



## Response of *Clarias gariepinus* to *Allium sativum*-based diet on growth performance and *Staphylococcus aureus* challenge infection

\*NWABUEZE, AA; EKELEMU, JK; OWE, OA

Department of Fisheries, Delta State University, Asaba Campus, Asaba, Southern Nigeria.

\*Corresponding Author Email: [aanwabueze2@gmail.com](mailto:aanwabueze2@gmail.com); Tel: +234 806 413 8580

**ABSTRACT:** The response of *Clarias gariepinus* to *Allium sativum* on growth performance and as anti-bacterial agent in *Staphylococcus aureus* challenge infection was evaluated. *A. sativum* was included at 0% (control), 1.5%, 3.0%, and 4.5% in fish diet. Twenty fish samples each were randomly distributed into four tanks (T1, T2, T3 and T4). Fish were fed twice daily at 5% body weight for twelve weeks. All fish were challenged with 0.5ml of pure culture of *S. aureus*. A significant ( $P<0.05$ ) increase in body weight, total length and standard length was observed. *A. sativum* at 3.0% inclusion promoted highest growth with feed conversion ratio (1.25-1.41), protein efficiency ratio (1.72-0.56), specific growth rate (1.92-2.09), condition factor (0.83-1.99) and survival rate (60-95). Fish-fed *A. sativum* diet showed that 4.5% inclusion had the least *S. aureus* activity. Bacteria load significantly ( $P<0.05$ ) decrease at week 12. Culture water had dissolved oxygen of 4.0 - 4.2 mg/L, pH 6.0 - 7.0 and temperature of 26.0-28.0°C. This study has shown that *A. sativum* supplemented diets enhanced growth of *C. gariepinus* and functioned as antibacterial agent against *S. aureus*. *A. sativum* at 3.0% inclusion in diet is recommended for better fish growth and 4.5% *A. sativum* for antibacterial action.

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*Clarias gariepinus* is a dominant freshwater fish and popular in commercial aquaculture due to its ability to grow rapidly and high tolerance to environmental conditions (Mohamed *et al.*, 2017). However, *C. gariepinus* has been found to be susceptible to both microbial and parasitic infections particularly in intensive culture systems (Sudheesh *et al.*, 2012; Opiyo *et al.*, 2018). For decades antibiotics have been used for the treatment of bacterial infection in fish. The adverse effects associated with the use of antibiotics include drug residue, bioaccumulation and resistance of pathogens, which threaten human consumers (Lee and Gao, 2012). Hence, herbs are now being used as probiotics in preventing bacterial infections and are gaining success because they are cost effective, eco-friendly and have minimal side effects (Carusol *et al.*, 2013). Herbs exhibit anti-microbial, anti-stress, appetite stimulation, immunostimulation, and

aphrodisiac and antipathogenic effects which facilitate growth and maturation of cultured species (Harikrishnan *et al.*, 2011). *Allium sativum* is a pungent herb and has been reported to inhibit bacterial growth, promote fish growth and enhancement of blood parameters (Nya and Austin, 2009; Dikel, 2015). In aquaculture, *A. sativum* has been observed to promote growth, enhance immunity, stimulate appetite and strengthens the control of bacteria and fungi pathogens (Harris *et al.*, 2001; Lee and Gao, 2012). *S. aureus* is one of the major bacterial agents causing food-borne diseases in humans worldwide and has been found on skin of healthy people and animals including fish (Fetsch and Jöhler, 2018). The response of *Clarias gariepinus* to *A. sativum* based diet on growth performance and *Staphylococcus aureus* challenge infection was investigated.

\*Corresponding Author Email: [aanwabueze2@gmail.com](mailto:aanwabueze2@gmail.com); Tel: +234 806 413 8580

## MATERIALS AND METHODS

**Sampling:** One hundred and sixty six weeks old fingerlings of *C. gariepinus* with a mean total length of  $11.47 \pm 0.24$ cm and a mean body weight of  $11.85 \pm 0.24$ g were purchased from Ben Fish Farm, in Asaba and transported to Fisheries Laboratory of the Department of Animal Science and Fisheries, Delta

State University, Asaba Campus, for the study which lasted 12 weeks in 2019.

**Preparation of *A. sativum* extracts:** *A. sativum* bulbs obtained locally were washed thoroughly under running tap water and aqueous extract obtained according to Mikail (2010). The phytochemical properties of *A. sativum* are documented (Charkraborty and Hancz, 2011).

**Table 1.** Composition of Experimental Diets (g).

Ingredients	T1 (0%, Control)	T2 (1.5%)	T3 (3%)	T4 (4.5%)
Fish meal	20.57	20.57	20.57	20.57
Groundnut cake	20.57	20.57	20.57	20.57
Soya bean meal	20.57	20.57	20.57	20.57
Yellow maize	23.30	23.30	23.30	23.30
Vitamin premix	3.00	3.00	3.00	3.00
Salt	0.50	0.50	0.50	0.50
Bone meal	1.00	1.00	1.00	1.00
Vegetable oil	9.50	9.50	9.50	9.50
Methionine	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50
Garlic	0.00	1.50	3.00	4.50

**Table 2:** Proximate Composition of Experimental Diet

	T1(0% Control)	T2 (1.5%)	T3 (3.0%)	T4 (4.5%)
Crude protein (%)	30.32	32.55	34.12	35.43
Moisture content (%)	8.48	7.50	7.46	5.12
Ash content (%)	2.48	2.97	2.99	3.01
Crude fiber (%)	3.47	4.21	4.94	5.01
Nitrogen free Extract (NFE)	55.31	53.00	51.04	51.87

**Acclimation and Experimentation:** Fish specimens were acclimated for 14 days in a stock tank (45 cm x 45 cm x 90 cm) containing 120 L of bore hole water. Fish were fed commercially available fish feed at 5% body weight twice daily. Stock tank was well aerated and water temperature maintained at a ranged of 27.0 °C – 28.0 °C. Twenty experimental fish each were randomly distributed into four tanks, T1 as control (0%) and T2 (1.5%), T3 (3.0%) and T4 (4.5%) as treatments dietary inclusion of *A. sativum*. The experiments were in duplicates and maintained in a weekly half renewal static bioassay. Experimental diets (Table 1) were formulated according to Ndong and Fall (2011). Prior to administration of experimental diet, fish were starved for 24 hours. Experimental diets were analyzed (AOAC, 1984) and proximate composition as presented in Table 2.

**Growth Parameters and Nutrients Utilization:** Growth parameters were calculated following the method described by Bagenal (1978). Growth indices considered were percentage weight gain and total length gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor, K and survival rate, SR.

$$SGR = 100 \left[ \frac{\log W_f - \log W_i}{T} \right]$$

Where  $W_f$  = final weight,  $W_i$  = initial weight; T = Time (days)

$$FCR = \frac{T_f}{W_g}$$

Where  $T_f$  = Total feed (g);  $W_g$  = Weight gain (g)

$$K = 100 \frac{W}{L^3}$$

$$PER = \frac{W_g}{P_i}$$

Where  $P_i$  = Protein intake (g)

$$SR = 100 \left[ \frac{FS_i - M}{F_i} \right]$$

Where  $FS_i$  = initial number of fish stocked; M = mortality;  $F_i$  = Initial number of fish

**Bacterial Studies:** Bacterial isolates were obtained from diseased *C. gariepinus* (Plate 1 a, b & c) collected from Obinna Farms in Ugbolu, Delta State. Isolates were obtained by macerating aseptically 1cm of skin of the diseased fish. Nutrient and MacConkey agar

media used were prepared according to the manufacturer's instruction. *S. aureus* isolates were cultured and sub-cultured to obtain pure cultures. Stock culture of the isolates were prepared by making slants of nutrients agar in Mac-Cartney bottles for modulation of pure distinct colonies. Serial dilution was made to achieve  $10^{-9}$  dilution factor. All culture water: T1, T2, T3 and T4 were infected with 0.5 ml respectively.

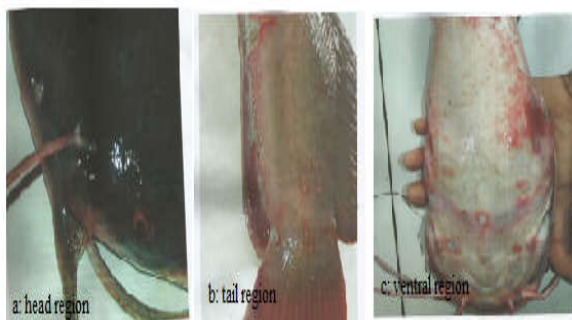


Plate 1 a, b, c : Diseased *Clarias gariepinus* with *Staphylococcus aureus* obtained from Obinna farm, Ugbolu Delta State

Gram Staining and biochemical characterization were done according to Olutiola *et al.* (1991). The isolates were identified by comparing their characteristics with those of known taxa, as described by Oyeleke and Manga (2008). Experimental fish were sampled biweekly for a period of six months for bacterial examination for possible infection. The average count on plates was multiplied by dilution factor and expressed as colony forming unit per milliliter ( $\text{Cfu} \cdot \text{g}^{-3}$ ) of the original factor. Water quality parameters such

as temperature, dissolved oxygen, hydrogen ion concentrations, pH and ammonia were analyzed according to (ALPHA, 2010).

**Data Analysis:** Data collected were subjected to one way analysis of variance with significant means ( $p < 0.05$ ) separated using Duncan's Multiple Range Test.

## RESULTS AND DISCUSSION

Mean growth in body weight, standard length and total length were progressive during the experimental period (Figure 1).

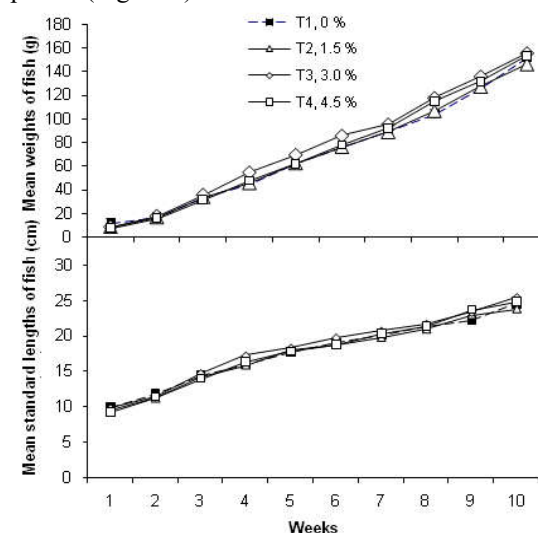


Fig 1. Weekly mean weight and standard length of *C. gariepinus*-fed different dietary inclusion of *A. sativum*

**Table 3.** Nutrient utilization of *C. gariepinus*-fed dietary inclusions of *A. sativum*

Parameters	T1 (0%)	T2 (1.5%)	T3 (3.0%)	T4 (4.5%)
Feed conversion ratio, FCR	1.35	1.41	1.25	1.30
Protein efficiency ratio, PER	1.49	1.52	1.73	1.69
Protein intake	14.2	16.9	18.6	17.5
Specific growth rate, SGR	1.95	1.92	2.06	1.99
Condition factor, K	0.83	1.91	1.99	1.85
Survival rate. %	60	70	80	95

**Table 4.** Biweekly mean of *Staphylococcus aureus* load ( $\text{cfu/g}$ ) of *C. gariepinus*-fed dietary inclusion of *A. sativum*.

Weeks	T1 (0%)	T2 (1.5%)	T3 (3.0%)	T4 (4.5%)
Week 2	127.50 $\pm$ 2.50 <sup>c</sup>	112.50 $\pm$ 1.50 <sup>c</sup>	112.50 $\pm$ 2.50 <sup>b</sup>	99.50 $\pm$ 0.50 <sup>a</sup>
Week 4	131.50 $\pm$ 1.50 <sup>d</sup>	86.00 $\pm$ 1.00 <sup>c</sup>	77.70 $\pm$ 1.00 <sup>b</sup>	69.50 $\pm$ 0.50 <sup>a</sup>
Week 6	144.00 $\pm$ 1.00 <sup>c</sup>	73.00 $\pm$ 3.00 <sup>b</sup>	69.00 $\pm$ 2.00 <sup>b</sup>	58.00 $\pm$ 1.00 <sup>a</sup>
Week 8	152.00 $\pm$ 3.00 <sup>c</sup>	57.50 $\pm$ 2.50 <sup>b</sup>	47.50 $\pm$ 0.50 <sup>a</sup>	43.50 $\pm$ 1.50 <sup>a</sup>
Week 10	156.00 $\pm$ 2.00 <sup>c</sup>	50.00 $\pm$ 3.00 <sup>b</sup>	36.50 $\pm$ 2.50 <sup>a</sup>	30.50 $\pm$ 1.50 <sup>a</sup>
Week 12	174.50 $\pm$ 4.50 <sup>c</sup>	45.00 $\pm$ 4.00 <sup>b</sup>	33.50 $\pm$ 2.50 <sup>ab</sup>	26.50 $\pm$ 1.50 <sup>a</sup>

Means on the same row with the same superscript are not significantly different ( $P > 0.05$ )

This study shows that fish fed (3.0%) of *A. sativum* inclusion in diet had the highest specific growth rate. This finding is in agreement with the study of Javadzadeh *et al.*, (2012) who observed high specific growth rate in *L. vannami* when fed *Artemia nauplii* enriched with 200 mg garlic extract/ *L. Jasour et al.*,

(2018) reported that biogenic diet increased feed intake, feed conversion ratio (FCR) and PER in fish which could have attributed to the better growth performance observed in fish fed 3.0% of garlic. PER and FCR are utilized as quality indicators for fish diet and its amino acid balance. Therefore, these factors are used to evaluate protein utilization and turnover (Shalaby *et al.*, 2006). Table 3 shows the nutrient

utilization of *C. gariepinus*-fed dietary inclusions of *A. sativum*. The biweekly mean bacteria load of fingerlings of *C. gariepinus*-fed dietary inclusion of *A. sativum* is presented in Table 4. This study revealed a significant decrease ( $P < 0.05$ ) of bacteria load of *C. gariepinus*-fed experimental diet. This finding is in line with the report of Rahman *et al.* (2008) who found that young Thai silver barb, *Barbonymus gonionotus* fed a diet supplemented with 8 mg mL<sup>-1</sup> garlic showed the best recovery rate (90%) during the 10-days experimental period. Deresse (2010) reported that dilute solutions of garlic completely inhibited the growth of *S. aureus* at concentrations greater than 7.50 mg mL<sup>-1</sup>. *C. gariepinus* in treatment tanks, were positively infected with *S. aureus*. However, 4.5% garlic inclusion was observed to be more effective in reducing *S. aureus* counts/loads. Aly and Mohamed (2010) found that *O. niloticus*-fed 3% garlic supplemented feed showed a significantly increased survival rate (85%) even after infection with *A. hydrophila*. The water quality parameters were within the levels recommended for fish culture (Boyd and Lichtkoppler, 1990).

**Conclusion:** This study has shown that garlic supplemented diets in fish enhanced growth performance and reduced bacterial load in *Clarias gariepinus*. Three percent (3.0%) inclusion of garlic in diet is recommended for increase in fish growth performance while 4.5% garlic inclusion can be used to reduce *Staphylococcus aureus* counts/loads in *Clarias gariepinus* fish culture.

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