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Research Article

Dietary Black Seed (*Nigella sativa*) Promotes Growth Performance of African Catfish (*Clarias gariepinus*) in Aquaculture

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Abstract:

This study evaluated the effects of dietary black seed as growth promoter in the diet of Clarias *gariepinus* juveniles. Five iso-proteinous diets were formulated and supplemented with black seed at 0.0%, 1.0%, 1.5%, 2.0% and 2.5% to make four treatments and one control diets. Completely randomized design (CRD) was employed. One hundred and fifty (150) C. *gariepinus* juveniles were used for the experiment. Ten fish were randomly assigned to a 1m2 Hapa net. A total of 15 Hapa nets were used in an outdoor earthen lined pond of 10m × 7m (l × b) and depth of 1.5m, and the five formulated diets were fed to the experimental fish at 5% body weight for a period of 8 weeks. Highest mean weight gain of 49.66±4.52g was obtained in the fish fed 2.5% followed by 47.66±4.52g obtained in the fish fed 2.0%. The least mean weight gain of 38.33±4.52g was recorded in the fish fed 1.0%. The highest feed conversion ratio of 2.63±0.09 was recorded in the fish fed 1.0% while the least (2.43±0.09) was recorded in the fish fed 2.5% dietary black seed which differed significantly ($P \leq 0.05$) with the other treatments. In conclusion, the result of this study elucidated that the fish fed 2.5% dietary black seed had the best growth performance, revealing the positive effects of black seed on the growth of C. *gariepinus.* Therefore, black seed can be used as natural growth promoter in the diet of C. *gariepinus.*

Key words: aquaculture; natural growth promoter; african catfish; black seed

Introduction

Aquaculture development today is imbalanced and there are multiple opportunities for the expansion of aquaculture in less developed areas with suitable natural resources, particularly in Africa and specifically in areas not otherwise farmable such as arid zones, alkaline land or open oceans [1]. Aquaculture production in Western Africa increased from 32 146 tonnes in 2000 to 388 375 tonnes in 2021 [2]. The 12.6 percent annual growth was higher than regional and world averages. The production increased in all 15 aquaculture countries in the sub-region. Nigeria contributed most of aquaculture production in the sub-region. Ghana, Mali, and Côte d'Ivoire were another three countries in the sub-region with relatively large (> 5 000 tonnes) aquaculture production [3]. In 2021, aquaculture was practiced in 15 out of 17 countries in the sub-region. Yet the effective number of countries was only 2.4, reflecting a concentration of aquaculture production in Nigeria and Ghana [2].

African catfish (*Clarias gariepinus*) is one of the most cultured fish species in Nigeria [4], it has a high growth rate, resistant to diseases and characterized with high fertility and can survive in a high stocking density. These characteristics have determined its commercial use and made it to be an excellent fish species for aquaculture. Nigeria is the world's largest producer of African catfish on which millions of people's livelihoods depend [5], it is widely distributed in Africa (i.e. from Nile to West Africa and from Algeria to Southern Africa) and middle East, and it inhabits freshwater ecosystem such as reservoirs, lakes and rivers as well as culture ponds.

Black seed (*Nigella sativa*) is a medicinal plant belonging to the family ranunculaceae and it has been known as far back as 1400 years ago [6]. Due to its numerous therapeutic properties, black seed is widely cultivated

and used in different regions of the world [7]. Black seeds contain many bioactive constituents, such as antioxidant compounds (mainly represented by thymoquinone and dithymoquinone), flavonoids, sterols, and polyunsaturated fatty acids [8]. Interestingly, black seeds are rich in bioactive compounds. In general, it contains about 32-40% fixed oils, 0.4-0.45% volatile oil 8-9 types of essential amino acids beside some carbohydrates and vitamins [9]. The pharmacological properties of this seeds are mainly attributed to having several bioactive constituents such as thymoquinone, dithymoquinone, thymol, nigellicine and nigellidine [10]. Positive effects of dietary black seed have been shown on the growth performance, biochemical and immuno-haematological parameters of *Oreochromis niloticus* [11] and *Cyprinus carpio* [12]. Therefore, this study aimed to evaluate the potential of black seed as a growth promoter in the diets of African catfish (*C. gariepinus*).

Materials and methods

Study Area

The study was conducted at fish nutrition unit of the Department of Fisheries, Faculty of Agriculture, University of Maiduguri, Nigeria. The University is located along Bama road, Maiduguri, Borno state and lies between latitude 11°29N and longitude 13°70E. The mean monthly temperature is highest (40.2°C) prior to the onset of the rain in June and the lowest (31.3°C) during the peak of the rainy period of August.

Experimental fish

One hundred and fifty *Clarias gariepinus* juveniles were procured from Garbati fish farm in Maiduguri, Borno state, Nigeria and transported in an oxygenated container to the experimental site.

Feed formulation and compounding

The experimental diets were formulated using soybeans, fishmeal, maize, oil and black seed. Other ingredients were premix, lysine, methionine, salt and binder. The soybeans was toasted and ground into powdered form separately. The black seed was ground and supplemented at different levels of 0, 1.0, 1.5, 2.0 and 2.5% to make one control and four experimental diets, respectively. The experimental feed ingredients were ground separately into a powdered form. Pearson's square method was used to calculate the inclusion levels of each ingredients as presented in Table 1. After the measurement, all the ingredients were mixed thoroughly to obtain a homogeneous product and water was added to form dough. The dough was pelleted using pelleting machine. The pelleted feeds were sun-dried and package in polythene bag in well ventilated room under ambient temperature.

Ingredient	BS 0.0%	BS 1.0%	BS 1.5%	BS 2.0 %	BS 2.5 %
Maize	15.84	15.84	15.84	15.84	15.84
Frish meal	24.72	24.72	24.72	24.72	24.72
Soyabean meal	49.44	48.44	47.94	47.44	46.94
Premix	0.7	0.7	0.7	0.7	0.7
Palm oil	3.5	3.5	3.5	3.5	3.5
Lysine	2	2	2	2	2
Methionine	2	2	2	2	2
Salt	0.8	0.8	0.8	0.8	0.8
Binder	1	1	1	1	1

Table 1: Feed Composition

Experimental design

Completely randomized design (CRD) was employed. One hundred and fifty (150) *C. gariepinus* juveniles were used for the experiment. Ten fish were randomly assigned to a $1m^2$ Hapa net. A total of 15 Hapa nets were used in an outdoor earthen lined pond of 10m × 7m (l × b) and depth of 1.5m, and the five formulated diets were fed to the experimental fish and the pond water was monitored daily.

Feeding trials

The fish were fed at 5% body weight twice daily, morning (8:00 – 9:00 a.m.) and evening (5:00 – 6:00 p.m.). Fish in each experimental Hapa was collectively weighed and both the total and standard lengths were measured biweekly using weighing balance and measuring board respectively, throughout the experimental period. The experiment lasted for 2 months.

Determination of proximate composition

The proximate composition of the diets and carcass composition of the experimental fish were determined using the methods of the AOAC (2019).

Moisture content

A clean crucible was dried to a constant weight in an air oven at 110°C, cooled in a desiccator and weighed (W_1). 2g of finely pulverized sample was weighed in the crucible and then re-weighed (W_2). The crucible and its content will be dried in an oven to a constant weight (W_3). The percentage moisture was calculated thus % Moisture content = $\{(W_2-W_3)/(W_2-W_1)\} \times 100$

Ash content

Porcelain crucible was dried in an oven at 100°C for 10 minutes, cooled in a desiccator and weighed (W_1). 2g of finely pulverized sample was weighed (W_2) into the previously weighed clean crucible which was ignited in the muffle furnace at 550°C for 1 hour and cooled in a desiccator. The crucible and its content was transferred into the muffle furnace and the temperature was gradually increased until it reached 550°C. The sample was left in the furnace for 8 hours to ensure proper ashing. The crucible containing the ash was allowed to cool to 200°C, the crucible was removed and cooled in a desiccator until constant weight is obtained (W_3). % Ash content = $\{(W_2-W_3)/(W_2-W_1)\} \times 100$

Crude lipid content

Four grams of sample was weighed (W_1) into a clean, dried 500 mL round bottom flask containing few antibumping granules was weighed (W_2) and 300mL of petroleum ether (40°C-60°C) for the extraction was poured into

the flask fitted with soxhlet extraction unit. The round bottom flask and a condenser was connected to the soxhlet extractor, and cold water circulation was put on. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for 6 hours. The solvent was recovered and the oil was dried in the oven at 70°C for 1 hour. The round bottom flask and oil was cooled and then weighed (W_3).

% Crude Content = $\{(W_2-W_3)/(W_2-W_1)\} \times 100$

Crude fibre

Two grams of finely pulverized sample was weighed into an extraction apparatus, fat was extracted with liquid petroleum spirit (40°C-60°C) the extracted was removed and dried at 105°C for 30 minutes. Two grams of the defatted sample was weighed into a dry 600 cm round bottom flask. 100 cm3 of (0.023M) sulphuric acid was added and the mixture boiled under reflux for 30 minutes. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it is acid free. This was quantitatively transferred into the flask and 100 cm3 of hot (0.312) sodium hydroxide solution was added and the mixture boiled under reflux for 30 minutes and quickly filtered under suction. The insoluble residue was washed with boiling water until it was base free. It will be dried to constant weight in the oven set at 100°C, cooled in a desiccator and weighed (C_2). The weighed residue was incinerated in a muffle furnace at 550°C for 2 hours, cooled in a desiccator and reweighed (C_3).

The loss in weight on ashing (incineration) = $C_2 - C_3$
Weight of original sample = W % Crude Fibre = $\{C_2-C_3)/W\} \times 100$

Crude protein

Two grams of the sample was weighed into 100cm3 Kjeldahl digestion flask and about lg of catalyst mixture (K_2SO_4 and $CuSO_4$) was added to speed up the reaction. 25mL of concentrated sulphuric acid was added into the flask. The content in the Kjeldahl digestion flask was heated slowly at first in Kjeldahl heating unit frotting subsides and then more vigorously with occasional rotation of the flask to ensure even digestion and avoid over heating of the content. The heating continued until a clear solution is obtained. After cooling, the solution was transferred into 100cm3 volumetric flask and diluted to mark with distilled water. 10mL aliquot of the diluted solution or digest was pipette into Markham semi macro nitrogen steel and 10cm3 of 40% sodium hydroxide solution will be added. The liberated ammonia was trapped in a 100cm3 conical flask containing l0cm^3 of 40% boric acid and 2 drops of methyl red indicator. Distillation was allowed to continue until pink colour of the indicator turn green. The content of the conical flask was titrated with 0.1M HCl, with end point indicated by a change from green to pink colour. The volume of the acid used for the distillate as well as the blank was noted.
% Nitrogen = $\{(0.014 \times M \times (V_1-V_0)\}/ \{\text{weight of test sample}\} \times 100$
where M = actual molarity of acid; V_1 = volume of HCl required for 10 mL sample solution, V_0 = volume of HCl required for the blank.
Atomic weight of nitrogen = 0.014
% Crude = % Nitrogen (N2) × 6.25

Nitrogen free extract

The total carbohydrate content was determined by different methods. The sum of the percentage moisture, % ash, %crude lipid, % crude protein and % crude fibre was subtracted from 100 as described by Abdullahi *et al*. [13].
NFE = 100-(ash+ crude lipid + crude protein + crude fibre)

Determination of growth performance and nutrient utilization parameters

The data was obtained on the growth performance and nutrient utilization of *Clarias gariepinus* fed the experimental diets was determined as following the methods of Abdullahi *et al*. [14].

Mean weight gain (MWG) (g)

Mean Weight Gain (MWG) = W2 - W1

Where W1 = initial mean weight (g)

W2 = Final mean weight (g)

Specific growth rate (SGR%/day)

$$\text{SGR \%} = \frac{\log of\ W2 - \log of\ W1}{T2-T1} X\ 100$$

Where W1 = initial mean weight (g)

W2 = Final mean weight (g)

T1 = initial time (g)

T2 = Final time (g)

Condition factor (CF)

$$\text{CF} = \frac{100\ (\text{Weight gain})(g)}{(Final\ Length)3\ (cm)}$$

Survival rate (%)

$$\text{SR} = \frac{Number\ of\ Fish\ that\ remain\ at\ the\ end\ of\ the\ experiment}{the\ initial\ number\ of\ fish\ stock} X\ 100$$

Protein efficiency ratio (PER)

$$\text{PER} = \frac{\text{Total weight gain (g)}}{Crude\ Protein\ fed\ (g)}$$

Feed conversion ratio (FCR)

$$\text{FCR} = \frac{Total\ weight\ of\ diet\ fed\ (g)}{Total\ weight\ of\ fish\ (g)}$$

Mortality

$$\text{M} = \frac{Number\ of\ fish\ dead\ at\ the\ end\ of\ experiment}{The\ initial\ number\ of\ fish\ stocked} X\ 100$$

Water quality parameters

Water quality parameters were determined weekly, before feeding the fish. The dissolved oxygen level of the water was measured using a digital water dissolved oxygen meter (Smart Sensor AR8210 model) while pH and temperature were measured using a digital pH/Temperature meter (HI-98127 model).

Data Analysis

All data collected from the experiment were subjected to one-way analysis of variance to test for significant differences among treatment means using XLSTAT version 2022, followed by Duncan pairwise comparisons which was used to separate significantly different means at a confidence interval of 95%.

Results and Discussion

Proximate composition of the experimental diets

Proximate composition of the experimental diets is shown in Table 2. There was no significant difference (P>0.05) in the crude protein content of all experimental diets. The moisture content in the Experimental diets ranges from 8.16 to 8.67%, the highest was recorded in the diets with 2.0% of inclusion level while the least was recorded in the diets with 1.5% of black seed meal. Highest value (19.03%) of ether extract and the least value of (17.63%) were recorded in the fish fed 2.0% and 0.0% black seeds, respectively. The crude protein content of all the experimental diets

met the required amount necessary for the optimal growth of African catfish (*C. gariepinus*) which ranged from 35-55% crude protein. There was no significant difference (P>0.05) in the crude protein content of the experimental diets showing uniformity of all the diets, therefore, was considered as iso-proteinous diets. The ether extract, ash content and nitrogen free extract revealed no significant difference (P>0.05) among all the diets with the highest value of 19.03%, 8.99% and 18.51%, respectively.

Parameter	BS 0.0%	BS 1.0%	BS 1.5%	BS 2.0%	BS 2.5%
Moisture	8.06±0.59^{a}	8.40±0.59^{a}	8.16±0.59^{a}	8.67±0.59^{a}	8.62±0.59^{a}
CP	39.91±0.92^{a}	41.75±0.92^{a}	42.45±0.92^{a}	41.47±0.92^{a}	42.15±0.92^{a}
Crude fibre	7.60±0.85^{a}	5.80±0.85^{ab}	7.11±0.85^{a}	5.57±0.85^{ab}	4.03±0.85^{b}
Ether Extract	17.67±0.82^{a}	18.00±0.82^{a}	18.01±0.82^{a}	19.03±0.82^{a}	18.34±0.82^{a}
Ash	8.32±0.66^{a}	8.36±0.66^{a}	7.60±0.66^{a}	7.64±0.66^{a}	8.99±0.66^{a}
Nitrogen free extract	18.43±1.54^{a}	17.67±1.54^{a}	16.66±1.54^{a}	17.60±1.54^{a}	18.51±1.54^{a}

Mean with the same superscript across the same row were not significantly different (P>0.05)

Key: BS=Black seed, CP=Crude protein

Table 2: Proximate composition of the experimental diets

Growth performance and nutrient utilization of *Clarias gariepinus* fed experimental diet

Growth performance of *Clarias gariepinus* fed experimental diets is presented in Table 3. The results revealed that dietary inclusion of different levels of black seed has positive effect on the growth rate of *Clarias gariepinus*. A significant highest value of mean weight gain was recorded in 2.5% level (49.66g) followed by 2.0% (47.66g), 0.0% (47.33g), 1.5% (44.33g) and 1.0% (38.33g) was recorded. The highest mean final length was observed in 2.5% (20.00cm) followed by 2.0% (18.33cm) while the least mean final length of 14.66cm was recorded in the fish fed control diet. The highest specific growth rate was recorded in 2.5% followed by 0.0% and the least value in the fish fed 1.0% with a values of 1.01g, 0.99g and 0.88g, respectively. There was no significant difference (P>0.05) in initial weight (16g) among all the experimental fish at the onset of the experiment showing uniformity in their sizes and unbiasedness. This study revealed that the fish fed 2.0% and 2.5% supplementation levels of black seed had the highest final weight, mean weight gain and specific growth rate. This finding is inline with the finding of Latif *et al*. [15] who documented that 2.5% black seed supplementation is suggested in rohu diet to increase it growth and avoid oxidative stress related losses. Diab [16] also reported that the growth rate of *Oreochromis niloticus* fed on 2.0% black seed supplemented diet improved the growth performance. These positive growth responses revealed in this study and that of the aforementioned authors could be attributed to chemical composition of the black seed making it an excellent grow promoter in the diets of fish. The Nutrient utilization of *Clarias gariepinus* fed experimental diets is presented in Table 4. There was significant different (P≤0.05) in the protein efficiency ratio and feed conversion ratio among the treatments and the control. Apparent net protein utilization also differed significantly (P≤0.05) with the highest value (76.81) in 2.5%, followed by 2.0% (71.17), 0.0% (64.96), 1.0% (60.38) and 1.5%, (55.31). The condition factors (1.39 to 1.53) of fish fed the dietary black seed were not significant different (P>0.05) indicating that dietary inclusion of black seed did not influence the welfare of the experimental fish negatively. Survival rate of *C. gariepinus* juveniles fed the experimental diets showed similar values (83.33-96.66%) among the treatment there was no significant difference (P>0.05) observed.

Parameter	BS 0.0%	BS 1.0%	BS 1.5%	BS 2.0%	BS 2.5%
IW	16.00±0.48^{a}	16.00±0.48^{a}	16.00±0.48^{a}	16.00±0.48^{a}	16.00+0.48^{a}
FW	63.33±4.52^{a}	54.33±4.52^{a}	60.33±4.52^{a}	63.66±4.52^{a}	65.66+4.52^{a}
IL	11.33±0.61^{b}	12.00±0.61^{ab}	12.33±0.61^{ab}	12.00±0.61^{ab}	13.50+0.61^{a}
FL	14.66±0.86^{c}	16.33±0.86^{bc}	17.33±0.86^{abc}	18.33±0.86^{ab}	20.00+0.86^{a}
MWG	47.33±4.52^{a}	38.33±4.52^{a}	44.33±4.52^{a}	47.66±4.52^{a}	49.66+4.52^{a}
SGR	0.99±0.05^{a}	0.88±0.05^{a}	0.95±0.05^{a}	0.98±0.05^{a}	1.01+0.05^{a}
SR	96.66±5.37^{a}	83.33±5.37^{a}	90.00±5.37^{a}	93.33±5.37^{a}	93.33+5.37^{a}
PWG	74.66±2.02^{a}	70.29±2.02^{a}	72.69±2.02^{a}	74.59±2.02^{a}	75.51+2.02^{a}
PER	1.34±0.12^{b}	1.09±0.12^{d}	1.26±0.12^{c}	1.36±0.12^{b}	1.41±0.12^{a}
FCR	2.62±0.09^{a}	2.63±0.09^{a}	2.58±0.09^{a}	2.48±0.09^{b}	2.43±0.09^{c}
ANPU	64.96±1.54^{c}	60.38±1.54^{c}	55.31±1.54^{d}	71.17±1.54^{b}	76.81±1.54^{a}
NNR	50.58±1.94^{b}	61.09±1.94^{a}	55.80±1.94^{ab}	56.52±1.94^{ab}	54.58±1.94^{ab}
CF	1.43±0.16^{a}	1.39±0.16^{a}	1.44±0.16^{a}	1.43±0.16^{a}	1.53+0.16^{a}

Mean with the same superscript across the same row were not significantly different (P>0.05)

Key: BS=Black Seed, MWG=Mean weight gain, SGR=Specific growth rate, SR=Survival range, PWG=Percentage weight gain, PER=Protein efficiency ratio, FCR=Feed conversion ratio, ANPU=Apparent net protein utilization, NNR=Net nitrogen retention, CF=Condition factor.

Table 3: Growth performance and nutrient utilization of *Clarias gariepinus* fed experimental diets

Carcass proximate composition of *Clarias gariepinus* fed experimental diets

The carcass proximate composition of *Clarias gariepinus* fed experimental diets is shown in Table 4. Highest value of carcass crude protein was 61.33g/100g was recorded in the fish fed 2.5% followed by 2.0%, 1.5%, 1.0% and 0.0% with the mean value of 60.70, 59.75, 56.17 and 55.18g/100g, respectively. All the mean crude protein value obtained in the final carcass differed significantly (P>0.05) with initial carcass crude protein. There was no significant different in the value of ether extract among all the treatments and the control. The moisture content, ether extract and nitrogen free extract of all the experimental diets recorded no significant different (P>0.05). The moisture ranged from 6.45 to 8.06%, ether extract ranged from 10.66 to 16.56%. The highest crude protein observed in the study revealed that the growth performance of the experimental fish was due to protein as high amount of crude protein was recorded in the carcass of all the experimental fish.

Parameter	Initial	0.0	1.0	1.5	2.0	2.5
Moisture	6.65±0.75^{a}	6.45±0.75^{a}	8.06±0.75^{a}	6.69±0.75^{a}	7.24±0.75^{a}	7.19±0.75^{a}
CP	45.18±1.47^{d}	55.18±1.47^{c}	56.17±1.47^{bc}	59.75±1.47^{ab}	60.70±1.47^{abc}	61.33±1.47^{a}
Ash	5.26±0.72^{ab}	5.06±0.72^{ab}	3.42±0.72^{b}	7.04±0.72^{a}	6.66±0.72^{a}	3.38±0.72^{b}
EE	16.07±1.17^{a}	18.07±1.17^{a}	15.77±1.17^{a}	14.07±1.17^{a}	14.72±1.17^{a}	17.40±1.17^{a}
NFE	27.22±2.30^{a}	15.22±2.30^{b}	16.56±2.30^{b}	12.43±2.30^{b}	10.66±2.30^{b}	10.68±2.30^{b}

Mean with the same superscript across the same row were not significantly different (P>0.05)

Key: CP=Crude protein, EE=Ether extract, NFE=Nitrogen free extract

Table 4: Carcass Composition of *Clarias gariepinus* fed experimental diet

Water quality parameters of culture medium

The summary of the mean values of the water quality parameters of the culture medium is presented in Table 5. There was no significant difference (P>0.05) in the physico-chemical parameters observed in this study. The temperature ranged from 29.55 to 32.38°C, the pH ranged from 5.70 to 6.16, while the dissolved oxygen ranged from 4.99 to 5.85mg/L. The results revealed that the values of the water quality parameters throughout the experimental period did not differ significantly with each other, and these physico-chemical parameters values were within the acceptable and optimum range for the culture of African catfish (*C. gariepinus*).

Treatments	T°C	pH	DO (mg/L)
BS 0.0%	32.38^{a}	6.10^{a}	5.30^{a}
BS 1.0%	31.28^{a}	5.90^{a}	5.61^{a}
BS 1.5%	31.74^{a}	5.78^{a}	5.45^{a}
BS 2.0%	29.71^{a}	5.70^{a}	5.85^{a}
BS 2.5%	29.55^{a}	6.16^{a}	4.99^{a}
P-values	0.51	0.86	0.35

Mean with the same superscript across the same column were not significantly different (P>0.05)

Key: BS=Black seed, T= Temperature, DO= Dissolved oxygen

Table 5: Shows the water quality parameters of culture medium

Conclusion

In conclusion, the result of the study elucidated that the supplemental level of black seed at 1.0 to 2.5% are safe and have positive effect on the growth performance of *Clarias gariepinus* juvenile. The best inclusion level was at 2.5% dietary black seed. Therefore, black seed can be used as an excellent natural growth promoter in the diet of African catfish (*C. gariepinus*).

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