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Effect of dietary inclusion of garlic as additive on growth, heamatology and histological changes of intestinal mucosa of *Clarias gariepinus* (Burchell 1822) fingerlings

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Abstract

A 72-day experiment was conducted in outdoor of 0.9cm^3 square concrete tanks filled with fresh water to evaluate the effect of dietary inclusion of garlic as additive on growth performance, hematology and histological changes of intestinal mucosa of Clarias gariepinus fingerlings. 150 C. gariepinus *fingerlings at mean weight (3.13 ± 0.07g) and length 6.08±0.03cm were randomly distributed into five dietary treatments and stocked at 10fingerlings per tank (120L) and replicated twice. They were fed with 45% crude protein diets varied garlic inclusions at 0.00,* 0.5, 0.75, 1.00 and 1.5 g/kg ad libitum *twice daily. Data collected on growth performance indices, nutrient utilization 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 increases in diets and utilized the feed efficiently than the control. Significant differences (P < 0.05) were observed in all the blood parameters due to garlic inclusions. The histological examinations indicated mild focal area of inflammatory cell aggregate, mild infiltration of inflammatory cell (IIC) for 0.75 to 1.00g/kg and severe effect of ulceration and focal area of necrotic debris (ND) of the mucosa was observed in control diet and inclusion levels above 1.00g/kg. Result obtained in this study indicated that garlic inclusion in a compounded fish diets up to 1.00g/kg balances the feed with no negative severe effect to the tissue. Dietary garlic inclusion at 1.00g/kg had the best performance in growth and blood values. Therefore, from the findings of this research, garlic is encouraged to be included in fish diet from 0.75-1.00g/kg.*

Key words: *Garlic inclusion, growth performance, hematology, intestinal mucosa, Clarias gariepinus, fingerlings.*

Introduction

The supply of qualitative animal protein in sufficient quantity and at affordable cost has continued to remain a dream yet to be realized. It is a perennial problem and a major challenge to the livestock industry in most developing countries. High costs of feed due to shortage and unavailability of conventional feedstuffs for compounding livestock rations has been the major cause of rising cost of animal products Sarkiyay and Agar (2010). Feed additives are a group of nutrient and non-nutrient compounds which helps 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, use of antibiotics is not only limited but their use in livestock and aquaculture industry also have

been banned in many countries due to the reasons like alteration of natural gut micro-biota and drug resistance in bacteria and humans. One approach is to include new substances into fish diets to improve feed conversion efficiency or elevate general conditions for fish growth and maintenance (Fernández-Navarro *et al.,*2006). Plants are natural source of safer and cheaper chemicals. Beneficial effects of bioactive plant substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune responses and antibacterial, antiviral and antioxidant actions (Citarasu,2010). Garlic is a rich source of calcium, phosphorus and vitamin B1; it has a high content of carbohydrates and as a consequence a high nutritive value. Garlic also contains iodine salts which have a positive effect on the circulatory system and rheumatism, silicates which have a positive effect on the skeletal and circulatory system and sulfur salts with positive effects on the skeletal system, cholesterolemia, and liver diseases. Garlic also contains vitamin complex B, vitamins A, C and F (Drăgan, *et al.,* 2008). Garlic has also been shown to have antioxidant properties, which could have a protective nature against gastrointestinal neoplasias, against blood clots (anti-platelet action) due in part to the compounds alliin and ajoene, which have fibrinolytic activity. Ajoene inhibits thromboxane synthesis through the inhibition of the cyclo-oxygenase and lipoxygenase enzymes (Schulz *et al.,* 2004). Histological alterations in fish liver, kidney and intestinal mucosa are important indicators of chemical toxicity, and it is away to know the effects of exposure of aquatic animals to toxins present in the aquatic environment (Thopon *et al.,* 2004 and Loganathan *et al.,* 2006). Little or no work has been carried out on the effect of dietary garlic on intestinal mucosa of African catfish. Therefore, the study investigated the effect of dietary garlic on growth performance, hematology and intestinal mucosa changes of *Clarias gariepinus* fingerlings.

Materials and Methods

Area of the Study

The experiment was carried out in wet laboratory of Department of Fisheries and Aquaculture Ebonyi State University, Abakaliki. 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 117m.

Experimental Materials

Materials that were used for the experiment include: Garlic, *Clarias gariepinus* fingerlings, weighing balance, hand gloves, scoop net, mechanical pellet machine, plastic bowls, dissecting kit, sample bottles, normal formal saline solution and water testing kits.

Procurement of experimental fish and preparation of diets:

One hundred and fifty (150) fingerlings of *Clarias gariepinus* were collected from Thy Grace Aqua-tech fish farm in Ebonyi State University in a plastic container. The fish were acclimated for 14 days during which period they were fed twice daily (0800 h and 1600 h) at 5% body

weight with 45% crude protein pelleted feed. During this period, dead and abnormal individuals were removed. The purpose of the acclimation was to enable them recover from stress and stabilized to the experimental environment. Crude protein of 45% iso-nitrogenous diet was formulated to contain garlic as additive in the diets for the experimental fish at different levels of inclusion for the diets. The entire feed ingredients were sourced from the nearest market in Abakaliki, Ebonyi State. Garlic and other ingredients were processed before use to remove chaffs and ensure homogeneous size profile. The ingredients for each diet were mixed thoroughly in a bowl and pelletized with mechanical pelleting machine in the department. The moist pellets were sun dried and stored in dry place at ambient room temperature. The proximate composition of each diet was analysed to determine the nutrient composition.

Experimental procedure and design:

This research was carried out in five treatments each was replicated thrice (30 fish/treatment) using complete randomize design (CRD) as the experimental design. At the start of the feeding trial, the acclimated fish were not fed for 24 hours. The initial body weight and initial total length was measured to the nearest 0.01g and 0.1 cm,

Growth indices

To determine the growth response of the fish, the following parameters were calculated (Ogunji, 2004)

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 fishes

Feed Conversion Ratio (g) (FCR)

$$FCR = \frac{\text{weight of feed given (g)}}{\text{Fish weight gain}}$$

Protein Efficiency Ratio (PER)

$$PER = \frac{\text{fish Weight Gain (g)}}{\text{Protein intake (g)}}$$

Specific Growth Rate (SGR)

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

Where; Log Wf = logarithm of final weight of the fish

Log Wi = logarithm of initial weight of the fish

Survival Rate (%)

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

Sample Collection of Intestinal mucosa and blood

Immediately after the feeding trial confinement, the fish samples from each experimental unit were collected then sacrificed by cervical section. Section of intestine was excised, rinsed in physiological saline and fixed in sample bottle containing normal formal- saline fluid for histological analysis. Blood samples for hematology were collected at the end of the feeding trial from the caudal peduncle of both the test and control fish with new 2ml syringe. The blood samples were dispensed into a tube containing Ethylene diamine tetra acetate (EDTA) to avoid clotting of the blood sample preparation. The samples were preserved with ice cubes and taken to the laboratory for analysis of Packed cell volume, White blood cell, Red blood cell and Hemoglobin (Blaxhall and Daisley 1973). The mean cell volume (MCV), mean hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated using the method described by (Merghami, 2010).

Monitoring of Water Quality:

Water quality parameters like pH, temperature, and dissolved oxygen (DO), conductivity, and ammonia, nitrate and

nitrite level were monitored and measured daily.

Data 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 (REF).

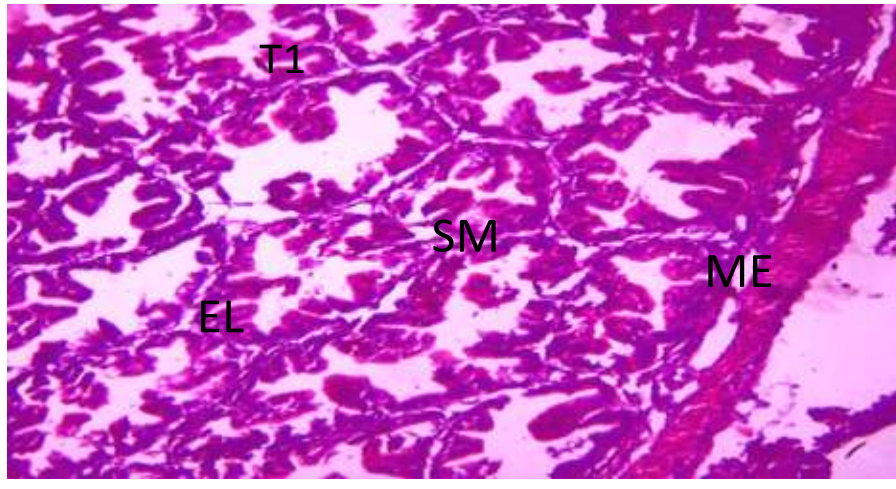
Result

The contributory levels of ingredients used in formulating the iso-nitrogenous diets and as well the inclusion levels of garlic powder from diet1 (T_1) to diet 4 (T_4) at 0.5g/kg to 1.5g/kg for the experimental fish are shown Table 2.

Proximate compositions of the five diets formulated and prepared for the feeding trial are presented in Table 3. The percentage proximate composition of the dietary treatments varied which was relative to the inclusion levels of garlic powder as additive. The record showed proportional increment of percentage crude protein with increase in inclusion levels of garlic powder, where moisture, fibre, carbohydrates and fats varied but ash had the same trend with crude protein, among the treatments. Crude protein of 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%. The nutrient and some mineral composition of garlic are shown in Table 5. The growth performance and nutrient utilization of *C. gariepinus* fed garlic diets at four varying levels of dietary supplementation and the control is shown in Table 2. There were differences in the mean weight gain (WG) among fish fed garlic as additive. There were significant differences in the mean weight gain among fish fed with the garlic diets when compared to the fish fed with the control diet. The highest WG (744.00g) was recorded in fish fed with T_1 (0.5g/kg) while the lowest WG is in T_5 control (359.00 g). The highest feed conversion ratio, FCR (0.03) was observed on fish fed with 0.5 and 1.00g/kg garlic diets while the lowest FCR was recorded in fish fed with control diet. There were no significant differences in the protein efficiency ratio among fish fed with diets of garlic inclusions. Hematological results of the experimental fish are presented in Table 6. Highest PCV (30%) was observed in treatments (T_3 and T_5) while the least PCV value (26%) was recorded with T_4. Treatment three (T_3) has the highest WBC (96.00cmm) while treatment four (T_4) has the least value (85.00cmm). The findings of the present study on the hematology revealed that *Clarias gariepinus* juveniles fed with garlic meal of 1.0g and control (T_3 andT_5) produced the highest Hb (10.1gm/%) while fish that was fed 1.5g (T_4) produced the least (8.6gm/%) value. Highest values (7.0% and 34.32Fl) of RBC and MCV were observed in treatments (T_3) and (T_1), while the least values (6.6% and 28.16Fl) were observed in treatments (T_4) and (T_2) respectively. Treatment two (T_2) recorded high MCH (18.38Pg) while treatment one (T_1) recorded the least value (6.72Pg). Treatments two (T_2) and one (T1) recorded the highest and least values of MCHC (0.48g/dl) and (0.19g/dl) respectively.

Histological analysis of intestinal mucosa of *Clarias gariepinus* fingerlings fed diets with garlic powder inclusions are shown in figures 1-4 and figure 5 as the control diet indicated some changes. The intestinal mucosa of fish fed diet (0.5g/kg) showed muscular external (ME) and epithelia lining (EL) with the overall features

appearing normal. Fish on $diet_2$ (0.75g garlic/kg diet) as in figure 2) indicated mild focal area of inflammatory cell aggregates (ICA) with well outlined intestinal mucosa and epithelia lining (EL), where $diet_3$ 1.00g/kg inclusion (fig.3) showed a section of intestinal mucosa of the fish with well outlined intestine and epithelia lining (EL) with mild infiltration of inflammatory cells (IIC). $Diet_4$ (1.5g/kg) inclusion (fig. 4) a section of intestine of fish showed moderate focal area of inflammatory cells (IC) of the intestinal mucosa. $Diet_5$ without inclusion of garlic indicated a section of intestine of the fish with severe effect of ulceration and focal area of necrotic debris (ND) of the mucosa.



Fg1 0.5g/kg A section of intestine of fish shows normal and well outlined intestinal mucosa with muscular external (ME), sub-mucosa (SM) and well arranged epithelia lining (EL)

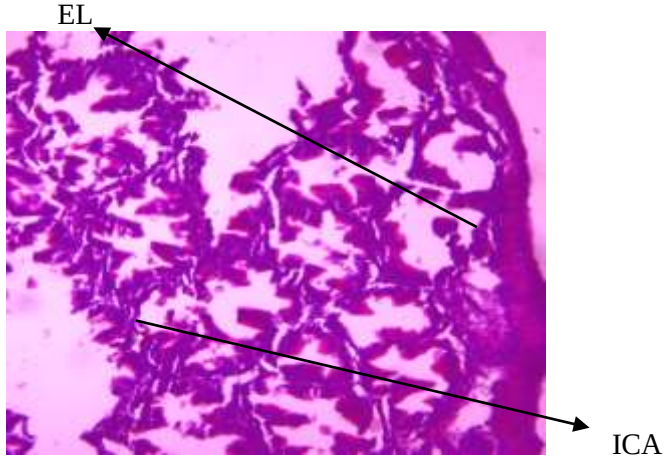


Fig.2: 0.75g/kg. A section of intestine of fish showed mild focal area of inflammatory cell aggregate (ICA) with well outlined intestinal mucosa and epithelia lining (EL)

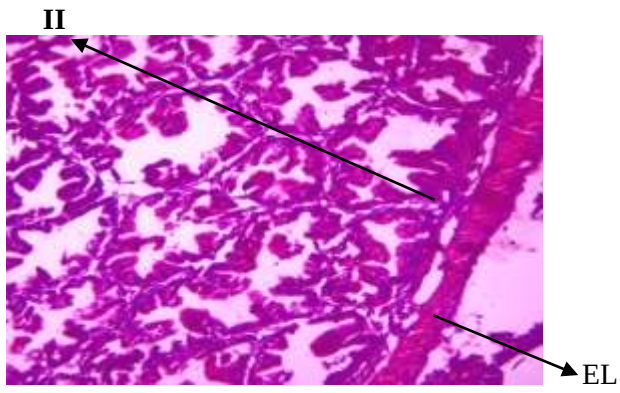
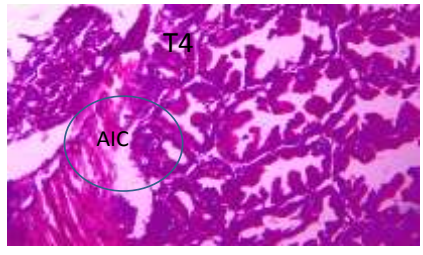


Fig. 3: 1.00g/kg. A section of intestine of fish showed well outlined intestinal mucosa and epithelia lining (EL) with mild infiltration of inflammatory cells (II)



Photomicrograph of Fig.4 1.5g/kg. A section of intestine of fish showed intestinal mucosa with moderate focal area of inflammatory cell (AIC)

Discussion

The values of the physico-chemical parameters observed in the experimental tanks during this study were within the range recommended for *C. gariepinus* (Adekoya, *et al.,* 2004 and Omotayo *et al.,* 2006). The achievement of this was as a result of optimum water management practices. There was a general increase in weight gain with the highest growth performance observed in fish fed 0.5g and 0.75g garlic powder. This is in agreement with the work of (Bello *et al.,* 2014), who recorded similar increase in weight gain of fish fed diets supplemented with Walnut leaf and Onion bulb residues. This is in agreement with Onibi *et al.* (2009) that suggested ginger and garlic supplements collectively or individually improved growth performance of broilers. This was corroborated by Haghighi and Rohani (2013).There was a variation in the total feed intake of garlic powder diets; this could probably due to lower palatability of the two diets as a result of the presence of tannin in the garlic powder diets. The work of Fasasi *et al.* (2005) 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. Also, Azaza *et al.* (2009) were of the opinion that the presence of 2.4 % tannin in faba beans (*Viciafaba* L. *var. minuta*) might be responsible for low palatability and consequently low feed intake in Nile tilapia. The increased FCR as revealed in fish fed garlic powder at 0.5g higher than the fish fed control diet is similar to the report of (Onibi, *et al.,* 2009), who revealed that inclusion of 1.5% walnut leaf increased FCR

in the supplemented groups than the control. This was also corroborated by the work of (Isaac *et al.*,2013) 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 result of this study disagreed with the findings of Zomrawi *et al.* (2011), who reported that no significant differences ($p > 0.05$) in FCR among all dietary *Z. officinale* root-powder treatments. The better SGR recorded in the supplemented diets is in correlation with the result of (Etim *et al.*, 1999) who reported that *Allium sativum* supplementation positively affected *O. niloticus* biomass and Specific Growth Rate (SGR).There is a reduction in survival rate in fish fed the *Z. officinale* root-powder diets as seen in this experiment and could be as a result of some phytochemicals present in the phytobiotic. Fish fed 1.00g and 1.5g *Allium sativum* powder recorded the highest mortality rate. The findings of Sotolu and Faturoti (2011) also revealed that the mortality rate of fish fed untreated ginger peel increased with respect to the different concentrations and the highest concentration having more mortality rate.

Blood is a vital special circulatory tissue, composed of cells suspended in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis (Soetan *et al.,* 2013). Packed Cell Volume (PCV), also known as haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF) is the percentage of red blood cells in blood and is involved in the transport of oxygen and absorbed nutrients (Soetan *et al.,* 2013). In this present study, PCV values increased from 28% in fish fed the 0.5g garlic diet (T_1) to 30.00% in fish fed 1.00g garlic diet (T_3).The results obtained falls within the range of 20% and 50% in agreement with Ayoola, (2011), who also stated that PCV values above 50% are rarely reported. Increased PCV results to an increased primary and secondary polycythemia (Soetan *et al.,* 2013), however, (Alkahem *et al.,*1998) reported that lower PCV values is attributed to anaemia, thus the present study reveals that there is no tendency for anaemia. Red blood cell is involved in the transport of oxygen carried to the tissues and carbondioxide returned to the lungs in the body (Alkahem *et al.,* 1998; Ugwuene, 2011andSoetan *et al.,* 2013). The red blood cell (RBC) increased from the value of 6.7% in the fish fed 0.5g garlic diet (T1) to 6.9% in fish fed control diet (T_5). These values were higher than $1.9x10^{12}L^{-1}$ reported for *Clarias gariepinus* juveniles (Iwuji and Herbert 2012). The final white blood cell counts (WBC) of *C .gariepinus* fingerlings fed garlic included diets ranged between 8.50cmm in fish fed 0.5g garlic based diet (T_3) and 96.00cmm in fish fed 1.00g garlic-based diets (T) and were significantly different from the control of WBC count (95.00cmm; T_5). Kabir *et al.*, (2011) stated that increasing or decreasing numbers of WBC are normal physiological reactions to toxicants and these showed the response of the immune system under toxic conditions. However, (Kabir *et al.,* 2011 and Anyanwu *et al.,* 2011), stated that animals with low white blood cells are exposed to high risk of disease infections while those with high counts are capable of generating antibodies in the process of phagocytocis and have high degree of resistance to diseases. They also have high degree of resistance to entrance adaptability to local environmental and disease prevalent conditions (Soetan *et al.,* 2013; Kabir *et al,*2011; Iwuji and Herbert 2012). 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 result gotten from the present study

showed that fish fed with 0.5g garlic diet (T_1), gave the highest volume (34.32Fl) while the fish fed 0.75g garlic diet (T_2), had the least (28.16Fl) MCV value. These values are higher when compared to 79.20-105.32µg/ml reported for Hetero clarias (Anyanwu *et al.*,2011). 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 (Larsson *et al.*, 1985). Mean corpuscular haemoglobin (MCH) values were significantly different. The highest value (18.38pg) was recorded in fish fed 0.75g garlic-based diet (T_2) while the least (6.72pg) was recorded in fish fed 0.5g garlic-based diet (T_1). These values agreed with Olasunkanmi, (2011) 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 haemoglobin concentration (MCHC) (0.19g/dl, 0.48g/dl, 0.45g/dl, 0.34g/dl and 0.37g/dl) compared, very far with 33.97% recorded by Adeyemo, (2007) and values ranging between 28.75 and 37.62% recorded for fish fed *M. Oleifera* leaf meal-based diet Dienye and Olumuji (2014). The alterations found in these organs are normally easier to identify than functional ones (Fanta *et al.*, 2003), and serve as warning signs of damage to animal health (Hinton and Laurén, 1990). The result of the experiment on the histology of the intestinal mucosa disagreed with the findings of Ayotunde *et al*. (2011), who observed no visible lesion on control fish in the work on histological changes in *O. niloticus* exposed to Aqueous extract of *Moringa oleifera* seeds powder and Agbebi *et al.* (2012) who also reported normal gut, no visible lesion and alteration of gut of catfish fingerlings fed levels of garlic diets.

Conclusion and Recommendation

The result obtained in this study revealed that, inclusion of 1.00g/kg garlic in the diet of African catfish generally increased growth and blood values optimally. It is adjudged that garlic can be used as additive in fish feed formulation. Generally, the results obtained showed no negative effect on histological changes of the viscera organs suggesting that it is essentially good for growth and utilization.

Farmers are encouraged to include not more than 1.00g/kg of garlic powder as additive in the diet of African catfish *Clarias gariepinus.*

Table 1: Composition of diets (45% CP)

Ingredients	T_1 0.5g/kg	T_2 0.75g/kg	T_3 1.00g/kg	$T_4$1.5g/kg	$T_5$0.00g/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	100.00	100.00	100.00	100.00	100.00

Table 2: Growth performance of *Clarias gariepinus* fingerlings fed diets mixed with graded levels of garlic as additive

Growth indices	T_1 (0.5g)	T_2 (0.75g)	T_3 (1.00g)	T_4 (1.5g)	T_5 (0.00g) 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
Identify abbreviations

Table 3: Proximate composition of experimental fish diets

Parameters (%)	T_1 (0.5g)	T_2 (0.75g)	T_3 (1.00g)	T_4 (1.5g)	T_5(0.00)
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
Carbohydrate	38.05	38.46	32.80	34.90	29.89

Table 4: Nutrient composition and some minerals of garlic

Parameters	Composition %
Moisture	4.89
Ash	8.72
Fats	4.20
Protein	13.16
Fibre	1.51
Carbohydrate	67.52
Zinc	2.42
Phosphorus	1.85
Iron	1.35

Table 5: Mean value of heamatological result of *C. gariepinus* fed varied inclusion of garlic

Parameters	T_1 (0.5g/kg)	T_2(0.75g/kg)	T_3 (1.00g/kg)	T_4 (1.5g/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	8700±36.28[a]	9000±38.23[b]	9600±40.01[ab]	8500±38.42[b]	9500±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 = Hemoglobin, WBC = White Blood Corpuscle, RBC = Red Blood Corpuscle, MCV = Mean Corpuscle Volume, MCH = Mean Corpuscle Hemoglobin, MCHC = Mean Corpuscle Hemoglobin Count.

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