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Original Article

Evaluation of soybean meal replacement with sesame seed meal using activated charcoal as an additive in the diet of African catfish juveniles, *Clarias gariepinus*

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Abstract: This study evaluated the effects of 0.25% activated charcoal added to sesame seed meal (SSM) in partial replacement of soybean meal (SBM) of juvenile *Clarias gariepinus* diet on growth and haematological parameters for 70 day experimental period. Six experimental diets were formulated as: control diet 1 (100% SSM without activated charcoal), control diet 2 (100% SSM plus 0.25% activated charcoal), diet 3 (50% SMB+50% SSM), diet 4 (50% SBM+50% SSM+0.25% AC), diet 5 (30% SBM+70% SSM) and diet 6 (30% SBM+70% SSM+0.25AC). The inclusion of activated charcoal recorded significant improvement in mean weight gain, mean feed intake, specific growth rate (SGR), protein intake (PI) and protein efficiency ratio (PER). The control diet 2 recorded the highest values for growth and nutrient utilization parameters while diet 5 recorded the least values for these parameters. The best values for mean weight gain (65.33±5.57 g), specific growth rate (2.56±0.12% / day) and feed conversion ratio (0.71±0.05) were recorded in fish fed control diet 2 while the worst values (41.30±3.82 g, 1.92±0.11% / day, and 1.07±0.07, respectively) for these parameters were observed with diet 5. However, haematological parameters did not differ significantly across the diets. Thus, from this study 0.25% activated charcoal could favorably be added to the feed of *C. gariepinus* for optimum performance without any adverse effect on the health status of the fish.

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Introduction

Plant protein could be an alternative to expensive fishmeal; however, the inclusion of plant ingredients like soybean meal, groundnut seed meal and cotton seed meal in fish feed has been reported to reduce fish growth rate in comparison to fish meal (Imorou-Toko et al., 2008; Li et al., 2010). This reduced growth rate of fish fed with plant proteins inclusion could be due to several factors such as poor digestibility (Lech and Reigh, 2012), imbalanced amino acid composition (Xie et al., 2001) and inherent anti nutritional factors (ANF) in most plant ingredients (Francis et al., 2001).

Soybean has been the major plant ingredient used in livestock and fish feeds (Shipton and Hecht, 2005), but a sharp price pressure in recent times ensued by increasing utilization by livestock feed production and human being nutrition (Hardy, 2010). The search for alternatives to soybean is thus important for sustainable aquaculture industry and its profitability.

Sesame (*Sesamum indicum* L.) plant is a xerophyte adapted to many soil types (Ram et al., 1990). According to Ahmed (2005), there are about 335,000 hectares of land under sesame cultivation in Nigeria with yields of between 1.5-2.0 tonnes/hectare. Sesame seed has nutrient composition similar to other oilseed proteins including soybean meal and other conventional legumes (Sintayehu et al., 1996) and its potential as dietary protein source is well-recognized (Olvera-Novoa et al., 2002).

Full-fat sesame seed contains 22% crude protein and the meal after oil extraction about 44% crude proteins (Mamputu and Buhr, 1995). The seed contains 50-60% oil compared to 20% in soybean

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(Kato et al., 1998; Ahmed, 2005). Hossain and Jauncey (1989) found that sesame oilseed meal can be included up to 25% in common carp, *Cyprinus carpio* L, however, Tacon (1993) suggested that maximum level of its inclusion is 35% in both omnivorous and herbivorous fish species. Whereas, Mukhopadhyay and Ray (1999) suggested that fermented sesame seed meal protein can replace up to 50% of fish meal protein in the diets for rohu fingerling.

Limitation to the effective utilization of sesame seed include high phytic acid up to 50 $\mu g\ g^{-1}$, tannin and oxalic acid which reduces the biological availability of zinc, calcium, magnesium and iron (Nahm, 2007), the following treatment methods already suggested; adequate heat treatment, soaking in water, fermented with lactic acid bacteria and extraction of oil, could reduce seed toxicants (Tanin, oxalic and phytic acids) and crude proteins and lipids level improvement (Mukhopadhyay and Ray, 1999; Diarra and Usman (2008). Effective approach to the reduction of anti-nutritional factors in sesame seed could be the use of non-nutritive and inert adsorbents additives which could bind ANF and reduce the absorption of ANF in the gastrointestinal tract.

Activated charcoal (AC) is a form of carbon with increased surface area for absorption. Adsorption therapy with activated charcoal as a non-digestible carrier is one of the important methods of preventing the ingested toxicants or noxious substances formed in the gastrointestinal tract (Van et al., 2006). The major advantages of AC include safety, easy administration and control of pathogenic bacteria through addition to the animal feed (Dalvi and Ademoyero, 1984; Nikoleavia et al., 1994). The present study therefore, looked at the possibility of using bamboo activated charcoal to enhance sesame seed meal utilization on growth performance and haematology of African catfish, *Clarias gariepinus*, juveniles.

Materials and Methods

The experimental catfish, *C. gariepnus* were purchased from a fish farm at Cele, Ikotun-Egbe, Lagos State. The fish were transported in a 25 litres plastic container with water to the Nutritional Unit of the Department of Marine Sciences, University of Lagos. The fish were kept in plastic tank to acclimatize for two weeks. During the acclimatization period, they were fed with 2 mm coppens fish feed (Coppens International bv, the Netherlands), thereafter 10 juveniles of an average weight 20.9 g were allocated to each of the twenty-four plastic containers making a total of two hundred and forty *C. gariepinus* juveniles.

All the ingredients, including soybean meal (SBM) and activated charcoal (AC) used for the experiment except sesame seed were purchased at Soleace Nigeria limited, Oko-Oba, Agege, Lagos, Nigeria. The sesame seed (*Sesamum indicum*) was bought from Bariga market, Lagos, Nigeria. Ingredients were separately milled, screened to fine particle size (< 250 μm mesh size) and stored for future use.

The sesame seeds were screened, winnowed and cleaned to remove dirt, sand, stones and other undesirable particles. Using fire wood as a source of heat, the Sesame seeds were placed in a wide open aluminum pan and toasted for about 15 minutes by stirring continuously to prevent the burning of the seed coat and enhance even distribution of heat. The toasting was stopped when pop sound was produced and the seeds slightly turned brown to produce sesame seed meal (SSM) according to Yakubu and Alfred (2014).

Six experimental feeds were formulated (Table 1), including: the positive control (CTR) diet 1 (100% SSM without activated charcoal); the negative control (CTR) diet 2 (100% SSM plus 0.25% activated charcoal), diet 3 (50% SMB + 50% SSM), diet 4 (50% SBM + 50% SSM + 0.25% AC), diet 5 (30% SBM + 70% SSM) and diet 6 (30% SBM + 70% SSM + 0.25AC). Ingredients were carefully weighed, mixed and water was added to form dough. The dough was pelletized with 3 mm die using locally fabricated pelletizing machine and sundried.

The study, which lasted for a period of 10 weeks (70 days), was conducted in twenty-four (55 x 33 x

Table 1. Composition of experimental diets.

Ingredients	100% SSM (CTR 1)	100% SSM + AC (CTR 2)	50% SSM (Diet 3)	50% SSM + AC (Diet 4)	70% SSM (Diet 5)	70% SSM + AC (Diet 6)
Fish meal	20.0	20.0	21.0	21.0	24.0	24.0
Groundnut cake	22.0	22.0	25.0	25.0	24.0	24.0
Noodle waste	25.0	25.0	21.0	21.0	19.0	19.0
Soybean meal	NIL	NIL	15.0	15.0	9.0	9.0
SSM	30.0	30.0	15.0	15.0	21.0	21.0
Oil	1.0	1.0	1.0	1.0	1.0	1.0
Premix	0.25	0.25	0.25	0.25	0.25	0.25
Calcium monohydrogen phosphate	1.0	1.0	1.0	1.0	1.0	1.0
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin C	0.25	0.25	0.25	0.25	0.25	0.25
AC	NIL	0.25	NIL	0.25	NIL	0.25
Crude protein (%)	41	41	40	40	42	42
Crude energy	3046	3046	3189	3189	3117	3117

Table 2. Growth and nutrient utilization parameters of *Clarias gariepinus* fed experimental diets.

Diets	CTR Diet 1	CTR Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
IMW (g/fish)	21.30±0.02	21.00±0.20	21.30±0.17	21.17±0.21	21.33±0.06	19.93±2.45
FMW (g/fish)	86.63±5.57^{b}	112.57±3.58^{a}	64.17±4.66^{c}	68.87±4.18^{c}	62.63±3.76^{c}	66.87±9.43^{c}
MWG (g/fish)	65.33±5.57^{b}	91.47±3.62^{a}	42.87±4.62^{c}	47.70±3.99^{c}	41.30±3.82^{c}	46.93±7.50^{c}
MFI (g)	52.93±7.73^{ab}	64.44±4.14^{a}	42.19±14.02^{b}	44.11±7.46^{b}	44.01±1.54^{b}	44.89±6.55^{b}
SGR (%/day)	2.56±0.12^{b}	2.99±0.06^{a}	1.97±0.13^{cd}	2.10±0.09^{cd}	1.92±0.11^{d}	2.16±0.13^{cd}
FCR	0.81±0.07^{bc}	0.71±0.05^{c}	0.97±0.23^{ab}	0.92±0.09^{ab}	1.07±0.07^{a}	0.96±0.01^{ab}
PI	44.75±3.63^{ab}	51.21±3.38^{a}	38.37±8.60^{bc}	39.32±3.75^{bc}	33.66±1.99^{c}	37.59±0.53^{bc}
PER	1.47±0.21^{ab}	1.79±0.12^{a}	1.17±0.39^{b}	1.23±0.21^{b}	1.15±0.04^{b}	1.25±0.18^{b}

31 cm^3) rectangular plastic tanks. The tanks were covered with nets to prevent fish from jumping out. The experiment was run in triplicate, making a total of 24 tanks. The fishes were hand fed to satiation three times a day (9:00 am, 1:00 pm and 5:00 pm). The water was changed every other day to ensure the fish were kept under standard condition; temperature (27.5-29.5ºC), dissolved oxygen (4.5-4.8 mg/l), and pH (7.3-8.0) as described by Aderolu and Akpabio (2009). The weekly weight gain of the fish was measured by bulk-weighing the fish and the weekly feed intake also recorded. After acclimatization, initial weight of fish was taken using an electronic scale (OHAUS CS5000) and recorded. Fish weighing was done every week.

Growth and nutrient utilization parameters: these parameters were calculated using following formulas:

(1) Mean Weight Gain (MWG) (g) = Final mean weight (FMW) – initial mean weight (IMW)

(2) Feed Conversion Ratio (FCR) = (Feed intake (g)) / (Weight gain (g))

(3) Specific Growth Rate (SGR) (%/day) = (Log_e W_2 – Log_e W_1x 100) / (T_2 – T_1 (day))

Where, $_e$ is natural logarithm, T_2 – T_1= experimental period, W_1 = initial weight, and W_2 = final weight.

(4) Protein efficiency ratio (PER) = (Mean weight gain) / (Protein intake)

(5) Mean feed Intake (MFI) = Feed intake for Experimental period) / (Number of days in the period)

(6) Protein intake (PI) = (Total feed intake) / (Protein content of feed)

Haematological analysis: The blood samples were

Table 3. Haematological indices of *Clarias gariepinus* fed experimental diets.

Parameters	CTR diet 1	CTR diet 2 + AC	Diet 3	Diet 4	Diet 5	Diet 6
RBC (10^{12}/L)	10166666 ±6048415.79	5300000 ±1137610.0	7050000 ±1060660.1	6950000 ±2050609.6	6300000 ±141421.35	6000000 ±565685.42
PVC (%)	35.33±16.92	18 ±0	28 ±2.83	26 ±8.49	25.5 ±0.71	24 ±2.83
WBC (10^9/L)	12000±4000	11000 ±0	12000±2828.43	9500 ±2121.32	13500±2121.32	11000±5656.85
Hb (g/L)	11.53 ±5.56	5.9 ±0	9.05 ±92	8.4 ±2.69	8.25 ±0.21	7.85 ±0.78
MCHC (g/L)	32.63 ±0.33	32.78 ±0	32.32 ±0.02	32.34 ±0.22	32.35 ±0.07	32.75 ±0.62
MCV (fl)	0.000036 ±0.000004	0.000034 ±0	0.00004 ±0.00002	0.000037 ±0.000012	0.00040 ±0.0000002	0.00004 ±0.0000009

collected from the caudal peduncle as described by Joshi et al. (2002) in a 2 ml syringe ad mixed with Ethylene-diamine-tetra-acetic acid (EDTA) for haematological analysis at Bioassay diagnostic laboratory, Cele-Egbe, Ikotun, Lagos. Haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV) were analyzed using the methods described by Svobodova et al. (1991).

Data analysis: All the data were subjected to analysis of variance (ANOVA) and Duncan's multiple range tests were used to compare means among the treatments.The computations were performed using the package SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

Results

The results of the growth and nutrient utilization parameters are shown in Table 2. The final mean weights (FMW) were higher in the groups with the inclusion of 0.25% activated charcoal (AC) across the experimental diets. The highest FMW (112.57±3.58 g) was recorded for fish fed control 2 diet (100% SSM plus 0.25% AC) while the lowest value (62.63±3.76 g) was recorded in diet 5 (70% SSM). The partial substitution of soybean meal with roasted sesame seed meal actually resulted in inferior (P<0.05) mean weight gain; however, the inclusion of AC led to significant (P<0.05) improvement in mean weigh gain (MWG), mean feed intake (MFI) and specific growth rate (SGR). The CTR diet 2 recorded highest values for MWG (91.47±3.62 g), MFI (64.44±4.14 g) and SGR (2.99±0.06% day-1). The lowest values for these parameters MWG (41.30±3.82 g), MFI (44.01±1.54 g) and SGR (1.92±0.11% day-1) were recorded in fish fed diet 5.

Furthermore, the nutrient utilization parameters tested recorded significant differences (P<0.05) across diets, the inclusion of AC positively (P<0.05) affected feed conversion ratio (FCR), protein intake (PI) and protein efficiency ratio (PER). The best value (0.71±0.05) for FCR was recorded in fish fed CTR diet 2 while the worst value (1.07±0.07) was recorded by the group fed diet 5. Similar results were observed with the protein intake (PI) and protein efficiency ratio (PER). The CTR diet 2 recorded highest values for PI (51.21±3.38) and PER (1.79±0.12) while diet 5 recorded least values (33.66±1.99, 1.15±0.04, respectively) for these parameters. No significant differences (P>0.05) were recorded in FMW, MWG, MFI and PER among diets 3, 4, 5 and 6. Also, significant differences (P<0.05) were not recorded between the graded levels (50 and 70% substitutions) of sesame seed meal with activated charcoal. Amongst the experimental diets, the diets with activated charcoal inclusion had better growth and nutrient utilization performances than the other dietary groups (Table 2).

The results of the haematological parameters showed no significant differences (P>0.05), in Hb, RBC, WBC, PCV, MCHC and MCV across the experimental diets despite the inclusion of 0.25% activated charcoal (Table 3).

Discussion

The reduction in growth parameters recorded at the inclusion of toasted sesame seed meal without activated charcoal in the diet of *C. gariepinus* could be due to dietary amino acid profile imbalance and the presence of anti-nutritional factors in test ingredient (sesame seed). Sesame seed is reported to contain high amount of oxalate and phytic acid (Narasinga Rao, 1985). Oxalate acid reduces the physiological availability of calcium from the seed (Salunkhe et al., 1991). Also, phytic acid reduces bio-availability of minerals, impairs protein digestibility by formation of phytic acid-protein complexes and depresses absorption of nutrients due to damage to pyloric caeca region of the intestine (Francis et al., 2001). The above suggested reasons could then account for the resultant poor nutrient utilization and growth rate on the substitution of soybean meal for sesame seed meal in *C. gariepinus* (Jimoh and Aroyehun, 2011).

However, the inclusion of activated charcoal along with the sesame recorded better growth performance and improved nutrients utilization, especially in the negative control diet. This result is corroborated by the works of Yoo et al. (2005) on juvenile Japanese flounder and Thu et al. (2009) when they fed Tiger fish, *Takifugu rubripes*, with graded levels of bamboo charcoal. They reported improved growth and nutrient utilization which was attributed to increase availability of certain macronutrients, particularly proteins, leading to a better nutrient accumulation. Also, Jahan et al. (2014) attributed the enhanced growth and nutrient utilization of fish fed activated charcoal feed to the presence of nutrient enhancing microbes along the gastrointestinal tract and possibly the thinner and lighter surface area of the intestinal villi of animal. The increased weight gain might also be due to the fact that the activated charcoal has the capacity to improve apparent nutrient digestibility, reduce toxins in diets by adsorbing it and thereby preventing its absorption from the intestine and selectively inhibit the harmful coliforms (Anjaneyulu et al., 1993; Choi et al., 2009).

The improved FCR, SGR and PER were similar to previous studies when activated charcoal was included in the feed of chicken as reported by Moe et al. (2009) and Jahan et al. (2014) on *Pangasius hypophthalmus* catfish, respectively. The result was attributed to the absorbent effect of activated charcoal which has the potential to condition the intestinal cell membranes to reduce surface tension by eliminating noxious substances along the intestine and the utilization and absorption of nutrients across the cell membranes.

Blood is an important index of physiological, pathological and nutritional status in the organism (Olorode et al., 2007). It is an index and a reflection of the effects of dietary treatments on the animal in terms of the type, quality and amounts of the feed ingested and was available for the animal to meet its physiological, biochemical and metabolic necessities (Ewuola et al., 2004). The haematological parameters measured did not show any significant difference across all experimental diets, despite the inclusion of activated charcoal. This result is supported by the findings of Thu et al. (2009), when they recorded no significant difference in the values of heamatocrit and heamoglobin concentration across the different diets after feeding dietary bamboo charcoal to Tiger puffer fish. Probable implication of these results is that the experimental fish heamatology parameters were by no means affected by the dietary inclusion of activated charcoal.

In conclusion, from the present study 0.25% AC could favorably be added as natural substance to the feed of *C. gariepinusfor* optimum performance without any adverse effect on the health status of the fish.

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چکیده فارسی

ارزیابی جایگزینی پودر سویا با پودر کنجد با استفاده از زغال فعال به‌عنوان یک افزودنی در خوراک گربه ماهی نوجوان آفریقایی، *Clarias gariepinus*

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گروه علوم دریایی، دانشگاه لاگوس، آکوکا، یابا، نیجریه.

چکیده:
این مطالعه اثرات افزودن ۰/۲۵ درصد زغال فعال (AC) به پودر دانه کنجد (SSM) به‌عنوان جایگزینی بخشی از پودر سویا (SBM) در خوراک گربه‌ماهی نوجوان آفریقایی، *Clarias gariepinus* بر رشد و پارامترهای خون‌شناسی را در یک دوره آزمایش ۷۰ روزه مورد ارزیابی قرار داد. شش خوراک آزمایشی شامل (۱) تیمار شاهد ۱ حاوی ۱۰۰٪ SSM بدون AC، (۲) تیمار شاهد ۲ حاوی ۱۰۰٪ SSM به علاوه ۰/۲۵٪ AC، (۳) خوراک ۳ شامل ۵۰٪ SMB + ۵۰٪ SSM، (۴) خوراک ۴ شامل ۵۰٪ SMB + ۵۰٪ SSM + ۰/۲۵٪ AC، (۵) خوراک ۵ شامل ۳۰٪ SMB + ۷۰٪ SSM و (۶) خوراک ۶ شامل ۳۰٪ SMB + ۷۰٪ SSM + ۰/۲۵٪ AC فرموله شدند. افزودن زغال فعال بهبود افزایش وزن، میانگین جذب غذا، نرخ رشد ویژه (SGR)، جذب پروتئین (PI) و نسبت پروتیئن موثر (PER) را ثبت کرد. خوراک شاهد ۲ بالاترین مقادیر رشد و پارامترهای بهره‌وری تغذیه‌ای را نشان داد در حالی‌که خوارک تیمار ۵ کمترین مقادیر این پارامترهای تغذیه‌ای را داشت. بهترین مقادیر میانگین افزایش وزن (۶۵/۳۳±۵/۵۷ g)، نرخ رشد ویژه (۲/۵۶±۰/۱۲ درصد در روز) و نرخ تبدیل غذا (۰/۷۱±۰/۰۵) در ماهیان تغذیه شده با خوراک شاهد ۲ بدست آمد، در حالی‌که بدترین مقادیر برای پارامترهای فوق (به‌ترتیب ۴۱/۳۰±۳/۸۲ g، ۰/۰۷±۰/۱۱ درصد در روز و ۱/۰۷±۰/۰۷) در خوراک تیمار ۵ مشاهده شد. پارامترهای خون‌شناختی در بین تیمارها تفاوت معنی‌داری را نشان نداد. بنابراین براساس نتایج این مطالعه ۰/۲۵ درصد زغال فعال به غذای گربه‌ماهی آفریقایی می‌تواند برای کارایی مناسب تغذیه‌ای بدون هیچ گونه اثر زیانباری بر وضعیت سلامت ماهیان اضافه شود.

کلمات کلیدی: پودر سویا، کارایی رشد، زغال فعال، گربه‌ماهی آفریقایی.