



EFFECT OF REPLACEMENT OF SOYBEAN MEAL WITH BENISEED (*Sesamum indicum*) MEAL ON THE GROWTH AND HAEMATOLOGY OF AFRICAN CATFISH (*Clarias gariepinus*)

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ABSTRACT

A 56 day feeding trial was conducted to investigate the suitability of sesame seed meal (SSM) as an alternative to soybean meal (SBM) in the diet of *Clarias gariepinus* juveniles. Five isonitrogenous, (41% C.P) diets were prepared with SSM replacing 25, 50, 75 and 100% SBM component of the diets. Diet with 0% SSM served as the control. The fishes with mean initial weight of 28.7 ± 0.83 g were fed 5% of their body weight daily. The highest mean weight gain was in the control diet and differed ($p < 0.05$) significantly with other diets except for 25% SSM. Food conversion ratio was lowest in the control diet with 1.4 ± 0.17 and highest in 100% SSM with 7.09 ± 0.28 . The relative growth rate of the control was comparable to 25% SSM. However, they differed ($p < 0.05$) significantly from other treatments. The haematological analysis showed significant ($p < 0.05$) difference over the fish fed control diet with respect to Packed cell volume (PCV), haemoglobin concentration (Hb), white blood count (WBC), red blood count (RBC), mean corpuscular haemoglobin (MCH) and Erythrocyte sedimentation rate (ESR). Whereas, PCV, Hb, WBC and RBC values decreased with increase in the levels of sesame seed meal in the diets, MCH and ESR increased. Hence, the inclusion of sesame seed meal up to 25% is recommended in the diet of *Clarias gariepinus* juvenile, since this inclusion level did not exhibit any negative effect on the fish health.

Keywords: Sesame seed meal, Soybean meal, Growth response, *Clarias gariepinus*, Haematology

INTRODUCTION

Aquaculture with 8–10 % annual growth rate is the fastest growing agricultural sector (FAO, 2013). More than half of world food fish are produced through aquaculture which in turn is heavily dependent on aquafeed input (FAO, 2012). Feed production must be able to sustain growing world fish demand. It is dependent on a number of protein and energy ingredient sources like fishmeal, fish oil and soybean meal which has become costly in international markets (Naylor *et al.*, 2009, Hardy, 2010). Fishmeal is an excellent protein source for fish feed and it provides essential amino acids and omega 3 and 6 fatty acids (Lech and Reigh, 2012). However, the supply of fishmeal is adversely affected by overfishing, and aquatic pollution (Hardy, 2010).

Soybean is presently the most used plant protein in fish feed production and also for African catfish, *Clarias gariepinus* (Shipton and Hetch, 2005). High-protein soybean meal contains around 48 % crude protein while soy protein concentrate contains about 65 % crude protein (Gatlin *et al.*, 2007; Salze *et al.*, 2010). Soybean has a good amino acid profile, although it is poor in sulphur amino acids like lysine and methionine (Cai and Burtle, 1996, Gatlin *et al.*, 2007). Although soybean meal is

very high in protein, its use is restricted by high price (Gatlin *et al.*, 2007). Soybean is used for human food, making it even less attractive as aquafeed ingredient. Conversely, multi usages of this ingredient in human and animal feed item, vegetable oil production, recently as biofuel have made this ingredient costly (Hill *et al.*, 2006; Hardy, 2010). Hence, soybean as a multipurpose raw material is competitively scarce and expensive for aquaculture in sub-Saharan African (Shipton and Hecht, 2005; Ayinla, 2007; Azaza *et al.*, 2009). Consequently, depending on the region, there may be need to seek alternatives to both fishmeal and soybean meal.

Sesame (family: Pedaliaceae) grown all over tropical and sub-tropical world (Sen and Bhattacharyya, 2001; El-Adawy and Monsour, 2011) are source of essential amino acids and sulphur amino acids like methionine (Lee *et al.*, 2003; Hahm *et al.*, 2009). Furthermore, the seeds have about 41–58 % oil, 18–25 % protein and 13–17 % carbohydrates (Bahkali *et al.*, 1998; Kang *et al.*, 2003; Yusuf *et al.*, 2008). They have good production base (Sen and Bhattacharyya, 2001), readily available (El-Adawy and Monsour, 2011) and thus cheaper than soybean meal.

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 of fish health (Adeparusi and Ajayi, 2004; Bello-Olusoji *et al.*, 2006). 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 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 (Maxwell *et al.*, 1990). More so, Animashahun *et al.* (2006) suggested that the comparison of haematological profile with nutrient intake may provide a base line for either increasing or reducing of certain nutrients for different population groups. Hence, this study investigates the effect of sesame seed meal based diet on the nutrient utilization and haematological characteristics of *Clarias gariepinus*.

MATERIALS AND METHODS

Procurement and preparation of experimental diets

The feed ingredients to be used in the feeding trial; fish meal, soybean meal (SBM), corn, vitamin-mineral premix, vegetable oil and starch were bought from Meat market, Abakaliki, Ebonyi State, Nigeria. These ingredients were separately milled, screened with fine mesh sieve to fine particle size (< 250 µm) and triplicate samples were analysed for proximate composition using the method of AOAC (2005). Sesame seed were obtained from Ikwo market, Ikwo Local Government Area of Ebonyi State, Nigeria. The seeds were ground in a hammer mill and the oil was removed from the seed meal using a volatile catalyst; n-Hexane as recommended by Enujiugha and Akanbi (2005). After 72 hours, the defatted cake and soybean meal were analyzed for its proximate composition (AOAC, 2005) as presented in Table 1.

Based on the nutrient composition of the protein feedstuff (Table 1), five isonitrogenous and isocaloric experimental diets were formulated. The experimental diets were formulated to produce diets in which 0% (SSM0), 25% (SSM25), 50% (SSM50), 75% (SSM75), and 100% (SSM100) of proteins from SBM were replaced with that from SSM (Table 2). The feedstuffs were finely grounded and mixed in plastic bowl into dough form using hot water, with cassava starch as binding material. The mixture was then pelleted by passing it through a mincer of 2 mm die to produce 2 mm diameter size of pellets. These

were sundried to about 10% moisture content, packed in polythene bags and kept safe dry for use.

Experimental design

Three hundred (300) fingerlings of *Clarias gariepinus* of 28.7 g ± 0.83 mean weight were purchased from Kelchez Fish Farm in Abakaliki, Ebonyi State, Nigeria and were transported to the Department of Fisheries and Aquaculture (the experimental site) using an oxygen air bag. They were acclimated for one week in plastic holding tanks of 3.0 m x 0.5 m x 0.5 m, and with a feed of 35% crude protein in the Laboratory.

Fish were sorted, weighed with an electronic balance (Metler Toledo 320) and randomly stocked into 70 litre plastic experimental tanks using three tanks per treatment at the rate of 15 fingerlings per tank. They were starved overnight before the commencement of the feeding trials (Ayinla, 2007). Fish were fed diets at the rate of 5% of their body weight per day, administered in two equal portions between 8.00-9.00 h and 18.00-19.00 h. The quantity of feed was adjusted fortnightly based on the weight gain of fish throughout the 8 weeks duration of the feeding trial. The fish were monitored daily for mortality. Dead fish were removed, counted and recorded. Growth response and feed utilization indices were estimated according to Jimoh and Aroyehun (2011).

Haematological studies

The blood analysis was determined according to the method described by Svobodova *et al.* (1991). The following was done.

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:

$$\text{RBC} = \text{No of cells counted} \times 10 \times 200 \text{ (} 10^6 \text{ mm}^3 \text{)}$$

$$\text{WBC} = \text{No of cells counted} \times 0 \times 25 \times 10 \times 20 \text{ (} 10^4 \text{ mm}^3 \text{)}$$

Haemoglobin estimation: Haemoglobinometer was used for haemoglobin estimation based on acid haematin method

$$\text{Haemoglobin} = \text{value obtained} / 100 \times 17.2 \text{ mg} / 100 \text{ (mL)}$$

Packed cell volume: the packed cell volume was measured after placing sealed micro-haematocrit tube in a centrifuge at 10,500 rpm using micro-haematocrit reader and expressed as percentage.

Erythrocyte sedimentation rate (ESR): ESR was determined using the procedure of Svobodova *et al.*

(1991). The volume of ESR within the given time interval is the difference between 100% and percentage part presented by the corpuscle volume.

The remaining haematological indices such as mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH) and mean cell volume (MCV) were calculated as described Dacie Lewis (2001), using the total red blood cell count (RBC), haemoglobin concentration (Hb), and hematocrit (Hct).

$$\text{MCHC (g l-1)} = [\text{Hb (g dl-1)} \times 10] / \text{Hct} \times 100$$

$$\text{MCH (pg)} = [\text{Hb (g dl-1)} \times 10] / \text{RBC (106 } \mu\text{l-1)}$$

$$\text{MCV (fl)} = \text{Hct} / \text{RBC (106 } \mu\text{l-1)}$$

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) test using SPSS 17.0 version. Where ANOVA revealed significant difference ($P < 0.05$), Duncan multiple – range test was applied to

characterize and quantify the differences between treatments.

RESULTS

The crude protein content of soybean, 43.72% used for this experiment is higher than that of sesame seed meal, 41.64%, though, they were not significantly ($p > 0.05$) different. However, sesame seed meal recorded significantly ($p > 0.05$) higher values of crude fat, crude fiber and ash than soybean meal (Table 1).

The crude protein of the test diets ranged between 40.09% - 41.54% and was not significantly different ($P > 0.05$). The highest crude fat 4.03% was in 50% sesame seed meal inclusion diet while the lowest value of 3.41% was in the 100% sesame seed meal diet (Table 2). Table 3 shows the proximate composition of experimental diets prepared with sesame seed meal; there was no significant difference ($p > 0.05$) for crude protein levels across diets.

Table 1: Proximate Composition of soybean meal (SM) and sesame meal (SSM) meal used in diet formulation

Composition	Soybean meal	Sesame meal
Crude protein	43.72 ^a	41.64 ^a
Crude fat	3.69 ^b	6.81 ^a
Crude fibre	4.40 ^b	7.04 ^a
Ash	5.91 ^b	11.88 ^a
Moisture	7.27	6.25
Nitrogen free extract	5.01	26.13

All values on the same row with the different superscripts are significantly different ($P < 0.05$).

Table 2: Ingredient composition of diets containing sesame meal for *Clarias gariepinus*

Ingredients (g/100g)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal (64% CP)	36.00	36.00	36.00	36.00	36.00
Soybean meal (44% CP)	36.00	27.00	18.00	9.00	0.00
Sesame seed meal (44% CP)	0.00	9.00	18.00	27.00	36.00
Maize	13.00	13.00	13.00	13.00	13.00
Wheat meal	5.00	5.00	5.00	5.00	5.00
Vegetable oil	3.00	3.00	3.00	3.00	3.00
Fish oil	3.00	3.00	3.00	3.00	3.00
Vitamin/mineral premix	2.00	2.00	2.00	2.00	2.00
Binder	2.00	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated crude protein level (%)	40.57	40.57	40.57	40.57	40.57

Table 3: Proximate composition of experimental diets containing sesame meal for *Clarias gariepinus*

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Crude Protein	41.23 ^a	40.21 ^a	41.32 ^a	41.54 ^a	40.09 ^a
Crude Fat	3.95 ^a	3.23 ^b	4.03 ^a	3.78 ^a	3.14 ^b
Fibre	2.78 ^b	2.88 ^b	2.66 ^b	2.55 ^b	3.08 ^a
Ash	9.91 ^a	8.75 ^b	9.86 ^a	8.84 ^b	9.44 ^b
Moisture	6.36	7.17	6.29	6.51	7.28
Nitrogen free Extract	35.77	37.76	45.84	36.78	36.97

All values on the same row with the different superscripts are significantly different (P<0.05).

The highest and lowest mean weight gain, 62.25 g/fish and 12.25 g/fish were recorded from 0% (Control diet) and 100% sesame seed meal diets respectively. The mean weight gain and relative growth rate of the control diet differed (P < 0.05) significantly with 50%, 75% and 100% sesame seed meal diets. However, they were statistically comparable with 25% inclusion of sesame seed meal based diet (Table 4). The highest relative growth rate

of 213.18% was recorded from 0% (control diet) sesame seed meal diet while the lowest value of 35.96% was recorded in 100% sesame seed meal diet. The highest FCR, 7.09 was observed in 100% sesame seed meal diet whereas the least, 1.46 was recorded in 0% (control diet). There was significant difference (P < 0.05) between the FCR of fish fed the control diet and the test diets.

Table 4: Growth response of *Clarias gariepinus* fed diets containing sesame meal for 56 days, mean ± S.E. of three replicates

	Diet1	Diet 2	Diet 3	Diet 4	Diet5
Initial Weight (g)	29.20±1.22 ^b	30.55±2.83 ^b	29.89±1.75 ^b	27.02±0.98 ^c	34.07±3.53 ^a
Final Weight (g)	91.45±4.72 ^a	88.50±4.58 ^a	53.33±2.73 ^c	69.98±3.13 ^b	46.31±1.53 ^d
Mean Weight Gain (g)	62.25±3.50 ^a	57.95±1.75 ^a	24.40±0.298 ^c	42.95±2.15 ^b	12.25±0.50 ^d
Relative growth rate	213.18±5.46 ^a	189.69±4258 ^a	81.63±1.90 ^b	158.10±3.00 ^b	35.96±0.53 ^d
Feed Conversion Ratio ¹	1.46±0.17 ^a	2.25±0.14 ^b	3.37±0.77 ^c	2.18±0.25 ^b	7.09±0.28 ^d
Survival (%)	75.0	66.7	62.2	53.3	64.4

All values on the same row with the different superscripts are significantly different (P<0.05).

The highest survival rate of 75% was recorded in control while the lowest of 53.3% was recorded in 75% sesame seed meal diets. There was significant difference (P<0.05) between the percentage survival of fish fed sesame seed meal based diets and the control (Table 4).

The Packed cell volume (PCV) and Haemoglobin concentration (Hb) ranges between 24.04% and 8.10g/dl in fish fed control diet to 15.23% and 5.10g/dl in fish fed 100% sesame seed meal diet respectively (Table 5). PCV and Hb of the fish decrease with increase in the inclusion levels of sesame seed meal. They were significantly (p<0.05) different from the control diet. However, the PCV and Hb of the fishes receiving 50%, 75% and 100% sesame seed diets were not significantly (p > 0.05) different. The Erythrocyte sedimentation (ESR), increases with increments in levels of sesame seed meal in the test diets. Hence, diet with 100% sesame meal has the highest ESR (12.01 mm/hr) and is significantly (p < 0.05) different from the control diet. The blood cells (WBC and RBC) of the fish fed

control diets were found to be significantly (p < 0.05) higher than those fed test diets. They were observed to be negatively correlated with increase in the level of sesame seed meal in the diets. However, the WBC and RBC of the fishes receiving the test diets were not significantly (p > 0.05) different. The mean corpuscular haemoglobin (MCH) ranges from 13.97 (pg) in control fish to 17.00 (pg) in the fish receiving 100% sesame seed meal diet. Whereas, the MCH increases with increase in the level of sesame seed meal inclusion and differed (p < 0.05) significantly with the control diets, they were found to be statistically (p > 0.05) the same among the fish receiving the test diets. The mean corpuscular haemoglobin concentration (MCHC) were found to be quite variable among the fishes, but were not significantly (p > 0.05) different except for fish receiving 50% sesame seed meal.

Table 5: Haematology of *Clarias gariepinus* fed diets containing sesame meal for 56 days, mean \pm S.E. of three replicates

	PCV (%)	Hb (g dl ⁻¹)	ESR (mm/hr)	WBC (x10 ³ /μl)	RBC (x 10 ⁶ /μl)	MCH (pg)	MCHC (g l ⁻¹)	MCV (fl)
Initial	20.00 \pm 6.03 ^b	6.70 \pm 2.00 ^b	4.00 \pm 1.20 ^{a,b}	6.40 \pm 1.40 ^b	4.00 \pm 0.64 ^b	16.75 \pm 0.09 ^{b,c}	335.00 \pm 3.20 ^a	5.00 \pm 0.19 ^{a,b,c}
Diet 1	24.04 \pm 0.33 ^a	8.10 \pm 0.03 ^a	3.00 \pm 0.33 ^c	8.70 \pm 0.03 ^a	5.80 \pm 0.06 ^a	13.97 \pm 0.16 ^c	337.50 \pm 8.10 ^a	4.14 \pm 0.60 ^c
Diet 2	19.01 \pm 0.00 ^b	6.52 \pm 0.03 ^b	6.02 \pm 0.00 ^{a,b}	6.22 \pm 0.00 ^b	3.93 \pm 0.00 ^{a,b}	16.67 \pm 0.02 ^a	342.11 \pm 2.30 ^a	4.87 \pm 0.00 ^{a,b}
Diet 3	16.13 \pm 0.33 ^c	5.33 \pm 0.03 ^c	7.110 \pm 0.06 ^{a,b}	4.50 \pm 0.03 ^{a,b}	3.32 \pm 0.03 ^{a,b}	16.06 \pm 0.06 ^a	279.95 \pm 7.10 ^b	4.85 \pm 0.39 ^{b,c}
Diet 4	17.33 \pm 0.33 ^c	5.10 \pm 0.06 ^c	8.04 \pm 0.4 ^b	4.32 \pm 0.03 ^b	3.03 \pm 0.07 ^b	15.43 \pm 0.06 ^{a,b}	340.00 \pm 9.50 ^a	5.00 \pm 0.22 ^a
Diet 5	15.23 \pm 0.33 ^c	5.41 \pm 0.06 ^c	12.01 \pm 0.33 ^a	4.71 \pm 0.06 ^{a,b}	3.50 \pm 0.06 ^{a,b}	17.00 \pm 0.11 ^a	317.65 \pm 0.06 ^{a,b}	4.86 \pm 0.06 ^{a,b}

*Means with similar superscripts in a same column are not significantly different (p>0.05).

PCV = Packed cell volume; Hb = Haemoglobin concentration; ESR = Erythrocyte sedimentation; WBC = White blood count; RBC = Red blood count; MCH = Mean corpuscular haemoglobin and MCHC = Mean corpuscular haemoglobin concentration.

DISCUSSION

Fagbenro *et al.* (1992) and Eyo (1996) reported that the optimum protein requirement of the mud catfishes (*Clarias gariepinus*, *Heterobranchius bidorsalis* and their hybrids) falls within 40.0 to 42.5%, which is close to the crude protein recorded in this present study. The results showed that it is possible to replace soybean meal in *C. gariepinus* diet with raw sesame seed meal with optimum growth response at a 25% replacement level. These results also show a reduction in growth and feed conversion ratio as the raw plant protein increased beyond 25%. This growth reduction observed at higher inclusion levels of sesame seed meal could be related not only to dietary amino acid profile but also the presence of anti-nutritional factors. Sesame seed is reported to contain high amount of oxalate and phytic acid (Johnson *et al.*, 1979, Narasinga, 1985). Oxalic acid has been reported to reduce the physiological availability of calcium from the seed. However dehulling reduces the oxalic acid content of the seed (Salunkhe *et al.*, 1991).

Sesame seed meal was suggested by Tacon (1992) of its maximum level of inclusion in both omnivorous and herbivores fish species to be 35%. Hossain and Jauncey (1989) reported that Bangladeshi variety of sesame oil seed meal can be included up to 25% in raw condition in the diet of *Cyprinus carpio* L. Hossain *et al.* (1992) substituted fish meal with 20% sesame oilseed meal in the diets of catfish, *Heteropneustes fossilis* and reported promising result.

Sesame usually contains anti-nutritional factor, phytic acid which either forms complex with protein or binds metal ions such as calcium and magnesium inhibiting the absorption of these important minerals (Gohl, 1981). However, it seems that at a lower level of inclusion, there is a physiological mechanism in fish that could compensate for the presence of these anti-nutrients, hence their negative effect may not be felt; but at higher level of inclusion, when the limit might have been exceeded, then the negative effect of

these anti-nutrients will be well pronounced (Fagbenro *et al.*, 2010). Francis *et al.* (2001) postulated that below the 5 mg/g level, most cultured fish are able to compensate the presence of trypsin inhibitors by increasing trypsin production. The lowered growth performance of fish fed high phytate containing sesame diets; can be attributed to various factors, namely reduced bioavailability of minerals, impaired protein digestibility caused by formation of phytic acid-protein complexes and depressed absorption of nutrients due to damage to pyloric cecal region of the intestine (Francis *et al.*, 2001).

A measurable increase in the haematological parameters of *Clarias gariepinus* fed toasted sunflower seed meal was reported by (Akintayo *et al.*, 2008; Fagbenro *et al.*, 2012). Yue and Zhuo (2008) had similar report when they fed cotton seed meal to juvenile hybrid Tilapia. However, contrary to the observations of the above authors, this present investigation revealed a decrease in the haematological parameters of the fishes with increase in the inclusion level of sesame seed meal. This finding agrees with the reports of Adeparusi and Ajayi (2004) in *Oreochromis niloticus* fed Lima bean. Other researchers (Bloom *et al.*, 2001; Dabrowski *et al.*, 2001; Richard *et al.*, 2003) also reported a decrease in haematocrit and haemoglobin with increasing levels of ingredients. This decrease in the haematological profiles of fish with increase level of incorporation of sesame seed meal may probably be connected with the presence of tannin and phytate present in the seed meal. However, the presence of these anti-nutrients in the diets did not exert sufficient negative effects as to induce pathological changes in the fish.

CONCLUSION

This study showed that raw sesame seed meal can replace soybean meal up to 25% for optimum growth without negative effect on the health of the fish. It is therefore; recommended that processing of sesame seed by either soaking or toasting may reduce

the level of anti-nutrients present in the seed. Hence, increasing the nutritional value of its use in fish feed production with little or no negative effect on the fish.

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