Available online www.unicrossjournals.com

UNICROSS JOURNAL OF SCIENCE AND TECHNOLOGY, UJOST

RESEARCH ARTICLE VOL. 1(2) SEPTEMBER, 2022 ISSN:2814-2233

Date Accepted: 31st August, 2022 Pages 56 - 70

EFFECTS OF REPLACEMENT OF SOYBEAN MEAL (SBM) WITH DEFATTED *SESAME* SEED MEAL (SSM) ON GROWTH AND HAEMATOLOGY OF *CLARIAS GARIEPINUS* JUVENILES.

Faith Ugo Inya, Irom Bassey Okey and Stephen Ncha Ochang

Department of Fisheries and Aquatic Science, Cross River University of Technology, Obubra Campus, Cross River Nigeria.

Corresponding Author: piusbass@yahoo.com +234 7063302755

Abstract

This study investigated the effects of sesame seed meal (SSM) substitution for soybean meal (SBM) in 39% isonitrogenous diets on *Clarias gariepinus* juveniles for eight weeks to evaluate the changes in body indices and haematology. Three hundred (300) apparently healthy juveniles with an initial average weight of 80.60 ± 4.53g, were randomly stocked in 15 plastic tanks fed diets in which the soybean component was substituted at 0, 25, 50, 75 and 100% levels. During the feeding trials, body weights were taken fortnightly, feed intake was estimated and blood samples were obtained for haematological analysis using standard methods. The results show that the Mean weight gain (MWG), Protein efficiency ratio (PER), Specific growth rate (SGR) and Survival rate (SR) decreased with an increase in the inclusion level of SSM. The mean weight gain of 46.92 ±1.08g was recorded for fish fed diets with no soybean meal (D5, 100% SSM) whereas no significant ($p > 0.05$) MWG existed between fish fed up to 50% substitution and those of control (0%). Haematological parameters also showed that red blood cell (RBC), haemoglobin (HGB) and haematocrit (HCT) were decreasing while white blood cell (WBC), lymphocytes (LYM), mean cell haemoglobin concentration (MCHC) and platelets (PLT) increased with increasing inclusion level of SSM. The results also revealed that fish fed with 75 and 100% SSM showed significant changes ($P > 0.05$) in all the parameters studied when compared with the control (0%) SSM. Hence, the inclusion of sesame seed meal up to 25% is recommended in the diet of *Clarias gariepinus* juveniles, since this inclusion level fish did not exhibit any negative effect on growth and haematology.

Keywords: Dietary replacement, sesame seed, soybean, *Clarias gariepinus*, growth, haematology

Introduction

Fish nutrition experts the world over and particularly in Nigeria have shown considerable interest in the procurement of alternative protein sources for inclusion in the fish diets to partially or completely replace fish and soybean meals (Stickney *et al.,* 2006). More than half of world food fish are produced through aquaculture which in turn is heavily dependent on aqua feed inputs (FAO, 2012). Fish feed production must be able to sustain growing world fish demand and this is dependent on protein and energy sources like fishmeal, fish oil and soybean meal which has become costly in the international markets (Naylor *et al.,* 2009; Hardy, 2010). Fish meal and soybean are the main sources of animal and plant protein respectively used in fish feed due to their balanced amino acids and minerals profiles, but these are expensive and competitively demanded by man, animals and industries. This makes their prices to stay high and is the reason why fish feeds account for 60-70% of total operating costs in aquaculture (Collins *et al.,* 2007). Soybean is presently the most used plant protein in fish feed production and contains around 48 % crude protein (Gatlin *et al.,* 2007; Salze *et al.,* 2010). Soybean has a good amino acid profile, although poor in sulphur- rich lysine and methionine (Cai and Burtle, 1996; Gatlin *et al.,* 2007). Soybean as a multipurpose raw material is competitively scarce and expensive for aquaculture in sub-Saharan Africa (Ayinla, 2007; Azaza *et al.,* 2009), hence the need to seek alternatives to both fishmeal and soybean meal.

Sesamum indicum (family: Pedaliaceae) grown all over the tropical and sub-tropical world are the main sources of essential amino acids such as lysine and methionine (El-Adawy and Monsour, 2011). The seed is reported to have about 41–58 % oil, 18–25 % protein and 13–17 % carbohydrates (Kang *et al*., 2003; Yusuf *et al*., 2008). However, Ochang *et al.* (2014) reported about 41.60% crude protein composition in Sesame seed procured from Ikwo market, Ebonyi state, Nigeria higher than those reported for seeds in other parts of the world. The replacement of soybean meal with *Sesamum indicum* feed meal is expected to reduce the high cost of feed which is one of Nigeria's major problems in fish farming and also provide some health benefits to fish.

Blood analysis is a valuable means of evaluating the physiological condition of cultured aquatic animals with respect to determining the effect of diets and other stress factors on fish health (Adeparusi and Ajayi, 2004; Bello-Olusoji *et al.,* 2006). According to Adeyemo, (2007) haematology, growth rate, oxygen consumption, biochemical, behavioural and physiological changes of fish are used in determining the toxicity of pollutants. Bhatti *et al*. (2009) reported that the intake of various dietary components has measurable effects on blood constituents. According to Animashahun *et al*. (2006), nutrient levels in the blood and body fluids are considered to be proximate measures of the long-term nutritional status of an organism. Blood parameters have been adjudged as an important criterion for assessing the quality and suitability of feed ingredients in farm animals (Bhatti *et al*., 2009). Animashahun *et al.* (2006) suggested that the comparison of haematological profile with nutrient intake may provide a baseline for either increasing or reducing of certain nutrients for different population groups. The African catfish, *Clarias gariepinus* is a choice species for aquaculture because of its possession of several qualities such as the ability to tolerate low dissolved oxygen (DO), withstand handling stress, high disease resistance, rapid growth rate, and its acceptability of a variety of feed items (Adewolu, 2008). Fagbenro and Davies (2004) reported that *Clarias* species have high propensity to consume a wide variety of supplementary feeds which makes it possible to combine a variety of conventional and non-conventional ingredients for formulating their diet. In Nigeria, it is a very important freshwater fish and enjoys wide acceptability in most parts of the country for its unique taste, and has fewer bones in contrast to Tilapia (Aiyeloja *et al.,* 2011).

The general shortage of fish meal, groundnut cake and soybean meal necessitate the craving for alternative ingredients for use in aquaculture feeds. Of particular interest is the need to focus on plant products that are not in serious competitive demand by humans for other uses, in order to develop cost-effective feeds for the production of aquaculture organisms (Ingweye *et al*., 2010). This study investigates the effects of sesame seed meal diets on growth performances and haematological characteristics of *Clarias gariepinus* juveniles

Materials and methods

Experimental site

The study was carried out in the wet laboratory of the Department of Fisheries and Aquatic Science, Cross River University of Technology (CRUTECH), Obubra Campus.

Experimental fish

Three hundred and fifty apparently healthy juveniles of *C. gariepinus* were procured from Farmosas family farm in Calabar, Cross River State, Nigeria. They were put in oxygen bags and transported by Car to the Wet Laboratory, Department of Fisheries and Aquatic Science, CRUTECH, Obubra Campus. They were batch weighed and put in aquaria, acclimated for two weeks before the commencement of the research. During the period of acclimation, the fish were fed at 5% body weight with Top Feed with a crude protein level of 40% twice daily. Feeding was discontinued 24 hours prior to commencement of the experiment. This was done to eliminate variation in weight due to residual food in their gut, to prepare the gastrointestinal tract for the experimental diets and to increase the appetite of the fish.

Collection and preparation of sesame seed meal (SSM)

The sesame seed was purchased from Adun market, Ofodua, Obubra Local Government Area of Cross River State. The sesame seed was sieved to remove dirt and sand, washed and sun-dried for 3 days. The seed was ground with a hammer mill and the oil was removed using a catalyst n-hexane as recommended by Enujiugha and Akanbi (2005). The proximate composition of the Sesame seed meal before and after oil extraction is shown in table 1

Table 1. Proximate composition of sesame seed meal before and after oil extraction

Parameter (%)	**Before oil extraction**	**After oil extraction**
Moisture content	3.8 ± 0.56	2.6 ± 0.25
Crude protein	22.1 ± 3.54	28.2 ± 3.05

Crude fibre	5.9 ± 1.82	8.8 ± 2.75
Lipid	44.1 ± 5.74	16.8 ± 1.75
Total ash	4.6 ± 0.54	10.8 ± 1.05
Nitrogen Free Extract	19.5 ± 2.05	32.8 ± 3.10

Preparation of experimental diets

Five isonitrogenous diets of 39% crude protein each were formulated using Pearson's square method. The sesame seed meal was included to replace varying quantities of soybean in the diets Table 2. The feed ingredients were ground separately to a fine powder using the hammer mill. Each ingredient was weighed into a bowl and all were mixed properly. Boiled water was then added to the mixture and stirred to obtain a consistent dough which was passed through a pelleting machine to produce 0.5 mm pellets. Thereafter, the feed was sundried for 4 days, then stored in an airtight plastic container and kept until the time for use.

Table 2: Ingredients composition of formulated diets (% Dry weight)

Ingredient (g/100g)	D1 (0%)	D2 (25%)	D3 (50%)	D4 (75%)	D5 (100%)
Blood meal (80% CP)	10	10	10	10	10
Fishmeal (64%CP)	25	25	25	25	25
Soybean meal (42%CP)	30	22.5	15	7.5	0
Sesame seed meal (28%CP)	0	7.5	15	22.5	30
Maize (11%CP)	27	27	27	27	27
Soybean oil	3	3	3	3	3
Vit/min premix	3	3	3	3	3
Binder	2	2	2	2	2
Total	100	100	100	100	100
Calculated Crude protein	**39.36**	**39.37**	**39.17**	**38.97**	**38.92**

Experimental design and procedure

A completely randomized design (CRD) with five treatments in three replicates (5 x 3) was used. Twenty fish were introduced randomly into each of the 15 labelled aquaria into which the five treatments (D1, D2, D3, D4 and D5) were administered in triplicates. The initial mean total body weight of 80.60 ± 4.53g was noted and other measurements were taken fortnightly for 56 days. Feeding was carried out twice daily, 8:00 – 8:30 am and 5:00 – 5:30 pm and the daily ration was maintained 5% of their body weights.

Growth parameters

The growth parameters were calculated using the following relationships:

Mean weight gain (MWG) = Final mean weight of fish – Initial mean weight of fish (Adikwu, 2003)

Specific growth rate (SGR) = $\frac{\text{In (Final body weight)} - \text{In (Initial body weight)}}{\text{Time (days)}} \times 100\%$

Feed conversion ratio (FCR) = $\frac{\text{Weight of feed given (g)}}{\text{Fish weight gain (g)}}$ (Adikwu, 2003)

Protein efficiency ratio (PER) = $\frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$ (Wilson, 1989)

Survival rate (SR) (%) = $\frac{\text{Number of fish that survived}}{\text{Number of total fish stocked}} \times 100$

Protein Intake (PI) = Total fed intake x protein intake.

Blood collection and haematological analysis

Blood samples were collected at the end of the 8th week of the experiment following the procedure of Schmitt *et al.* (1999). Blood of 2ml was collected from the caudal peduncle with the aid of a 2ml sterile plastic syringe fitted with 0.8 x 38mm hypodermic needle. The blood was collected in triplicates into sample bottles containing ethylene diamine tetra acetic acid (EDTA) as an anti-coagulant. The blood was rocked gently in the bottle to allow thorough mixing of its content. The samples were preserved in a cooler containing an ice block and thereafter transported to University of the Calabar Teaching Hospital (UCTH), haematology Department for analysis within six hours of collection. The haematological parameters determined were Red blood cell (RBC), White blood cell (WBC), Platelet (Pt), Pack cell volume (PCV) Haemoglobin (Hb) and Differential counts. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) were calculated from the data using standard formulae (Lee *et al.,* 1998). The direct measurements of erythrocyte value (Packed cell volume PCV, Haemoglobin Hb, and Red blood cell RBC), absolute erythrocyte indices (MCH, MCV and MCHC) were calculated. The platelets, white blood cells and differential count (neutrophils and lymphocytes) were analysed as described by Dacie and Lewis (2001).

Statistical analysis

The data collected were subjected to one - way analysis of variance (ANOVA) using SPSS version 20. Comparison among diet means for the parameters was carried out using Turkey's honest significant difference (HSD) at the significant level of 0.05.

Results

Growth performance

The mean values of the growth parameters of *C. gariepinus* juveniles fed graded levels of sesame seed meal is presented in Table 3. The initial mean weight of the fish recorded in the experimental tanks was not significant ($p > 0.05$) while the mean final weight reveals a decrease from 146.71± 5.60 g to

124.96 ± 2.54g with an increase in SSM substitution level. However, fish fed D2 (25% SSM) diet showed no significant difference (p> 0.05) in weight compared to control, D1 (0%). Other growth parameters such as AWG 65.36 – 46.92, SGR 2.77 – 2.44, PER, 2.22 – 1.24 and SR 99.58 – 90.83% all decreased while FCR (2.44 – 4.36) increased with increasing substitution level. The least average weight of 46.92g was recorded for fish fed diets with no soybean meal (D5, 100% SSM) while no significant difference (p > 0.05) existed between fish fed 25% substitution and those fed the control diet (0% SSM). Fish fed with 0% SSM diets had the least FCR 2.44 which was not significant from those fed 25% (3.16). The results also revealed that fish fed with 75 and 100% SSM showed significant changes in all the parameters studied when compared with the control (0%) SSM.

Table 3: The mean values of some growth parameters of *C. gariepinus* fed graded levels of sesame seed meal (SSM) for 8 weeks

Parameters	**Diets (%)**				
	D1 (0%)	**D2 (25%)**	**D3 (50%)**	**D4 (75%)**	**D5 (100%)**
Initial weight (g)	81.35 ±2.25^{a}	82.33 ±1.65^{a}	82.44 ±1.44^{a}	78.82 ± 3.50^{a}	78.04 ±2.40^{a}
Final weight (g)	146.71±5.60^{a}	135.49±4.25^{ab}	130.8 ±3.05^{b}	129.6±2.10^{bc}	124.96±2.54^{c}
Mean Weight Gain (MWG)	65.36±2.18^{a}	53.16±1.16^{ab}	50.78±1.74^{ab}	48.36±1.89^{b}	46.92±1.08^{b}
Feed Conversion Ratio (FCR)	2.44± 0.76^{b}	3.16± 1.09^{ab}	3.50± 1.20^{ab}	4.29±3.03^{a}	4.36± 2.88^{a}
Protein Efficiency Ratio (PER)	2.22± 0.76^{a}	1.63 ± 0.85^{ab}	1.43± 0.85^{ab}	1.43± 0.84^{ab}	1.21 ± 0.79^{b}
Specific Growth Rate (SGR)	2.77±0.32^{a}	2.69±0.73^{a}	2.56±0.46^{ab}	2.52±0.45^{ab}	2.44±0.45^{b}
Survival Rate (%)	99.58±1.44^{a}	97.08±3.34^{ab}	95.50±5.00^{bc}	92.50±5.22^{c}	90.83±8.22^{c}

Mean with the same superscript under the same row are not significant at (P< 0.05)

Haematological parameters

The result of the haematological indices of *C. gariepinus* fed graded levels of sesame meal-based diets is presented in Table 4. The mean values of RBC (3.18 - 2.41 x 10^{12}cells/L), HGB (14.4 – 13.90 g/l) and HCT (32.12 – 28.82%) decreased from D1 (0%) to D5 (100%) of SSM substitution level. the values of WBC (5.16 – 99.01 x 10^{9}cells/L), platelets (9.00 – 12.67 x 10^{9}cells/L) and LYM (59.16 – 81.64%) increased progressively from fish fed D1 to D5 respectively. the values of the erythrocyte indices MCHC (43.93 – 56.73g/l), MCH (56.83 – 70.93 pg) and MCV (121.33 – 133.33fl) also increased with an increase in SSM substitution level. The study also revealed that fish fed with D5 (100% SSM) showed significant changes whereas those fed D2 could not show differences from those fed the control diet (D1).

Table 4: The mean values of some haematological parameters of *C. gariepinus* fed graded levels of sesame seed meal (SSM) for 8 weeks

Parameters	Diets (%)				
	D1 (0%)	D2 (25%)	D3 (50%)	D4 (75%)	D5 (100%)
Red blood cell (10^{12}cells/L)	3.18 ±0.2^{a}	3.71 ±0.91^{a}	2.57 ±0.13^{ab}	2.71 ±0.35^{ab}	2.41± 0.8^{ab}
Haemoglobin HGB (g/l)	14.4 ± 0.42^{ab}	16.2 ±0.23^{a}	15.17± 0.43^{a}	14.37±0.59^{ab}	13.9± 0.1^{ab}
Haematocrit (%)	38.12 ± 2.14^{b}	40.35 ±2.62^{ab}	35.13± 1.25^{a}	32.48±3.21^{ab}	28.82±3.38^{c}
White blood cell(10^{9}cells/L)	95.16 ±0.4^{b}	97.37 ±0.23^{ab}	98.12 ±0.43^{a}	98.12 ±1.0^{a}	99.01 ±0.1^{a}
Lymphocytes(%)	59.16±0.2^{c}	65.73 ±0.74^{bc}	69.26 ±0.8^{ab}	75.64±2.93^{ab}	81.64 ±1.0^{a}
Mean cell haemoglobin concentration (g/l)	43.93±1.92^{c}	45.23 ±1.26^{bc}	45.37±3.12^{bc}	47.5 ± 1.74^{b}	58.73 ±3.3^{a}
Mean cell haemoglobin (pg)	56.83±2.32^{c}	59.4 ±1.74^{bc}	59.13±3.73^{bc}	61.87± 3.82^{b}	70.93±2.25^{a}
Mean cell volume (fl)	121.33 ±2.32^{c}	125.67±2.24^{bc}	126 ±3.73^{b}	128.33±1.82^{b}	133.33±1.25^{a}
Platelets (10^{9}cells/L)	9.00 ±2.00^{ab}	9.33± 2.12^{ab}	10.67±1.22^{ab}	11.33 ±2.82^{a}	12.67 ±1.15^{a}

Mean with the same superscript under the same row are not significant at ($P< 0.05$)

Discussion

Growth parameter.

The mean weight gain (MWG), food conversion ratio (FCR) and protein efficiency ratio (PER) have been reported as the major indices for assessing the growth rate of fish. Adewolu (2008) stated that mean weight gain and protein efficiency ratio are known to be the most important indices for measuring fish response to experimental diets and very reliable indicators of growth. A reduction in the values of MWG and PER with increasing substitution levels is an indication that SSM diets affect the growth rate of fish. At 25% replacement of soybean with SSM, the various parameters examined were not significantly different from those of the control. This finding is in agreement with those of several researchers who have used plant-based proteins to replace soybean in fish diets (Ochang *et al.,* 2014; Hossain *et al.,* 2003). The significant reduction in growth in the 75% and 100% inclusion levels of sesame seed diets could be due to improper balance of essential nutrients such as amino acids, minerals and the presence of some anti-nutritional compounds in the diet. According to Sotolu and Faturoti (2009), decrease in palatability and indigestibility due to high fibre content and anti-nutritional factors may have led to reduced growth and poor feed efficiency in experimental fishes. Fagbenro *et al.* (2010) and Adewolu (2008) reported that high inclusion of plant protein in the diets of *Clarias gariepinus* suppressed growth significantly. The feed conversion ratio is a measure of diet efficiency, the smaller the value the more suitable the diet in bringing about a unit weight gain. The value of 3.16 obtained for fish fed 25% SSM diet was not significant from those fed 0% implying that the diet is also suitable. A similar increase in the values of FCR have also been reported by several researchers using plant protein as substitutes for fishmeal and soybean meal (Olivera *et al*., 1997; Thiessen *et al*., 2004; Barros *et al*., 2002; Nwanna, 2003). De Silva and Anderson (1995) reported that the protein efficiency ratio is a measure of how well the protein sources in a diet could provide the essential amino acid requirement of the fish fed for maximum growth. Protein efficiency ratio (PER) is also known to be regulated by the non-protein energy input of the diet and is a good measure of the protein-sparing effect of lipid and/or carbohydrates (Tibbets *et al*., 2005). The decreased value reported in this study may have been responsible for the poor growth in fish fed with substitution level above 25%. Sesame seed inclusions above 25% have been reported to negatively affect the growth rate of *C. gariepinus* (Ochang *et al.,* 2014). Hossain and Jauncey (1989) and Hossain *et al*. (1992) have reported that sesame inclusion of 25% and 20% had produced a promising growth result in *Cyprinus carpio* and *Heteropneustes fossilis,* respectively. However, the increased substitution of plant-based protein has been reported to produce poor growth in *C. gariepinus* (Okey *et al.,* 2018). This could be due to the presence of anti-nutrients and high fibre content in the feed as the percentage of the SSM increased in the diets. This corroborates the findings of Fagbemi (2007) on fluted pumpkin seed flour and Akande *et al*., (2010) on various sources of plant proteins fed to fishes. Although sesame seeds, like any other plant protein, contain an anti-nutritional factor which inhibits the absorption of some important minerals, 25% inclusion was still promising inferring that the physiological mechanism in fish could compensate for the presence of these factors (Gohl, 1981). According to Francis *et al*., (2001), the low growth performance of fish fed plant protein sources is attributed to various factors such as reduced bioavailability of minerals, impaired protein digestibility caused by the formation of phytic acid-protein complexes and depressed absorption of nutrients due to damage to the pyloric caecal region of the intestine.

Haematological parameters

The applications of haematological techniques have proved valuable for fishery biologists in assessing the health status of fish and monitoring stress responses (Osuigwe *et al*., 2007). Haematological parameters of fish are affected by a range of factors which includes size, age, physiological status, environmental conditions and dietary regime. The changes in the various parameters recorded (although some were still within the recommended level) showed that they were affected by the increase in the substitution level of the diet. This study showed a decrease in the values of RBC, HGB and HCT while the values of WBC, LYM, PLT, MCV, MHCH and MCH increased with increasing levels of SSM replacement. This was in agreement with the findings of some researchers who use plant sources on *C. gariepinus* (Ochang *et al*., 2014; Umaru, 2015; Adesina, 2017; Okey *et al*., 2018). However, Babale (2016) rather reported an increase in these parameters on *C. gariepinus* adults fed graded levels of watermelon, *Citrullus lanatus* seed cake diet. The range of the RBC, 3. 18 - 2.41 x 10^{12}cells/L, HGB, 16.20 - 13.90g/l and 28.82 – 40.35% recorded in this study compared favourably with those of some researchers who fed *C. gariepinus* with graded levels of plant-based diet (Babale, 2016; Umaru, 2015; Adesina, 2017). The increase in the red blood cell and haemoglobin concentration in fish fed a 25% SSM diet may be attributed to the increase in the size of the fish as a result of growth and metabolic activities. The increase might also 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). Conversely, haemoglobin contents and erythrocyte counts tend to increase with the length and age of the fish (Babale, 2015). The reduction in the RBC and HGB concentration in fish fed diets above 50% substitutions levels of SSM, contained lower quality protein, due to residual metabolites such as the anti-nutritional compounds which probably affected blood production. A reduction in the concentration of the PCV and RBC in the blood usually suggests the presence of a toxic factor which has adverse effect on blood formation resulting to anaemia (Oyawoye and Ogunkunle, 1998). It has been reported that haematocrit and haemoglobin values vary according to deficiency of essential nutrients, environmental conditions, growth status, and anti-nutritional factors (Garrido *et al*., 1990; Lim and Lee, 2009). An increase in the concentration of WBC, LYM, PLT, and all the erythrocyte indices in this study was in an agreement with the finding of several researchers who fed *C. gariepinus* with plant-based protein sources (Yue and Zhou, 2008; Fagbenro *et al*., 2010; Adesina, 2017). The increase in WBC, LYM and PLT as SSM increased in the diet could be attributed to residual anti-nutrients present in the feed, and also an increase in leucopoiesis as a way of fighting stress-induced substances in the body system of the fish (Gabriel *et al.,* 2004). Douglas and Jane (2010) stated that their amount has implications for immune responses and the ability of the animal to fight infection.

The mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) of fish in this study were significantly higher in fish fed with 100% SSM and are comparable with values earlier reported by previous researchers (Adedeji and Adegbile, 2011; Anyanwu *et al*., 2011; Umaru, 2015). The MCHC, MCH and MCV are useful in the diagnosis of anaemia in most animals (Cole, 1986). The observed lower haemoglobin levels and increased MCV, MCH and MCHC as the inclusion level of SSM increased in this study may be due to abnormal maturation of the red blood cell. The value of 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 conditions (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) and Alwan *et al.* (2009). Higher MCH indicates a good volume of haemoglobin which shows that there was effective oxygen transportation in the bloodstream for the healthy well-being of the fish (Diyaware *et al*., 2013).

Conclusion

The growth performance and feed utilization of *C. gariepinus* juveniles fed diets in which 25% of the soybean was replaced by SSM gave the best AWG, SGR, FCR and PER. Haematological analysis revealed that 25% and 50% substitution of soybean with SSM in the diets had no adverse effect ($p>0.05$) on the blood parameters and physiology of the fish. Therefore, the inclusion of SSM in the diets of *C. gariepinus* should not exceed a 50% level of substitution.

Acknowledgements

Many thanks to the Tertiary Education Fund (TetFund) for sponsoring this research through the Directorate of Research and Development, Cross River University of Technology (CRUTECH), Calabar. We also thank the Head, Department of Fisheries and Aquatic Science, CRUTECH Obubra Campus for the logistic support.

References

Adedeji, O.B. and Adegbile, A.F. (2011). Comparative haematological parameters of the Bagrid catfish (*Chrysichthys nigrodigitatus*) and African catfish (*Clarias gariepinus*) from Asejire Dam South-western Nigeria. *Journal of Applied Science Resources*, 7(7):1042-1046.

Adeparusi, E. O. and Ajayi, A. D. (2004). Haematological characteristics of Nile Tilapia (*Oreochromis niloticus*) fed differently processed lima bean (*Phaseolus lunatus* L.) diets. *Journal of Research in Science and Management,* 2: 73 – 80.

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

Adewolu, M. A. (2008). Potentials of potato leaf meal as dietary ingredient for *Tilapia zilli* fingerlings. *Pakistan Journal of Nutrition,* 6 (7):444-449.

Adeyemo, O. K. (2007). Haematological Profile of *Clarias gariepinus* (Burchell, 1822) Exposed to Lead. *Turkish Journal of Fisheries and Aquatic Sciences*, 7:163-169.

Adikwu, I. A. (2003). A Review of Aquaculture Nutrition in Aquaculture Development in Nigeria. In: *Proceeding of National Workshop on Fish Feed Development and Feeding Practices in Aquaculture*. Fisheries Society of Nigeria/NIFFR/FAO-NSPFS, New Bussa, Sept. 15-19, pp. 34-42.

Aiyeloja, A.A., George, F.A., Obasa, S.O. and Sanni, L.O. (2011). Effect of post-slaughter time intervals on the quality of the African catfish, *Clarias gariepinus* (Burchell, 1822). *Journal of Food Technology,* 6 (9):790-797.

Akande, K. E., Doma, U. D., Agu, H. O. and Adamu, H. M. (2010). Major anti-nutrients found in plant protein sources, their effects on nutrition. *Pakistan Journal of Nutrition,***7**(**9**):827-832.

Alkahem, H. F., Z. Ahmed, A.S. Al-Akel and M.J.K. Shamusi. (1998). Toxicity bioassay and changes in haematological parameters of *Oreochromis niloticus* induced by Trichlorfon. *Arabian Gulf Journal of Scientific Research*, 16 (3): 581-593.

Alwan, S. F., A. A. Hadi and A.E. Shokr. (2009). Alterations in Haematological Parameters of Fresh Water Fish, *Tilapia zillii*, Exposed to Aluminium. *Journal of Science and Its Applications,* 3 (1): 12-19.

Animashahun, R. A., Omoikhoje, S. O. and Bamgbose, A. M. (2006). Haematological and biochemical indices of weaner rabbits fed concentrates and *Syndrella nodiflora* forage supplement. *Proceeding of 11th Annual Conference of Animal Science Association of Nigeria.* Institute of Agricultural Research and Training, Ibadan, Nigeria, pp: 29 - 32.

Anyanwu, A. D. C., Udedibie, A. B. I., Osuigwe, D. I. and Ogwoa, V. O. (2011). Haematological responses of hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus* fed dietary levels of *Carica papaya* leaf meal. *World Rural Observations*, 3 (1): 9-12.

Ayinla, O. A. (2007). Analysis of feeds and fertilizers for sustainable aquaculture development in Nigeria. In: Hasan, M. R., Hecht, T., De Silva, S. S., and Tacon, A.G.J. (Eds.), *Study and analysis of feeds and fertilizers for sustainable aquaculture development*, FAO Fisheries Technical Paper No. 497, FAO, Rome, pp. 453 – 470.

Azaza, M. S., Wassim, K., Mensi, F., Abdelmouleh, A., Brini, B. and Kraïem, M. M. (2009). Evaluation of faba beans (*Vicia faba* L. *var. minuta*) as a replacement for soybean meal in practical diets of juvenile Nile tilapia *Oreochromis niloticus. Aquaculture,* **287**: 174 - 179.

Babale, M. Y. (2016). Growth performance at various growth stages of *Clarias gariepinus* (Burchell, 1822) fed various inclusion levels of processed water melon (*Citrullus lanatus,* mansf.) Seed cake diets. Published Ph.D Thesis, Ahmadu Bell University ABU Zaria, Nigeria. 164pp

Barros, M. M.; Lim, C. and Klesius, P. H. (2002). Effect of soybean meal replacement by cottonseed meal and iron supplementation on growth, immune response and resistance of channel catfish (*Ictaturus punctatus*) to *Edwarsiella ictaluri* challenge. *Aquaculture*, **207**: 263 - 279.

Bello-Oluesoji, O. A., Omoare, V. Y. and Nwana, L. C. (2006). Comparative studies on the haematological characteristics of pond-cultured and wild tilapia (*Oreochromis niloticus*) Linnaeus, 1857.*Nigerian Journal of Forestry,* **36**: 134 – 141.

Bhagwant, S. and M. Bhikajee. (2000). Induction of hypochromic macrocytic anaemia in *Oreochromis* hybrid (Cichlidae) exposed to 100mg/l (sublethal dose) of aluminium. *Journal of Science and Technology,* **5**: 10-20.

Bhatti, J. A., Younas, M., Abdullah, M., Babar, M. E. and Nawaz, H. (2009). Feed intake, weight gain and haematology in Nili Ravi buffalo heifers fed on Mot grass and Barseem fodder substituted with Saltbush (*Atriplex amnicola*). *Pakistan Veterinary Journal*, **29** (3): 133 - 137.

Cai, Y. and Burtle, G. J. (1996). Methionine requirement of channel catfish fed soybean meal-corn-based diets. *Journal of Animal Science,* **74**: 514 – 521.

Collins, J. K., Wu,G. and Perkins-Veezie, P. (2007). Water melon consumption increases plasma arginine concentrations in adults. *Journal of Nutrition,* **23**(3): 261-266

Dacie, J.V. and S.M. Lewis. (2001). *Practical Haematology*, 6th edn: University Press, 633 pp.

De Silva, S. S and Anderson, T. A. (1995). *Fish Nutrition in Aquaculture*. Chapman and Hall, London. 319pp.

Diyaware, M.Y., A.B. Haruna and K.A. Abubakar. (2013). Some haematological parameters of intergeneric hybrid of African catfish (*Clarias anguillaris* × *Heterobranchus bidorsalis*) juveniles and their pure lines in north-eastern Nigeria. *Journal of Fisheries and Aquatic Science*, **8** (1): 33-42.

Douglas, J.W. and K.W. Jane. (2010). In Schalm's Veterinary Haematology. John Wiley and Sons. Blackwell publishing Ltd. pp: 1232.

El-Adawy, A. T. and Mansour, H. E. (2011). Nutritional and physiochemical evaluations of tahina (sesame butter) prepared from heat-treated sesame seeds. *Journal of Science, Food and Agriculture,* **80**: 2005 – 2011.

Enujiugha, V.N and Akanbi, C.T. (2005). Compositional changes in African oil bean seeds during thermal processing. *Pakistan Journal of Nutrition,* **4** (1): 21 - 31.

Fagbemi, T. N. (2007). Effects of processing on the nutritional composition of fleeted pumpkin (*Telfaira accidentalis*) seed flour. *Nigerian Food Journal,* **2** (25)**:**1-22.

Fagbenro, O. A. and Davies S. J. (2004). Use of high percentages of soybean protein concentrate as fishmeal substitute in practical diets for African catfish, *Clarias gariepinus* (Burchell 1822): Growth, Feed Utilization and Digestibility. *Journal of Applied Aquaculture,* **16** (1-2): 113-124.

Fagbenro, O. A., Adeparusi, E. O. and Jimoh, W. A. (2010). Nutritional evaluation of sunflower and sesame seed meals in *Clarias gariepinus*: An assessment by growth performance and nutrient utilization. *African Journal of Agricultural Resources*, **5** (22): 3096-3101.

FAO (2012). The state of World Fisheries and Aquaculture. http://www.fao.org/docrep/016/i2727e/i2727e.pdF. (Accessed on 10th August 2020)

Francis, G.; Makkar, H. P. S. and Becker, K. (2001). Anti-nutritional factors present in plant derived alternative fish feed ingredients and their effects in fish. *Aquaculture,* **199**: 197 - 227.

Gatlin, D. M., Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aqaufeeds: A review. *Aquaculture Research*, **38** (6), 551-579.

Gohl, B. (1981). *Tropical feeds*. Food and Agricultural organization of the United Nations Rome.

Hardy, R. W. (2010). Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. *Aquaculture Research,* **41**: 770 – 776.

Hossain, M. A. and Jauncey, K. (1989). Nutritional evaluation of some Bangladeshi oil seed meals as partial substitutes for fish meal in the diets of common carp, *Cyprinus carpio* L. *Aquaculture and Fisheries Management*, **20**: 255 - 268.

Hossain, M. A., Focken, U. and Becker, K. (2003). Anti-nutritive effects of galactomannans rich endosperm of sebania (*Sebania acculeata*) seeds on growth and feed utilization in Tilapia, *Oreochromis niloticus*. *Aquaculture Research,* **4** (34):1171-1179.

Hossain, M. A.; Razzaque, A. Kamal, M. and Islam, M. N. (1992). Substitution of fish meal by various protein sources in combination in the diets of catfish (*Heteropneustes fossilis*). *Third Asian Fisheries Forum "Fisheries Towards 2000: 26 – 30 October, Asian Fisheries Society, Singapore.*

Ingweye, J. N., Kalio, G. A., Ubua, J.A. and Umorem, E. P. (2010). Nutritional Evaluation of Wild Sickle Pod (*Senna obtusifolia*) Seeds from Obanliku South-Eastern Nigeria. *American Journal of Food Technology,* **5** (1): 1-12.

Kang, M. H., Choi, J. S. and Ha, T. Y. (2003). Chemical properties of sesame seed cultivated in Korea and China. *Food Science and Biotechnology,* **12**: 621 – 624

Larsson, A., C. Haux and M. Sjobeck. (1985). Fish physiology and metal pollution: results and experiences from laboratory and field studies. *Ecotoxicology, Environment and Safety*, **9**: 250-281.

Lim, S. J. and Lee, K. J. (2009). Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish (*Oplegnatus faciatus*). *Aquaculture*, **290** (3-4): 283-289.

Naylor, R. L.; Hardy, R. W.; Bureau, D. P.; Chiu, A.; Elliott, M.; Farrell, A. P.; Forster, I. Gatlin, D. M.; Goldburg, R. J.; Hua, K. and Nichols, P. D. (2009). Feeding aquaculture in an era of finite resources. *Publication of National; Academy of Science USA,* **106**: 15103 – 15110.

Nwanna, L. C. (2003). Nutritional value and Digestibility of fermented shrimp head waste meal by African catfish (*Clarias gariepinus*). *Pakistan. Journal of Nutrition*, **2** (6): 339-345

Ochang, S. N., Ugbor, O. N., Ezeonwu, K. C., (2014). Effect of Replacement of Soybean meal with Beniseed (*Sesamumm indicum*) meal on the Growth and Haematology of African catfish (*Clarias gariepinus*). *Nigerian Journal of Fisheries*, **11** (1 & 2): 762 – 769.

Okey, I. B, Ochang, S. N and Ewelu, E. E. (2018). Feed utilization and Haematological parameters of *Clarias gariepinus* post fingerlings fed varying levels of maize offal diets. *J Marine Biology and Aquascape* , Doi: http://dx.doi.org/ 10.31579/1.10033.

Oliveira, A. C. B., Cantelmo, O. A., Pezzato, L. E., Ribeiro, M. A. R., Barros, M. M. (1997). Apparent digestibility coefficients of dendecae and coconut bran by pacu (*Piaractus mesopotamicus*). *Revista Unimar*, 19 (3): 897-903.

Osuigwe, D. I., ,Obejezie, A. I. and Onuoha, G. I. (2007).Some haemotological changes in hybrid catfish (*Heterobranchus longifilis x Clarias gariepinus*) fed different dietary levels of raw and boiled jackbean (*Canavalia ensiformis)* seed meal.*Afrcan Journal of Biotechnology,**8***(4**)**: 1017-1021.

Oyawoye E. O, Ogunkunle, M. (1998). Physiological and biochemical effects of raw jack beans on broilers. *Proceedings of annual Conference of Nigerian Society of Animal production.* 1998; 23:141-142.

Rottman, R.W., R. Francis–Floyd and R. Durborow. (1992). The Role of Stress in Fish Disease. *Southern Regional Aquaculture Centre* (SRAC) Pub. No. 474.

Salze, G., Mclean, E., Rush-Battle, P. Schwarz, M. H., Craig, S. R (2010). Use of soy protein Concentrate and novel ingredients in the total elimination of fish meal and fish oil in diets of juvenile Cobia, *Rachycentron canadum*. *Aquaculture*, **298**: 294-299

Schmidt, C. J., Blakker, V. S., and Dethloft, G. M. (1999). Biomonitoring of Environmental Status and Trends (BEST) program; field procedures for assessing the exposure of fish to environmental contaminants information and technology and report, USGS/RD 1999-007. US Geological Survey of Biological Resources.

Sotolu, A. O. and Faturoti, E. O. (2009). Growth performance and haematology of *Clarias gariepinus* fed varying inclusion of *Laucaena leucecophala* meal. *Revista UDO Agricola,* **9** (4): 979-985.

Sowunmi, A. A. (2003). Haematology of the African catfish, *Clarias gariepinus* (Burchell 1822) from Eleiyele reservoir, Ibadan, Southwest Nigeria. *The Zoologist,* **2** (1): 40 – 44.

Stickey, R. R., Hardy, R. W., Koch, K., Harold, R., Searight, D. and Massee, K. C., Tacon, A. G. J., and Jackson, A. J. (2006). Utilization of conventional protein sources in practical fish feeds. *Journal of Food Science,* ***2 (4):*** 45-56.

Thiessen, D. L., Maenzi, D. D., Newir, R. W., Classen, H. L., and Drew, M. D. (2004). Replacement of fishmeal by canola protein concentrate in diets fed rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutr*ition, 10: 379-388.

Tibbets, S. M., Lall, S. P. and Milley, J. E. (2005). Effects of dietary protein and lipid levels and dietary protein/dietary energy ratio on growth, feed utilization and hepatosomatic index of juvenile haddock, *Melanogramsa eglefonus. Aquaculture nutrition*, **11**: 67-78

Umaru, O. H (2015). Nutrient values of differently processed *senna obtusifolia* (linn) seeds and growth performance of *clarias gariepinus* (teugels, 1982) fingerlings fed the fermented meal. Published Ph.D thesis, Ahmadu Bell University ABU Zaria, Nigeria. 195pp

Wilson, R.P. (1989). Utilization of dietary carbohydrate by fish. *Aquaculture,* **124**: 67-80.

Yue, Y. R. and Zhou, Q. C. (2008). Effect of replacing soybean meal with cotton seed meal on growth, feed utilization and haematological indixes for juvenile tilapia, *Oreochromis niloticus x O. aureus. Aquaculture,* **284**: 185 – 189.

Yusuf, A. A.; Ayedun, H and Sanni, L. O. (2008). Chemical composition and functional properties of raw and roasted Nigerian beniseed (*Sesamum indicum*) and bambara groundnut (*Vigna subterranea*). *Food Chemistry,* 111: 277 – 282.