

NUTRACEUTICAL ROLE OF HONEY AND GARLIC (*Allium sativum*) ON HAEMATOLOGICAL AND PLASMA-ANTIOXIDANT PROFILE OF AFRICAN CATFISH (*Clarias gariepinus*)

ABSTRACT

Aims: This work aimed to evaluate the nutritional benefits of honey and garlic (*Allium sativum*) in five different dietary inclusions on the blood and plasma-antioxidant profile of *Clarias gariepinus*.

Methodology: Five treatments; control T1 (0%), T2 (0.5% honey), T3 (1% honey), T4 (0.5% garlic), and T5 (1% honey) were designed for a 30-days study.

Results: The results indicated a significant difference ($P < .05$) in the haematological profile of the T2-T5 treatment groups compared to the T1 control group, except for neutrophils, monocytes, eosinophils, and basophils. It was noted that the superoxide dismutase (SOD) and catalase (CAT) levels showed a significant difference ($P < .05$). However, malondialdehyde (MDA) and reduced glutathione (GSH), showed an insignificant variation ($P > .05$).

Conclusion: The results indicated that honey and garlic increased the haematological and antioxidant indices of the supplemented groups (T2-T5) compared with the control group (T1). As a result, the study suggests the use of honey and garlic in aquaculture should be adopted to boost fish well-being.

Keywords: Honey; garlic; Clarias gariepinus; haematology; antioxidant.

1. INTRODUCTION

The aquaculture industry is one of the fastest-growing sectors worldwide, aquaculture alone contributes to 47% of the world's aquatic animal production. Of this proportion, the majority of this sector is attributed to fish farming (54.3 million tons), followed by molluscs (17.7 million tons), and crustaceans (9.4 million tons) [1]. The intensification and demand for fish have led to further development in aquaculture. The recent growth in aquaculture can be attributed to many factors, such as the growing world population, an increase in the spending power of individuals in developing countries, and the varying tastes and preferences toward seafood over meat. Worldwide, fish is a common source of protein, with Nigeria leading in its consumption on the African continent [2].

According to the Food and Agricultural Organization [1] annual per capita fish consumption is estimated at 17.5 kg for Nigeria, while the projected fish demand for 2018 was

3.61 million metric tonnes (MMT) and a domestic supply of 2.13 MMT [3]. The situation creates a shortage of over 1.5 MMT that can only be provided through imports, as production from capture appears to have been static over the years. The substantial growth in investment into *Claria gariepinus* (African catfish) production in contemporary times has contributed tremendously to overall fish production. According to Edeh et al. [4], *C. gariepinus* is the most cultured species in Nigeria, with an annual production of 122330 metric tonnes in 2015. Hence, increasing the aquaculture production of these species will help bridge the gap between fish demand and supply and as such enhance food security.

However, aquaculture production faces several challenges, such as poor feed quality, diseases caused by excessive nutrient addition, and difficulties in maintaining sustainable fish production [5]. Since fish growth and health are often hampered by feed quality, nutrition is the most important factor through which farmed fish

express their genetic potential for growth and reproduction. Therefore, it is necessary to develop sustainable aquaculture, maintain fish health, and increase fish productivity. One of the ways to improve aquaculture is to introduce immunomodulators, such as probiotics and prebiotics, into the diet of farmed fish.

Prebiotics are beneficial natural products that promote growth, strengthen the immunity of fish under stressed conditions, and increase the production of antibodies, acid phosphatase, lysozyme, and antimicrobial peptides [6,7]. Research on the use of prebiotics has demonstrated the potential to resist pathogens, improve feed efficiency, and improve overall fish health [8]. Shubha et al. [9] also reported that the use of these prebiotics is inexpensive and environmentally friendly with minimal adverse effects compared to the bio-accumulative action of antibiotics. The bioactive substances of various prebiotics stimulate feed intake, enhance the secretion of digestive enzymes, and stimulate the immune response of fish [10]. In aquaculture, natural sources of prebiotics include ginger, onions, garlic, chives, tomatoes, honey, etc.

In modern aquaculture, the combination of fish feeds and prebiotic supplements are used as immunostimulators to resist disease attacks and improve haematological and antioxidant profiles. Honey is known as an immune booster because it increases the immune response to infection by stimulating the activity of lymphocyte cells, which are part of the white blood cells [11]. Honey is known to be a powerful antioxidant that protects cellular components from harmful substances by inhibiting the formation of free radicals [12]. Nutritionally, honey is rich in vitamins A, E, K, and B3, thiamin (B1), riboflavin (B2), ascorbic acid (C), pyridoxine (B6), and minerals such as sodium (Na), calcium (Ca), and potassium (K), magnesium (Mg), chloride (Cl), iron (Fe), zinc

(Zn), niacin, pantothenic acid, biotin, and folic acid [13].

Garlic (*Allium sativum*) is used as a spice and in traditional medicine. It is rich in calcium, phosphorus, and carbohydrates and, generally, has high nutritive salts that have positive effects on the circulatory system [14]. Garlic also contains many vitamins, such as vitamins A, C, and B complex, and linoleic acid [15,16]. Allicin is one of the essential constituents of garlic with an anthelmintic effect [17]. Garlic is considered an antimicrobial agent [18], an antioxidant, and an antihypertensive agent [16], thereby improving the welfare of fish [19].

Because fish are closely associated with the aquatic environment, their blood will reveal measurable haematological changes faster than any other biochemical assessment parameter [20]. The study of the haematological and antioxidant properties of farmed fish species is an important tool in the evaluation of the suitability of new and unconventional diets [21]. Thus, the use of prebiotics (immunomodulators) along with nutritional supplementation is a productive strategy to improve fish health, growth performance, and immunity to diseases [22, 23]. Therefore, the current study aims to assess the role of honey and garlic in the haematological and plasma antioxidant profiles of *C. gariepinus* without negatively impacting the fish environment.

2. MATERIALS AND METHOD

2.1 Experimental Fish

The experimental fish used for the study is the African catfish; *Clarias gariepinus* due to its local availability, and tolerance for experimental manipulation. Eighty (80) male and female healthy *Clarias gariepinus* of fairly uniform sizes of about 150 grams were obtained from a local fish farmer in Anambra State. The post-juveniles were transported in a 50-litre container

partially filled with oxygenated water. *Clarias gariepinus* post-juveniles were acclimatized to the laboratory conditions in fish aquaria containing 35 litres of water for fourteen days. They were fed 5% (a diet containing 41% crude protein) of their body weight twice daily at 9 am and 5 pm with a 12-hour photoperiod.

2.2 Preparation of Garlic Powder

Garlic (*Allium sativum*) bulbs used in this study were purchased from a local vegetable market in Ifite-Awka and identified at the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria. The fresh garlic was washed and air-dried for 12 days. Then, the dried garlic was ground in the laboratory using an electric mill and then passed through a fine mesh sieve to obtain a powder with a uniform particle size. The resulting powder was stored in an airtight container to prevent evaporation of the active ingredients and loss of effectiveness, as suggested by [24].

2.3 Preparation of Experimental Diets

The experiment used 15 experimental aquaria in five different treatments. The treatments were formulated as follows [25]: for the 0.5% honey, 50 ml of honey was measured using a graduated glass cylinder and mixed with 25 ml of water at a ratio of 2:1 and was then poured into a bowl containing 1kg of fish feed and mixed thoroughly. The mixture was sun-dried for 2 days. For 1% honey, 100ml of honey was measured using a graduated cylinder and mixed with 25ml of water in a ratio of 2:1 and was then poured into a bowl containing 1kg of fish feed and mixed thoroughly. The mixture was sundried for 2 days. For 0.5% garlic, 5g of garlic was weighed and mixed with water at a ratio of 2:1 and then poured into a bowl containing 1kg of fish feed and mixed thoroughly. The mixture was sundried for 2 days. For 1% garlic, 10g of garlic was weighed and mixed with water at a ratio of 2:1 and was poured into a bowl

containing 1kg of fish feed and mixed thoroughly. The mixture was also sundried for 2 days.

2.4 Experimental Design

A completely randomized design (CRD) was used in this study, in which the fish were randomly assigned to five treatment groups, 10 fish per treatment and 3 replicates per treatment, based on the dose of natural honey and garlic. In Treatment 1 (control 0 ml/kg), fish were fed diets with no honey or garlic, fish in Treatment 2 were fed the basal diet supplemented with 5% honey 50ml/kg; fish in Treatment 3 were fed the basal diet supplemented with 10% honey 100ml/kg; in Treatment 4, fish were fed with a basal diet supplemented with 0.5% (5 g/kg of diet) of garlic while in Treatment 5, fish were fed the basal diet supplemented with 1% (10 g/kg diet) garlic. Fish were randomly selected and fed diets containing garlic and honey with various inclusions. They were fed twice a day between morning (8 a.m.-10 a.m.) and evening (5 p.m.-6 p.m.) for 30 days, as suggested by [24]. The measured water parameters of pH, temperature, and dissolved oxygen (6.5-7.0, 26-28°C, 7.4-8.0ml/l respectively) were maintained by partially renewing the water in each aquarium with clean water daily throughout the experimental period to avoid contamination that could alter the outcome of the study as suggested by Edeh et al. [26].

2.5 Blood Collection and Hematological Analysis

Blood samples were taken from the caudal vein of a set of three *Clarias gariepinus* post juveniles, randomly selected for treatment, and collected into EDTA bottles using 2 ml syringes and needles, as described by Iheanacho et al. [27]. For Packed Cell Volume analysis, the fish blood sample is centrifuged in a

microhematocrit tube for 10 minutes, then the value is measured to determine the PVC concentration. Haemoglobin (Hb) concentration was measured by the Cyanmethemoglobin method, in which 2 μ l of blood was added to 5 ml of Drabkin solution and allowed to stand for 5 minutes according to the method described by Blaxhall and Daisley [28]. The formation of cyanomethemoglobin was read using a colourimeter to obtain the haemoglobin values. White blood cells (WBC) were determined using a Neubauer hemocytometer as described by Briggs and Bain [29].

2.6 Plasma Antioxidant Assay (SOD, CAT, MDA)

Plasma SOD activity was measured by the method, by Misra and Fridovich [30]. The assay was performed by adding 0.2 ml of supernatant to 2.5 ml of 0.05 M carbonate buffer (pH 10.2). The reaction was initiated by adding 0.3 ml of freshly prepared epinephrine as a substrate to the buffer supernatant mixture and mixing rapidly by inversion. The reference cuvette contains 2.5 ml of buffer, 0.3 ml substrate and 0.2 ml distilled water. The increase in absorbance at 480 nm due to adrenochrome formed was monitored every 30 seconds for 120 seconds. For the determination of catalase activity in blood plasma, the method of Beutler (1984) was adopted in which two millilitres (2 ml) of H₂O₂ were added to 1 ml of the sample in the reaction cuvette. The absorbance was read at 360 nm for 70 seconds. The reference cuvette contained 2 ml of H₂O₂ and 1 ml of water. The disappearance of hydrogen peroxide was calculated using the molar extinction coefficient, $\epsilon = 39.4 \text{ M}^{-1} \text{ cm}^{-1}$. Lipid peroxidation in the form of malondialdehyde (MDA) was determined in the liver and kidney according to the method of Sharma and Krishna-Murti [31]. One millilitre of the sample was added to 2 ml of TCATBA-HCL reagent [0.37% thiobarbituric acid (TBA), 15% TCA, and 0.24 N hydrochloric acid (HCl) of ratio 1:1:1. The tube was capped

loosely and immersed in boiling water for 15 minutes, gently rotated at intervals. The mixture was then cooled and centrifuged at 5000 rpm for 10 minutes. Absorbance was read at 532 nm using a reagent blank. Lipid peroxidation in units/g of wet tissue was calculated with a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}$.

2.7 Statistical Analysis

This study presented all data as mean \pm standard deviation. Comparisons were made between the control and experimental groups. One-way ANOVA and Duncan's multiple range test [32] were used on SPSS statistical software (Version 25 for Windows; SPSS Inc., Chicago, USA) to detect the significant differences among the control and experimental groups. Statistical significance was defined at $P < .05$.

3. RESULTS

3.1 Some Haematological Parameters of *C. gariepinus* Fed Different Inclusions of Honey and *Allium sativum*

The result of some haematological indices of *C. gariepinus* fed with Honey and garlic (*Allium sativum*) at different dietary inclusions is shown in Table 1. The haematological studies of catfish in the treatment groups showed T3 (39 ± 1.14) recorded the highest packed cell volume (PCV) while the least was found in T2 (35 ± 1.42). There was a significant difference ($P < .05$) among the fish in T1, T2, T3, T4, and T5 inclusion of honey and *Allium sativum* respectively. A similar result was obtained on the concentration of haemoglobin (Hb) which also showed a significant difference ($P < .05$) among the catfish in T1, T2, T3, T4, and T5 inclusion of honey and *Allium sativum* respectively. From the result of the haematological analysis of white blood cells of catfish, it was observed that T5 (10.82 ± 0.01) recorded the highest white blood cell (WBC) count while the least was recorded in T1 (9.385 ± 0.01) and there was a significant

difference ($P < .05$) among the catfish in T1, T2, T3, T4, and T5 inclusion of honey and *Allium sativum* respectively. Neutrophil concentration recorded the highest value in T3 (43.00 ± 1.34) while the least was recorded in T4 (40.00 ± 1.43). However, there was no significant difference among the catfish in T1, T2, T3, T4, and T5 inclusion of honey and *Allium sativum* respectively. The result obtained from haematological studies showed that lymphocyte concentration recorded the highest value in T1 (52 ± 1.213) and lowest in Treatment 4 (40 ± 1.03). Similarly, the monocyte concentration of fish in T5 (2.00 ± 1.41) had the highest concentration while there were no significant changes in T1, T2, T3, and T4. There was no significant difference ($P > 0.05$) among the catfish in T1, T2, T3, T4, and T5 inclusion of honey and *Allium sativum* respectively. Eosinophil and basophil concentrations were absent in the fish. The result obtained from the haematological analysis of the red blood cell count (RBC) of catfish in the experiment revealed that T4 (25.87 ± 0.014) recorded the highest RBC while T1 (24.13 ± 0.11) had the least. Nevertheless, there was a significant difference ($P < .05$) among the fish in T1, T2, T3, T4, and T5 inclusion of honey and *Allium sativum* respectively.

3.2 Plasma Antioxidant Indices in Fish Fed with Honey and Garlic (*Allium sativum*) at Varying Levels

The result recorded in Table 2 showed that the SOD value was significantly highest in T2 (0.91 ± 0.00), while the lowest was recorded in T3 (0.19 ± 0.00). The CAT level was also significantly highest in T4 (2.88 ± 0.02) and lowest in T3 (0.23 ± 0.30). Table 2 also revealed insignificant variation in MDA level with T4 (0.8 ± 0.127) having the highest MDA and T5 (0.4 ± 0.451) having the lowest. The results recorded for GSH level were insignificantly highest at T1 (0.37 ± 0.06) and lowest at T5

(0.27 ± 0.33). There were mixed variations in the plasma antioxidant assay of *C. gariepinus* fed with honey and *Allium sativum* at different levels of inclusion.

4.0 DISCUSSION

There has been a steady increase in aquaculture's contribution to fish production. The intensive fish farming environment further suppresses the immune response of fish, which leads to an outbreak of infection [33]. The use of immunostimulants is often employed in fish farming routine procedures as a prophylactic measure. These substances do not have any negative side effects that live vaccines and antimicrobials may have on consumers and the environment and are generally classified as a biological response [34]. Immunostimulants also known as immunomodulators can activate the fish's physiological functions, even in immunosuppressive conditions caused by any form of disturbance or toxic situations. In addition to being anti-suppressant, anti-inflammatory, antibacterial, antiprotozoal, and antifungal, garlic has also been shown to improve the immune and blood systems [25]. Garlic contains a therapeutic factor, Germanium, which enhances natural killer cell and macrophage activity in experimental animals [35]. Honey is rich in phenolic acid derivatives and polyphenolic compounds, especially flavonoids [36], which have metal chelation properties [37, 38] and sieve free radicals and carcinogenic or genotoxic substances [39]. Furthermore, flavonoids have additional important pharmacological and physiological activities, such as anti-inflammatory, antioxidant, and anti-carcinogenic

Table 1: Hematological parameters of *Clarias gariepinus* fed with honey and garlic at varying levels for 30 days

GROUP	PCV (%)	Hb (g/dl)	RBC (103/mn)	WBC (x103/mm3)	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
T1	37±1.23 ^{ab}	11.85±0.0 ^{ab}	24.13±0.11 ^a	9.385±0.01 ^a	41.00±1.32 ^{ab}	52±1.21 ^c	1.00±0.00 ^a	nd	nd
T2	35±1.42 ^a	11.2±0.14 ^a	25.67±0.03 ^d	9.89±0.01 ^b	39.00±1.32 ^a	40±1.17 ^a	2.00±1.41 ^a	nd	nd
T3	39±1.14 ^b	12.1±0.14 ^b	24.89±0.01 ^c	9.69±0.02 ^c	43.00±1.34 ^b	45±1.41 ^b	1.00±0.00 ^a	nd	nd
T4	37±1.47 ^{ab}	11.3±0.16 ^a	25.87±0.01 ^e	10.07±0.01 ^d	40.00±1.43 ^{ab}	40±1.03 ^a	1.00±0.00 ^a	nd	nd
T5	36±1.22 ^{ab}	11.7±0.11 ^b	24.79±0.02 ^b	10.82±0.01 ^e	41.00±1.43 ^{ab}	43±1.41 ^{ab}	2.00±1.41 ^a	nd	nd

Different superscripts within the same column indicate significant differences ($P < .05$), T1 (control), T2 (0.5% honey); T3 (10% honey); T4 (0.5% garlic), and T5 (1% garlic). nd; not detected.

Table 2: Antioxidant Assay (Superoxide Dismutase Activity (SOD), Malondialdehyde (MDA), Catalase Activity (CAT) and Glutathione Serum Level (GSH)) Of Catfish Fed with Honey and Garlic at Varying Levels for 30 days

Treatments	SOD (U/L)	CAT (U/L)	MDA (μ mol/L)	GSH (μ mol/ml)
T1	0.38±0.454 ^{ab}	1.34±0.027 ^c	0.41±0.507 ^a	0.37±0.057 ^a
T2	0.91±0.001 ^b	0.72±0.052 ^b	0.07±0.007 ^a	0.28±0.078 ^a
T3	0.19±0.001 ^a	0.23±0.294 ^a	0.42±0.482 ^a	0.30±0.092 ^a
T4	0.47±0.001 ^{ab}	2.88±0.015 ^d	0.8±0.127 ^a	0.35±0.007 ^a
T5	0.20±0.001 ^a	1.45±0.047 ^c	0.4±0.451 ^a	0.27±0.325 ^a

Different superscripts within the same column indicate significant differences ($P < .05$), T1 (control), T2(0.5% honey); T3 (10% honey); T4 (0.5% garlic), and T5 (1% garlic)

properties, and improve endothelial function due to their intrinsic reducing abilities [40]. Haematological indices act as physiological indicators of a changing environment, as a result of their relationship with energy, respiration and defence mechanisms [27, 40]. The haematological parameters which include, Hb, PCV, RBC and WBC counts have a close interaction with the external surrounding and are used frequently to detect the well-being of animals [42]. In this study, the haematological responses of RBC, Hb, PCV and WBC increased significantly ($P < .05$) in all dietary groups when compared to the control. These observations might indicate compensatory erythropoiesis which resulted in the production of more RBCs to replace the older ones which were probably rapidly destroyed due to a decrease in the oxygen-carrying capacity of the blood. Increased haematocrit is an indication of a stress response causing RBC swelling or haemo-concentration due to plasma volume reduction [43]. Erythrocyte count greater than $1.00 \times 10^6 \text{ mm}^{-3}$ is considered high and indicative of the high oxygen-carrying capacity of the blood, which is characteristic of fishes capable of aerial respiration and with high metabolic activity [44]. This increase indicates induced stimulatory effects on the immune system of the fish with the consequent release of lymphocytes from lymphomyeloid tissues. The observed increase in haematological indices could be a result of the honey and garlic supplements in the fish diets. Haematocrit values represent the abundance of red blood cells and Blaxhall and Daisley [28] reported the essence of using haematocrit values to detect or diagnose anaemic conditions in fishes. Piotr et al. [45] stated that an increase in PCV can result from the increased number of RBC, erythrocyte swelling (due to lower blood pH, respiratory acidosis) or decreased volume of water in the circulating blood (due to muscle tissue acidification following stress exposure).

However, Sotolu and Faturoti [46] reported lower PCV values which were attributed to anaemia resulting from shrunken red blood cells, a situation that probably resulted in fish asphyxiation and death as confirmed by Adeyemo (2005). Blaxhall [47] associated reduced PCV values with loss of appetite or disease.

In this study, monocytes, basophils and eosinophils were scarcely observed and there was no significant difference between the control and dietary groups. This indicated an extremely low concentration of these cell types. This observation is similar to the findings of Iheanacho and Odoh [48], who stated that it is hard to preserve basophils and this is the main reason why basophils are difficult to identify in fish blood. Modra et al. [49] reported a decreased concentration of eosinophils and the absence of basophils in several fish species, including *Cyprinus carpio*, *Tinca tinca*, *Silurus glanis* and *Oncorhynchus mykiss*. Romao et al. [50] also reported the absence of eosinophils, basophils and granulocytes in *Hoplias malabaricus* and *Geophagus brasiliensis* collected from the wild. White blood cells are known to play an important role in the immune system and responses of living organisms.

The low WBC count in the control group could be attributed to a reduction in the number of lymphocytes. Alkahem [51] also reported reduced WBC counts in *Oreochromis niloticus* and associated them with a generalized stress response resulting in an increased pituitary-interrenal activity. Alkahem [51] also linked reduced WBC count with a reduction in the number of circulating thrombocytes and lymphocytes due to a diminution in the delivery of lymphocytes to the circulatory system through reduced lymphocyte production and rapid destruction of cells which leads to an increased rate of peripheral removal of lymphocytes. However, Ajani [52] and Kori-

Siakpere et al. [53] stated that a high WBC count means a release of more cells to maintain homeostasis while a low WBC count is a common stress response. Therefore, increasing or decreasing numbers of WBCs are normal physiological reactions in fish and these show the response of the immune system under stress conditions. Many researchers have reported low values of leukocyte counts in the blood of fish exposed to different pollutants and attributed them to the reduction in the number of circulating lymphocytes and thrombocytes [48,51,54,]. This gives considerable support to the present investigation. Douglas and Jane [55] demonstrated that their amount has implications for immune responses and the ability of the animal to fight infection.

Honey and garlic dietary supplementation in this study, also stimulated changes in the antioxidant profile of *C. gariepinus*, leading to a significant increase in SOD, and CAT, levels and non-significant variation in the MDA and GPx levels. These results support those reported by Dogan et al. [56], who found that antioxidant enzyme activities showed a two-staged response to stress exposure: an initial induction followed by a sharp inhibition in the liver tissue and a sustained increase in GPx activity and slight stimulation in SOD activity in the brain tissue. Like all aerobic organisms, fishes are susceptible to attack by ROS, so their cells have an efficient antioxidant defence system [57], allowing a balance to exist between the formation and elimination of ROS under normal conditions. However, an excess of ROS formation or a deficiency in the antioxidant defence system can cause cellular oxidative stress to occur, which can also cause lipid peroxidation. This is a complex process resulting from free radical reactions in biological membranes, which are rich in polyunsaturated fatty acids [58]. In the present study, it was found that supplementing the diet with honey or garlic, significantly decreased MDA levels, suggesting that these

feed additives have an antioxidant function. Physiologically, all haematological and antioxidant parameters measured in this study were within the range reported for *C. gariepinus*. An improvement in the parameters observed indicated that honey and garlic improved blood formation and immunity in the fish.

5. CONCLUSION

According to the findings of this study, honey and garlic can be supplemented in the diet of *C. gariepinus* without having any negative effects on the haematological and antioxidant parameters. The intrinsic potential of honey and garlic to improve the health and overall well-being of fish makes their use in aquaculture recommended.

ETHICAL APPROVAL

All experimental procedures were performed in compliance with the Animal Care Use Committee Research Ethics of Nnamdi Azikiwe University, Awka (NAU/AREC/2021/00052) on the protection of experimental animals.

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