean values of PCV in *O. niloticus* obtained in this study were within the range of the corresponding values by Sotolu and Faturoti, (2011a) and Ada et al., (2012). This is also in agreement with the findings of Omitoyin et al., (2007) who reported the haematological and plasma biochemical parameters of farmed catfish *C. gariepinus*.

The haemoglobin concentration in fish fed RLBSM, ALBSM, FLBSM, GLBSM and control were within the range of 10.40 – 16.70g/dL. This is similar to the value of 6.80±0.17mg/100ml to 12.70±0.00gm/100ml obtained by Adesina et al., (2017) on haematological profiles of *C. gariepinus* juveniles fed on diets containing different inclusion level extracted in sunflower (*H annus*) seed meal. It is also similar to the range of 5.6 to 15.8g/100ml reported for *Heterotic niloticus* (Fagbenro and Davies, 2002).

(Ajiboye and Yakubu 2009) also recorded between 4.70 and 7.84gm/100ml in *Synodontis nigrita* fed differently dietary crude proteins levels. The present values were within the range of baseline values established by (Adedeji and Okocha et al., 2011) for Nigeria freshwater fishes. The values of RBC in this study ranged between (3.20 – 4.80 X 10^6/L). These values were greater than 2.11X10^{-12}/ml to 2.93X10-^{12}/ml reported by Ajiboye et al., (2009) and 3.01X10^{12}/l recorded for *Channa stratus* (Lawal et al., 2015). It was also higher than 1.9X10^{12}/l found for *Clarias gareipinus* juveniles (Ayoola et al., 2011). The increase RBC count in this study might be due to the release of new RBCs from the erythropoetic tissue to improve the oxygen carrying capacity of the fish blood with observed higher values of erythrocyte count as evidence by (Rottman et al., 1992) and (Alkahem et al., 1988).

The WBC values obtained in this study ranged between 5.00X10^3/mm^3 to 6.25X10^3/mm^3. All the values are not significantly different ($p<0.05$). The values are similar to what were obtained by Soyinka et al., (2015) with values ranging between 5.187X10^3/mm^3 and 7.167X10^3. The WBC counts of this study were also similar to 6.62X10^6/L for *Channa stratus* (Lawali et al., 2015) and higher than 1.61X10^6/mm^3 for *Ictalarus punctatus* (Klinger et al., 1996). However, (Ajani et al., 2006) and (Kori-Siakpere et al., 2009) stated that high WBC count indicated a release of more cells to maintain haemostatics while low WBC count is a common stress response. Thus, increase or decrease in number of WBC's are normal physiological reactions to the toxicants and response of immune system under toxic conditions. Thus, the decrease values observed in haematological indices (WBC) in this study may be due to stress stimulus (Rehulka et al., 2002; Chen et al., 2004; Matins et al., 2004).

The values of lymphocytes count in this study are not significantly different ($p<0.05$). It ranged between 26.51% and 30.65%. This value is far lesser to 76.49% in *Synodontis membranaces* (Owolabi et al., 2011). They were however, higher than 15.38% recorded for *Channa stratus* (Lawali et al., 2015).

RECOMMENDATION AND CONCLUSION

It is hereby recommended that inclusion level of 20% ALBSM and 10% FLBSM is support haematological profile of the fish. Since blood is the means through which general condition of the animal body could be well assessed.

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