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Effect of Dietary Inclusion of Garlic as Additive on Growth Performance and Heamatological Parameters of *Clarias gariepinus* (Burchell 1822) Fingerlings

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ABSTRACT

Background and Objective: Feed additives are a group of nutrient and non-nutrient compounds which help in improving the efficiency of feed utilization and thus reducing the high cost of feed. A 72 Day experiment was conducted outdoors of 0.9 cm^3 square concrete tanks filled with fresh water to evaluate the effect of dietary inclusion of garlic as an additive on growth performance and hematological characteristics of *Clarias gariepinus* fingerlings. **Materials and Methods:** As 150 *C. gariepinus* fingerlings at the mean weight (3.13±0.07 g) and length of 6.08±0.03 cm were randomly selected, divided into five dietary treatments and stocked at the rate of 10 fingerlings per tank (120 L) and replicated twice. They were fed with 45% crude protein containing five garlic-based diets of 0.5, 0.75, 1.00, 1.5 and 0.00 g/kg *ad libitum* twice daily. **Results:** Significant differences ($p<0.05$) occurred in the growth and nutrient utilization parameters. Data collected on growth performance indices, feed intake, percentage mortality and blood parameters were subjected to One-way Analysis of Variance (ANOVA) using Completely Randomized Design (CRD). The results revealed that fish increased significantly in weight ($p<0.05$) as the level of garlic inclusions increased in diets and utilized the feed more efficiently than the control. Significant differences ($p<0.05$) were observed in all the growth and blood parameters. Dietary garlic inclusion at 1.00 g/kg had the best performance in growth and as well blood build-up. **Conclusion:** Therefore, from the findings of this research garlic, is encouraged to be included in fish diet from 0.75-1.00 g/kg.

KEYWORDS

Garlic inclusion, growth performance, hematology, *Clarias gariepinus*, fingerlings, feed additives

INTRODUCTION

Feed additives are group of nutrient and non-nutrient compounds which help in improving the efficiency of feed utilization and thus reducing the high cost of feed. In the past, antibiotics were the most routinely used feed additives. However, nowadays use of antibiotics is not only limited but their use in livestock has been banned in many countries due to reasons like alteration of natural gut microbiota and drug resistance in bacteria and humans. One approach is to include new substances into fish diets to improve

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feed conversion efficiency or elevate general conditions for fish growth and maintenance[1]. Plant products have been reported to promote various activities like anti-stress, growth promotion, appetite stimulation and immunestimulation in aquaculture practices[2]. It appear to have medicinal herbs as a better option to pharmaceutical drugs because of their various effects which include growth enhancement, digestion promotion, appetite inducement, anti-depressant, immune-stimulation and anti-pathogenic characteristics and other varied biological active metabolites[3,4].

Blood is a vital special circulatory tissue, composed of cells suspended in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis[5]. Hematological parameters have been recognized as valuable tools for monitoring fish health[6,7]. The significant difference between the white blood cell components of neutrophils, lymphocytes and monocytes which are good indicators of the health status of sick and healthy fish was reported. Jawad *et al*.[8] Gabriel *et al*.[9]observed that many toxicants are known to alter the behavior and physiology of aquatic biota. Packed Cell Volume (PCV), also known as haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF)is the percentage of red blood cells in the blood and is involved in the transport of oxygen and absorbed nutrients[6].

Garlic, which is a squamous bulb belonging to the Liliaceae family, is one of the ancient herbs known by man and used as a spice, food and traditional medicine for years[10,11]. In aquaculture, it is one of the interesting research herbs with the utmost research consent on its ability to stimulate growth[12,13] induce feed utilization i.e. optimum feed conversion ratio and high feed efficiency ratio[14], enhance non-specific immune responses[15] and increase disease resistance[15,16], prevent infections caused by parasite[17] and maintain meat quality in fish.

Powdered garlic contains approximately 1% alliin (S-allyl cysteine sulfoxide) one of the most biologically active ingredients. Allicin (diallylthiosulfinate or diallyl disulfide) cannot be noticed in garlic until it is crushed or cut; if garlic bulb is injured alliinase enzyme is activated, which metabolizes alliin to allicin. Allicin is further metabolized to vinyl thinner. Garlic (*Allium sativum*) has been used to enhance the growth and resistance of a number of livestock and fish[18]. Significant weight gain, increase in feed efficiency, protein efficiency ratio (PER) and specific growth rate (SGR) in Nile tilapia (*Oreochromis niloticus*) were observed when fed with a diet containing 30 g/kg garlic powder diet[19].

Generally, garlic takes effect by facilitating the function of phagocytic cells and improving bactericidal activities; it can equally promote natural killer cells, support, lysozyme and the antibody responses of fish. The activation of these immunological functions is associated with increased protection against infectious diseases in fish. Garlic increases phagocytosis by macrophages[20]. Nya and Austin[21] stated that the inclusion of garlic in fish diets accelerates the erythrocyte number, hemoglobin content, hematocrit, leukocyte number and thrombocyte number. Garlic supplementation enhanced significant changes in serum total protein and globulin in rainbow trout[22]. The increases in the serum total protein, albumin and globulin contents showed strong innate immunity[23] were of the opinion that garlic enhanced the immune response of *Oreochromis niloticus* to a rapid increase in monocytes for a longer period of time; it enhances phagocytic activity, which affords accelerated protection against sudden challenge with ahydrophila, exemplifying the anti-infection characteristics of garlic. In spite of these valuable findings, in-depth studies are needed to permit the complete implementation of these solutions in aquaculture. Therefore, the study investigated the effect of dietary garlic inclusions on growth performance and heamatological parameters of *Clarias gariepinus* fingerlings.

MATERIALS AND METHODS

The experiment was carried out from August to October 2022 in the Wet Laboratory of Department of Fisheries and Aquaculture Ebonyi State University Abakaliki, Nigeria. Ebonyi State is located approximately within Latitude 6°20'N and Longitude 8°06'E in the derived savannah of South-Eastern part of Nigeria at an elevation of 117 m.

Materials used for the experiment include: Garlic, *Clarias gariepinus* fingerlings, weighing balance, hand gloves, scoop net, mechanical pellet machine, plastic bowls, dissecting kit, Ethylenediaminetetraacetic acid (ETDA) sample bottle, hypodermal syringe and water testing kits like thermometer, dissolve oxygen meter HANNA H19146, pH meter Multifunctional water quality test Model Number EZ-9901 and conductivity meter HANNA H18733 all were digital. As 150 fingerlings of *Clarias gariepinus* were collected from Thy Grace Aqua-tech fish farm at Ebonyi State University in a plastic container. The fish were acclimated for 14 days during which period they were fed twice daily (0800 and 1600 hrs) at 5% body weight with 45% crude protein pelleted feed. During this period, dead and abnormal individuals were removed. Crude protein of 45% Iso-nitrogenous diet was formulated to contain garlic as an additive in the diets for the experimental fish. The formulation was based on the proximate composition of the ingredients using Pearson's square method.

The entire feed ingredients were sourced from Margret Umahi International Market Abakaliki, Ebonyi State. Garlic and other ingredients were processed before use to remove chaff and ensure a homogeneous size profile. The ingredients for each diet were mixed thoroughly in a bowl and pelletized with the mechanical pelleting machine in the department. The moist pellets were sun-dried and stored in a dry place at ambient room temperature. The proximate composition of each diet was analyzed to determine the nutrient composition. This research was carried out in five treatments each was replicated thrice (30 fish/treatment) using a Complete Randomized Design (CRD) as the experimental design. At the start of the feeding trial, the acclimated fish were deprived of feed for 24 hrs. The initial body weight and total length were measured before distribution to treatments and replicates.

Growth indices: To determine the growth response of the fish, the following parameters were calculated[24]:

Mean weight gain (g) (MWG):

$$MWG = \frac{Wt_2 - Wt_1}{N}$$

Where:
Wt_2 = Final weight
Wt_1 = Initial weight
N = Number of survived fish

Feed conversion ratio (FCR):

$$FCR = \frac{\text{Weight of feed given}}{\text{Fish weight gain}}$$

Protein efficiency ratio (PER):

$$PER = \frac{\text{Fish weight gain (g)}}{\text{Protein intake (g)}}$$

Specific growth rate (SGR):

$$SGR = \frac{100 - (\text{Log Wf} - \text{Log Wi})}{\text{Time (days)}}$$

Where:
Log Wf = Logarithm of final weight of the fish
Log Wi = Logarithm of initial weight of the fish

Survival rate (%):

$$\frac{\text{Number of fish that survived}}{\text{Total number of fish stocked}} \times 100$$

Collection of blood sample: Five samples of Blood for hematological analysis were collected at the end of the feeding trial from the caudal peduncle of both the test and control fishes with a new 2 mL syringe. The blood samples were dispensed into a tube containing Ethylenediaminetetraacetic acid (EDTA) to avoid clotting in the blood sample preparation. The samples were preserved with ice cubes and taken to the laboratory for analysis.

Data collection and analysis: Data obtained was subjected to one-way ANOVA test, where ANOVA revealed significant differences ($p<0.05$), Duncan's multiple-range test was applied to characterize and quantify the differences between treatments using SPSS version. 20.0.

Ethical approval: All experimental trials were approved by the Ebonyi State University (EBSU) Abakaliki Ethics Clearance Committee. This was done in accordance with the university's Animal Welfare Act and the National Environmental Standard Regulations Enforcement Agency (NESREA) Act of Nigeria on the protection of animals against cruelty.

RESULTS

The iso-nitrogenous diets formulated at 45% crude protein with inclusions of garlic at t_1 (0.5), t_2 (0.75), t_3 (1.00), t_4 (1.5) and t_5 (0.00) g/kg as control are shown in Table 1. The growth performance and nutrient utilization of C. gariepinus fed garlic diets at four varying levels of dietary supplementation and the control are shown in Table 2. There were significant differences in the mean weight gain among fish fed with the garlic diet when compared to the control diet. The highest FW (744.00 g) was recorded in fish fed with T_1 (0.5 g/kg) while the lowest WG was in T_5 control (359.00 g). The best Feed Conversion Ratio, FCR (0.03) was observed in fish fed with 0.5 and 1.00 g/kg garlic diets while the lowest FCR was recorded in fish fed with the control diet. There were significant differences in the protein efficiency ratio among fish fed with the garlic diets.

Hematology of the experimental fish is presented in Table 3. Highest PCV (30%) was discovered in treatments (T_3 and T_5) while the lowest PCV value (26%) was found with T_4. Treatment three (T_3) had the highest WBC (96.00 cmm) while treatment four (T_4) had the least value (85.00 cmm). The findings of the

Table 1: Records of iso-nitrogenous experimental diets formulation (45% CP)

Ingredients	T_1 (0.5 g/kg)	T_2 (0.75 g/kg)	T_3 (1.00 g/kg)	T_4 (1.5 g/kg)	T_5 (0.00 g/kg)
FM	41.89	41.89	41.89	41.89	41.89
SBM	26.18	26.18	26.18	26.18	26.18
Maize	11.93	11.93	11.93	11.93	11.93
Wheat offal	15.50	15.50	15.50	15.50	15.50
Garlic	0.50	0.75	1.00	1.50	-
Lysine	0.3	0.3	0.3	0.3	0.3
Vit. C	0.5	0.5	0.5	0.5	0.5
Bone meal	0.3	0.3	0.3	0.3	0.3
Methionine	0.5	0.5	0.5	0.5	0.5
Fish premix	0.5	0.5	0.5	0.5	0.5
Oil	0.5	0.5	0.5	0.5	0.5
Salt	0.3	0.3	0.3	0.3	0.3
Binder	0.5	0.5	0.5	0.5	0.5
Total (kg)	100.00	100.00	100.00	100.00	100.00

FM: Fish meal, SBM: Soya bean meal and Vit. C: Vitamin C

Table 2: Records of mean values of growth performance of *Clarias gariepinus* fingerlings fed diets mixed garlic as additive

Growth indices	T_1 (0.5 g)	T_2 (0.75 g)	T_3 (1.00 g)	T_4 (1.5 g)	T_5 (0.00 g) control
No. of stock	30	30	30	30	30
T I W (g)	94.00±23.08^a	94.00±23.08^a	93.00±21.24^b	83.00±21.09^c	83.00±21.09^c
M I.W (g)	3.13±1.10^a	3.13±1.10^a	3.10±1.07^{ab}	2.77±1.05^b	2.77±1.05^b
TF I (g)	16.51±5.32^b	19.04± 5.78^{ab}	11.44±4.56^a	17.18±5.97^c	9.93±4.38^{ab}
TFW (g)	744.00±65.71^b	596.00±58.49^c	546.00±58.35^c	418.00±55.07^{ab}	359.00±50.97^c
TWG (g)	650.00±61.07^a	502.00±55..57^b	453.00±49.82^b	335.00±45.73^b	276.00±42.86^a
MFW (g)	43.76±18.15^a	37.25±15.32^b	42.00±18.12^a	38.00±15.45^b	29.92±13.21^{ab}
MWG (g)	40.63±18.54^b	34.12±14.68^a	38.90±14.97^c	35.23±14.09^a	27.15±13.46^{ab}
FCR	0.03±0.01^a	0.04±0.11^b	0.03±0.01^a	0.05±0.13^{ab}	0.04±0.11^b
PER (%)	14.44±4.30^a	11.16±3.76^b	10.07±3.89^b	7.44±2.78^c	6.13±2.98^{ab}
SGR (%)	91.55±22.17^a	70.70± 20.68^b	63.80±19.45^c	47.18±18.67^{ab}	38.87±15.75^{ab}
SR (%)	57.00±19.65^b	53.00±18.97^b	43.00±18.15^a	37.00±15.30^c	40.00±18.50^a

Means on a row with the same superscript did not differ significantly but means with different superscript differed significantly ($p<0.05$), Mean separation by Duncan's multiple range tests at 5% level of significance, TIW: Total initial weight, MIW: Mean initial weight, TFI: Total feed intake, TFW: Total final weight, TWG: Total weight gain, MFW: Mean final weight, MWG: Mean weight gain, FCR: Food conversion ratio, PER: Protein efficiency ratio, SGR: Specific growth rate and SR: Survival rate

Table 3: Mean value of hematological result of *C. gariepinus* fed varied inclusion of garlic

Parameter	T_1 (0.5 g/kg)	T_2(0.75 g/kg)	T_3 (1.00 g/kg)	T_4 (1.5 g/kg)	T_5 (control)
PCV	28±11.27^a	29±10.78^a	30±11.45^{ab}	26±9.65^b	30±8.54^b
Hb	9.3±1.23^b	9.5±2.42^c	10.1±2.58^{ab}	8.6±2.98^a	10.1±2.31^b
WBC	87.00±36.28^a	90.00±38.23^b	96.00±40.01^{ab}	85.00±38.42^b	95.00±36.47^a
RBC	6.7±2.11^a	6.8±2.53^a	7.0±2.65^a	6.6±1.89^b	6.9±1.76^b
MCV	34.32±12.45^a	28.16±11^b	32.43±16.^{a}	32.20±16^a	32.72±16.2^a
MCH	6.72±2.32^{ab}	18.38±8.2^a	14.58±6.3^b	10.88±4.2^c	12.18±3.46^c
MCHC	0.19±0.02^{ab}	0.48±0.03^a	0.45±0.02^a	0.34±0.02^b	0.37±0.03^b

Means on a row with the same superscript did not differ significantly but means with different superscript differed significantly ($p<0.05$), Mean separation by Duncan's multiple range tests at 5% level of significance, PCV: Park cell volume, Hb: Heamoglobin, WBC: White blood corpuscle, RBC: Red blood corpuscle, MCV: Mean corpuscle volume, MCH: Mean corpuscle heamoglobin and MCHC: Mean corpuscle heamoglobin concentrations

Table 4: Results of proximate composition of experimental fish diets

Parameter	T_1 (0.5 g)	T_2 (0.75 g)	T_3 (1.00 g)	T_4 (1.5 g)	T_5 (0.00 g) control
Moisture (%)	9.83	9.04	9.76	10.41	7.95
Ash (%)	8.22	9.04	10.13	11.40	14.11
Fats (%)	7.80	6.60	7.6	7.40	14.80
Protein (%)	26.96	27.86	28.85	29.16	25.14
Fibre (%)	9.14	9.00	8.76	8.83	8.12
CHO	38.05	38.46	32.80	34.90	29.89

present study on the hematology revealed that *Clarias gariepinus* juveniles fed with a garlic diet of 1.0 g and control (T_3 and T_5) produced the highest Hb (10.1 gm/%) while fish that were fed 1.5 g (T_4) produced the least (8.6 gm/%) value. The highest values (7.0% and 34.32 Fl) of RBC and MCV were observed in treatments (T_3) and (T_1) respectively, while the least values (6.6% and 28.16 Fl) were observed in treatments (T_4) and (T_2) respectively. Treatment (T_2) recorded the highest (18.38 Pg) MCH while treatment one (T_1) recorded the least value (6.72 Pg). Treatments two (T_2) and one (T_1) were recorded to have the highest (0.48g/dl) and least (0.19 g/dL) MCHC, respectively.

Proximate compositions of the five diets formulated and prepared for the feeding trial are presented in Table 4. Crude protein in the experimental diets is 45% among the five treatments, moisture content ranged between 9.83 and 10.41%, ash between 7.80 and 14.80%, fibre contents ranged between 8.12 and 9.14 %, protein 25.14 and 29.16%, fats 6.60 and 14.80% and CHO 29.89 and 38.46%.

DISCUSSION

The values of the physico-chemical parameters observed in the experimental tanks during this study were within the range recommended for *C. gariepinus*. The achievement of this was as a result of optimum water management practices. This was in line with the report of Onibi *et al.*[25].There was a general increase

in weight gain, with a better increase in weight gain observed in fish fed 0.5 and 0.75 g garlic powder inclusions. This was in agreement with the work of Fasasi *et al*.[26] who recorded a similar increase in weight gain in fish-fed diets supplemented with walnut leaf and onion bulb residues. It also was in agreement with the report of Abd-El-Rhman[27], who suggested ginger and garlic supplements collectively or individually improved growth performance of broilers. There was a variation in the total feed intake of garlic powder inclusions in the diets; this could be due to lower palatability of the two diets that have higher inclusion of garlic and equally as a result of the presence of tannin in the garlic powder. The result opined with the report of Zomrawi *et al*.[28] who stated that tannins interfere with digestion by displaying anti-trypsin and anti-amylase activity, forming complexes with vitamin B_{12} and interfering with the bioavailability of proteins. The increased FCR was observed in fish fed garlic diet at 0.5 g/kg higher than others including the fish-fed control diet similar to the report of Ashade *et al*.[29], who revealed that inclusion of 1.5% walnut leaf increased FCR in the supplemented groups than the control. It also corroborated with the work of Sotolu and Faturoti[30] which indicated that the inclusion of propolis-ethanolic extract and crude propolis increased the FCR, FER and PER in the supplemented groups when compared with the control. The good SGR results recorded from the inclusion of garlic in the experimental diets were in correlation with the result of Ayoola[31] who reported that *Allium sativum* supplementation positively affected *O. niloticus* biomass and (SGR). Fish fed 1.00 and 1.5 g/kg *Allium sativum* diets recorded the highest mortality rate. The findings of Kefas *et al*.[32] also revealed that the mortality rate of fish fed untreated ginger peel increased with respect to the different concentrations and the highest concentration had a higher mortality rate.

Blood is a vital circulatory tissue, composed of cells suspended in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis[6]. In this study, PVC values increased from 28% in fish fed the garlic diet (T_1) to a final value of 30% for fish fed 1.00g//kg garlic diet (T_3) the results obtained fall within the range of 20% and 50% in agreement with Anyanwu *et al*.[33], who also stated that PCV values above 50% are rarely reported. However, the lower PCV values are attributed to anemia, thus the study reveals that there is no tendency for anemia. The red blood cell (RBC) increased from the value of 6.7% for the fish-fed (T_1) to the final value of 6.9% for fish fed control diet. These values were higher than $1.9x10^{12}$/L reported for *Clarias gariepinus* juveniles[34].

The final white blood cell counts (WBC) of *C .gariepinus* fingerlings fed garlic-included diets ranged between 8.50 cmm to fish fed garlic based diet (T_1) and 96.00 cmm in fish fed 1.00 g/kg garlic-based diets (T_3) and were significantly different from the control of WBC count (95.00 cmm; T_5). These supported by Adeyemo[35] who stated that increasing or decreasing WBC are normal physiological reactions to toxicants and these show the response of the immune system under toxic conditions. According to Adeyemo[35], Dienye and Olumuji[36] animals with low white blood cells are exposed to a high risk of disease infections while those with high counts are capable of generating antibodies in the process of phagocytocis and have a high degree of resistance to diseases. They also have a high degree of resistance to entrance adaptability to local environmental and disease-prevalent conditions[35].

Mean corpuscular volume (MCV) indicates the status or size of the RBCs and reflects a normal or an abnormal cell division during the production of RBC. The results obtained from the present study showed that fish fed with (T_1) had the higher volume while the fish fed (T_2), had the lower MCV values. These values are higher when compared to 79.20-105.32 µg/mL reported for Heteroclarias, this increase may be attributed to the swelling of the RBCs as a result of low oxygen condition and impaired water balance in fishes exposed to metal pollution[36]. Mean corpuscular haemoglobin (MCH) values were significantly different. The highest value was recorded in fish fed garlic-based diet (T_2) while the least was recorded in fish-fed garlic-based diet (T_1). This value agreed with Dienye and Olumuji[36] who reported a significant increase in the final MCH values in *C. gariepinus* fed raw mucuna seed meal-based diets. The values

recorded for mean corpuscular hemoglobin concentration (MCHC) when compared with 33.97% recorded were far from[36] and values ranging between 28.75 and 37.62% recorded for fish fed *M. oleifera* leaf meal-based diet.

The implications are that the inclusion of garlic powder above 100 g/kg in fish feed especially *Clarias gariepinus* diet will affect the growth and may lower the blood profile of the species. From the results obtained, garlic inclusion as an additive in the diet of African catfish fingerlings must be in powdered form. It should be included very minimally. Therefore, it is recommended that garlic can be used as an additive in fish feed formulation by fish farmers. More research should be carried out on the inclusion of garlic powder as an additive in the diets of other species of fish. The study was set to find out the effect of garlic powder as an additive to the diets of African catfish fingerlings at different inclusions.

CONCLUSION

The result obtained in this study revealed that the inclusion of garlic in the diet of African catfish had good results with optimal growth. However, it is concluded that garlic can be included in the diet of African catfish fingerlings as an additive. The inclusion has a profound influence on the blood profiles of the researched fish. Therefore, it is adjudged and recommended that garlic can be used as an additive in fish feed formulation by fish farmers.

SIGNIFICANCE STATEMENT

Feed additives are a group of nutrient compounds which help in improving the efficiency of feed utilization and thus reducing the high cost of feed. The major challenges to the livestock industry in most developing countries are high costs of feed due to shortages and the unavailability of conventional feedstuffs like additives for compounding livestock rations. This has been causing the rising cost of animal products. Garlic has the following effects: Lowers cholesterol and triglycerides, ameliorates atherosclerosis, coronary dilator and antioxidant. Therefore, the study investigated the effect of dietary garlic on growth and heamatology of *Clarias gariepinus* fingerlings. The approach is that the included new substance into fish diets improves feed conversion efficiency, boosts blood and elevates general conditions for fish growth.

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