Dietary Supplement of Tumeric (Curcuma longa) Powder: Impact on Haematological and Biochemical Responses in Clarias gariepinus (Burchell, 1822) Fingerlings

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Abstract
Dietary supplementation with herbal extract in the diet of fish has been noted to increase aquaculture production through the improvement of immunity and other physiological dysfunctions related to fish health. A feeding trial was conducted to assess the impact of incorporating varied concentrations of turmeric powder (TP) on haematological and biochemical responses in Clarias gariepinus fingerlings. Five test diets were composed as T1 (control (basal diet + 0 % TP)), T2 (basal diet + 1 % TP), T3 (basal diet + 2 % TP), T4 (basal diet + 3 % TP) and T5 (basal diet + 4 % TP). Fish specimens were apportioned into five groups in triplicate. Significantly lower (P<0.05) red blood cell count was found in those fed control diet compared with the other inclusion. Highest values of white blood cell count were noticed in T5 when compared with the control. There were significant differences in haemoglobin between T1 and T5. Highest values of serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, glucose and cholesterol level were found in T2 when compared with the control. TP can be used as feed supplement in the diet of C. gariepinus without any adverse effect on physiological responses of the fish.

Introduction
Aquaculture constitutes a major segment of the animal production enterprises, due to the sustained advancement in culturing fishes and shellfish species and arguably the fastest growing industry with an annual production capacity of 800 million tonnes (Kolkovski and Kolkovski, 2011).

Nigeria is among the highest aquaculture production in sub Saharan African with an output of 21700 tonnes in 1999 to 316700 tonnes in 2015. Among other fishes, catfish is the most important cultured fish species that are usually grown in ponds and tanks; and constitutes more than half of the total aquaculture production in Nigeria.

The culture and production of the fish species may have greeted with high incidence mortality, high stress-related diseases, suppressed immune system and other physiological dysfunctions due to bacterial infections. Many types of antibiotics including oxytetracycline, chloramphenicol, sulfonamides and other anthelmintic agents have been applied to overcome or subdue bacterial infections in aquaculture production (Sahu et al., 2005: Fawole et al., 2016). Unfortunately, the continuous introduction of these drugs in fish production has resulted to the development of residual effects and resistivity risk to livestock as well as human health (Magrone et al., 2016, Arciuli et al., 2017). However, in order to ameliorate or overcome the ugly development, attention is being given to the use or
production of alternative medicinal plant or phytogenic as feed supplement in aquaculture practices which are potent in mitigating the impasse of diseases by increasing the immune system of fish (Fawole et al., 2020; Iheanacho et al., 2018).

Medicinal plants or phytogenic feed supplement are made up bioactive ingredients and also possesses antimicrobial and antioxidative properties that are usually incorporated in animals’ diets in order to improve their performances (Yang et al., 2015; Landy et al., 2011).

Many studies have reported the incorporation of different plant supplements or additives to improve the physiological dysfunctions of fish (Mahmood et al., 2014; Sahu et al., 2014) and elevated responses of fish in reaction to different diets (Jahanbakhshi et al., 2013). The information on the dietary supplementation of turmeric powder in the diet on the physiological responses of C. gariepinus is scanty. The study was planned to find out the haematological and biochemical reactions in C. gariepinus fed diet incorporated with varied levels of turmeric powder.

Materials and Methods

Collection of Turmeric Plant and Preparation

Fresh rhizome of turmeric plant, ginger (C. longa) was procured from National Root and Crops Institute Research Umudike, Abia State Nigeria. It was washed, cooked and sun dried in a shade. The dried rhizome was ground into fine powder using machine, sieved, using appropriate mesh size, packed in tight container and kept at room temperature till further use.

Experimental Diet

Five experimental diets were formulated as T1 (basal diet + 0 % TP), T2 (basal diet + 1 % TP), T3 (basal diet + 2 % TP), T4 (basal diet + 3 % TP) and T5 (basal diet + 4 % TP). The ingredient presented in Table 1 was used for the formulation of the diet. They were weighed accordingly based on the proportion of each ingredient presented in Table 1 was used for the formulation of the diet. They were weighed accordingly based on the proportion of each diet. The diet was stirred extensively, water was included, and the diet was made into pellets using machine. The pelleted diet was dried, packed in tight polythene bag and preserved for further use.

As presented in Table 1, proximate composition of the feed was done according to AOAC (2005).

Table 1. Ingredients and proximate composition of experimental diet.

| Ingredients (%) | T1 | T2 | T3 | T4 | T5 |
|----------------|----|----|----|----|----|
| Maize Grain    | 49.80 | 49.80 | 49.80 | 49.80 | 48.80 |
| Soy Bean       | 27.50 | 26.50 | 26.50 | 26.50 | 27.50 |
| Wheat Offal    | 12.00 | 11.50 | 11.00 | 10.00 | 9.00 |
| Palm kernel Cake | 5.10 | 5.10 | 5.10 | 5.10 | 5.10 |
| Fish Meal      | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Bone Meal      | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Vitamin Premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Methionine     | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Salt           | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Palm Oil       | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Turmeric Powder| 0.00 | 1.00 | 2.00 | 3.00 | 4.00 |
| Total          | 100 | 100 | 100 | 100 | 100 |

Proximate composition (%)

| Protein       | 30.42±1.36 | 32.57±2.64 | 31.13±1.18 | 30.76±1.71 | 31.27±1.23 |
| Fat           | 7.90±0.18  | 8.42±0.32  | 8.21±0.25  | 7.82±0.87  | 9.19±0.17  |
| Moisture      | 11.48±0.52 | 12.24±0.20 | 12.07±0.12 | 11.80±0.83 | 11.69±0.72 |
| Ash           | 9.42±0.40  | 9.61±0.65  | 9.78±0.71  | 10.39±0.32 | 10.18±1.23 |
| Fibre         | 7.71±0.76  | 7.85±0.81  | 7.92±1.90  | 8.20±1.23  | 8.34±0.30  |
Fish and Feeding Experiment

*Clarias gariepinus* (n=180, mean weight 5.14±0.13 g) procured from a fish farm in Umuahia Abia State was used for the study. The fish were transported in oxygen bags to Animal Science and Fisheries Laboratory, Abia State University, Uturu and held in a tank containing 500 L of dechlorinated tap water. Prior to feeding experiment, the fish were acclimatized for two weeks and were fed with commercial feed.

The fish were not fed 24 hours before the commencement of the study. They were randomly distributed into five experimental groups and stocked with fifteen fish per tank of 150 L capacity in triplicate for each treatment group and filled with 80% capacity of dechlorinated tap water. The fish were fed two times daily to satiation at 9:00 and 16:00 hrs. Water in each experimental tank was renewed every three days while the debris of uneaten feed was siphoned daily to disallow pollution of the tanks and the water replaced thereupon. Continuous ventilation was made available to all the experimental tanks. The investigation was carried out indoors under natural photoperiod of 12:12 light-dark cycle. Physicochemical parameters such as temperature, pH, dissolved oxygen, alkalinity, total hardness and free carbon (iv) oxide were analysed according to APHA (2005) and were maintained within the range as: temperature (27.50 -28.50°C), pH (6.8-7.6), dissolved oxygen (6.50-7.35 mg L⁻¹), alkalinity (15.81-18.22 mg L⁻¹), total hardness (228-238 mg L⁻¹), free carbon (iv) oxide (4.13 -4.28 mg L⁻¹). At the end of the feeding trial which lasted for 60 days, the fish were sampled for haematological and biochemical analysis.

Haematological Analysis

Feeding of the fish was stopped 24 hr before sampling. Before blood collection, fish were anesthetized with MS 222 in order to reduce stress. Heparinized plastic syringe rinsed with 10% ethylene diaminetetraacetic acid (EDTA solution) was used to collect blood from the fish (4 fish per tank, n = 12 per experimental group) through the caudal vein. The blood was stored in eppendorf tube rinsed with EDTA and later used to estimate red blood cell (RBC), white blood cell (WBC) packed cell volume (PCV) and haemoglobin (Hb). RBC and WBC expressed in cubic millimeter were analysed using Neubauer-type hemocytometer according to Russia and Sood (1992).

Hb level of blood was estimated following the cyanmethemoglobin method according to Blaxhall and Daisely (1973). Briefly, 0.02 mL specimen of blood was stirred with 4 mL Drabkin’s solution and left to stay for 10 min for appropriate development of colour due to absorbance which was read at 540 nm by Unican spectrophotometer against a blank. PCV was analyzed in heparinized glass capillaries after centrifugation in a microhematocrit centrifuge according to Nelson and Morris (1989). Eosinophils, neutrophils, monocytes, lymphocytes, and basophils in the blood were estimated according to Anderson (2003). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the formulae:

\[
\text{MCV(FL)} = \frac{\text{Haematocrit (\%)} \times 10}{\text{RBC (10}^{12}/\text{L)}
\]

\[
\text{MCH(pg)} = \frac{\left(\frac{g}{dL}\right) \times 10}{\text{RBC(x10}^{12} /\text{L)}
\]

\[
\text{MCHC (g/dL)} = \frac{Hb \left(\frac{g}{dL}\right)}{\text{Haematocrit}} \times 100
\]

Biochemical Analysis

Fresh fish blood was collected with syringe from another batches of 4 fish per tank (n = 12 per experimental group) and then poured into a dried Eppendorf tube to allow for the clotting of the blood. The samples were centrifuged at 3,000 rpm for 10 mins at 4°C to get the serum.

Glucose level was determined using O-toluidine method (Cooper and MacDaniel, 1970). Total protein was estimated spectrophotometrically according to Bradford (1976). Serum glutamic pyruvic transaminase (SGPT/ALT) and serum glutamic oxaloacetic transaminase (SGOT/AST) were determined following Reitman and Frankel (1957). Alkaline phosphatase (ALP) was determined according to Essey et al. (1946). Creatinine was determined according to Henry et al. (1974). Urea was assessed according to Coulombe and Favreau (1963) while cholesterol was determined according to Allain et al. (1974).

Statistical Analysis

Data are presented as mean ± standard error. Statistical package SPSS 20.0 computer program was used to evaluate the data. One-way analysis of variance was used to determine the variation among treatments and the reference, followed by Post hoc test using Duncan multiple tests at 5% level of significance.

Results

Haematological Responses

The results of blood parameters of *C. gariepinus* fed diet incorporated with varying levels of turmeric powder as presented in Table 2 revealed no significant difference (P>0.05) in those fish fed control diet (T1) and among the treatment groups. WBC in the T5 which contained the inclusion level (4% turmeric) was highest, followed by T4, T3, T2 and T1 (Control diet).

A significant difference (P<0.05) existed between the treatments and the control. Haemoglobin was
highest in the fish fed diet T2 (1% turmeric) compared with the other inclusion levels and was significant (P<0.05) when compared with the control diet. Treatment containing 0% turmeric powder (T0) control diet inclusion had the highest value of haematocrit while T5 containing (4% turmeric powder) inclusion had the lowest value. The values indicated no significant difference (P>0.05) among the treatments groups and the control. The results of both MCV and MCH of the fish showed that T2 which contained 1% turmeric powder inclusion had the highest values of 57.85 and 18.52 respectively and was found to be significant (P<0.05) when compared with other treatments and the control diet. Highest value of MCHC was recorded in T2 followed by control which was not significant (P>0.05) when compared with other treatments.

The results of white blood cell component including lymphocytes, monocytes, neutrophils, eosinophils and basophils as shown in Table 3 indicated no significant difference (P>0.05) in all the indexes tested between the fish fed varying levels of turmeric powder and the control, depleting that the diet can be included in their feed at the following inclusion levels (0%, 1%, 2%, 3% and 4%) without affecting their white blood cell components.

### Biochemical Parameters

The value of blood sugar as presented in Table 4 showed a decreasing trend from T1 to T5. Treatment means T1, T2 and T3 containing the inclusion levels of 0%, 1% and 2% respectively had significant difference (P<0.05) when compared with T0 (3% of TP) and T5 (4%) of TP inclusion levels. A significant (P<0.05) lowest value (68.10 mg/dl) of cholesterol was found in T2 containing inclusion level of 1% turmeric powder when compared with the control that had the highest value (83.40 mg/dl) of cholesterol. The highest value of urea was recorded in control diet which was not significant (P>0.05) when compared with other treatments while there was no significant difference (P>0.05) in value of creatinine in the fish fed both the varying levels of turmeric powder and the control. There was a significant (P<0.05) reduction in the levels of serum glutamic

### Table 2. Mean ± (SE) values of haematological parameters of C. gariepinus fingerlings fed diets incorporated with Turmeric powder.

| Parameters       | T0 0% (TP) | T1 1% (TP) | T2 2% (TP) | T3 3% (TP) | T4 4% (TP) |
|------------------|------------|------------|------------|------------|------------|
| RBC (x10^12/L)   | 5.73±0.35a | 5.30±0.15a | 5.87±0.29a | 5.97±0.25a | 5.47±0.18a |
| WBC (10^9/L)     | 13.03±0.02a| 16.67±0.13b| 16.63±0.75b| 16.27±1.44b| 16.70±0.55a|
| Haemoglobin (g/dl)| 6.83±0.76a | 10.10±0.15a| 8.73±0.48a | 8.23±0.19a | 8.60±0.23a |
| Haematocrit (%)  | 30.07±0.99a| 30.00±0.58a| 29.47±1.57a| 30.93±0.41a| 29.17±0.76a|
| MCV (FL)         | 46.13±1.67a| 57.85±2.72a| 46.88±3.04b| 43.73±2.78b| 38.78±1.69a|
| MCH (pg)         | 15.07±0.65a| 18.52±0.96a| 14.91±0.93a| 13.89±0.96a| 13.51±0.60a|
| MCHC (g/dL)      | 32.65±0.57a| 32.99±1.62a| 31.80±0.06a| 31.74±0.24a| 32.26±0.38a|

RBC = Red blood cell, WBC = White blood cell, MCV= mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration Mean values with different letter superscripts along the rows differ significantly (P<0.05), n=15

### Table 3. Mean ± (SE) values of differential WBC counts (%) in C. gariepinus fingerlings fed diet incorporated with Turmeric powder.

| Parameters      | T0 0% (TP) | T1 1% (TP) | T2 2% (TP) | T3 3% (TP) | T4 4% (TP) |
|-----------------|------------|------------|------------|------------|------------|
| Lymphocytes     | 2.28±0.21a | 2.80±0.89a | 2.37±0.82a | 2.43±0.09a | 2.30±0.76a |
| Monocytes       | 1.80±0.06a | 1.93±0.09a | 2.47±0.26a | 2.40±0.43a | 2.30±0.03a |
| Neutrophil      | 8.15±0.09a | 8.33±0.54a | 8.10±0.86a | 8.27±0.90a | 8.17±0.33a |
| Eosinophil      | 1.37±0.09a | 1.53±0.05a | 1.33±0.16a | 1.37±0.33a | 1.23±0.03a |
| Basophils       | 0.80±0.56a | 1.00±0.12a | 0.97±0.09a | 0.80±0.06a | 0.90±0.06a |

### Table 4. Mean ± (SE) values of serum biochemistry in C. gariepinus fingerlings fed diets incorporated with Turmeric powder.

| Parameters  | T0 0% (TP) | T1 1% (TP) | T2 2% (TP) | T3 3% (TP) | T4 4% (TP) |
|-------------|------------|------------|------------|------------|------------|
| Blood sugar (mg/dl) | 72.73±0.64a | 71.47±1.41a | 70.97±1.77a | 66.40±1.30a | 60.40±1.36a |
| Cholesterol | 81.40±0.62a| 72.10±0.12a| 78.30±1.13a| 78.27±1.13a| 76.53±0.92a|
| Urea (mg/dl) | 12.53±12a  | 12.23±0.29a| 13.03±0.84a| 12.80±0.15a| 12.80±0.42a|
| Creatinine  | 0.13±0.05a | 0.12±0.04a | 0.13±0.03a | 0.14±0.03a | 0.13±0.02a |
| SGPT /ALT (U/l) | 53.30±0.91a| 42.47±6.86a| 44.53±6.69a| 45.50±9.72a| 52.43±3.32a|
| SGOT /AST (U/l) | 61.32±0.62a| 50.48±5.16a| 46.22±6.65a| 55.53±3.11a| 62.43±1.18a|
| ALP (U/l)    | 56.27±0.98a| 54.33±6.85a| 56.53±6.65a| 55.56±6.72a| 57.43±3.32a|
| Glucose (mg/dl)| 23.77±1.31a| 28.87±0.84a| 27.80±0.87a| 26.83±0.58a| 24.77±0.18a|
| Total protein (mg/dl) | 7.27±0.15a  | 7.42±0.05a  | 7.33±0.22a  | 7.36±0.38a  | 7.43±0.09a |

SGPT/ALT= Serum glutamic pyruvic transaminase, SGOT/AST = Serum glutamic oxaloacetic transaminase, ALP = Alkaline phosphatase. Mean values with different letter superscripts along the rows differ significantly (P<0.05), n=15
pyruvic transaminase (SGPT/ALT) and serum glutamic oxaloacetic transaminase (SGOT/AST) in the fish fed T2 in comparison with the control. The activities of alkaline phosphatase in the fish revealed no significant difference (P>0.05) when compared with the control. A significant (P<0.05) high level of glucose was found in T2 when compared with other treatments and the control while the lowest value was recorded in T1 (control diet). There was no significant increase (P>0.05) in the level of serum protein when compared with the control (Table 4). The highest value was found in T1 while the lowest was recorded in control group.

**Discussion**

The study has demonstrated that supplementation of turmeric powder in the feed of *C. gariepinus* at various inclusion levels can improve the haematological, biochemical parameters as well as the cellular immune system of the fish including the phagocytic cells.

Examination of blood and biochemical biomarkers are useful diagnostic tools used in evaluating the physiological status of fish (Banaee et al., 2011; Pandey et al., 2017) and also in assessing the effect of replacement of ingredient on fish health (Fagbenro et al., 2013; Fawole et al., 2016).

Increase in RBCs and WBCs counts noticed in the investigation for African catfish fed diets containing varying levels of turmeric powder when compared with the control suggested that turmeric powder elevated the haematological profile of the fish.

Also WBC function is majorly to battle against infectious diseases, safeguard the body against infiltration by foreign substances and distribute antibodies as well as immune response (Ajima et al., 2013). Increase in WBC of the fish fed diet incorporated with turmeric powder may be the immune-modulatory potential of the powder. Increase in WBCs count has been reported in Green terror (*Andinocara rivulatus*) fed diet incorporated with varying level of turmeric powder (Mooraki et al., 2019). Increase in WBC has been also reported in *C. gariepinus* juveniles fed ginger (*Zingiber officinale*) supplemented diets (Iheanacho et al., 2018) and in Wuchang Bream (*Megalobrama amblycephala*) fed diets supplemented with curcumin (Xia et al., 2015). Elevated WBCs as well as other immunological parameters are shown to be valuable prove of the health condition of fish (Kumar et al., 2013).

The blood indexes including MCV, MCH and MCHC play an important part in the assessment of anaemic condition in fish as well as in most other animals and are suggestive of the erythrocyte significance and oxygen-carrying potential of the blood in fish (Houston, 1997). We found increment in MCV and MCH level in fish fed diet supplemented with turmeric powder with no significant difference in MCHC; indicating that the oxygen-carrying abilities of blood for different metabolic processes were not weakened. Similar to our report, Fawole et al. (2020) noticed increment in MCV and MCH in *C. gariepinus* fed diet supplemented with pawpaw seed and onion peel powder.

The differences noted between the dietary groups in Hb and PCV indicated the ability of the fish to utilize diets incorporated with turmeric powder without compromising the oxygen-carrying capacity to the premier organs of the fish.

Leucocyte differentials including neutrophils, lymphocytes, monocytes and basophils, eosinophils play important part in cellular immune responses and are valuable indicator of health status of fish (Emeish and El-deen, 2016; Fazlolahzadeh et al., 2011; Jeney, 2017). No significant different found in the leucocyte differentials indicated that supplementation of turmeric powder in the diet of *C. gariepinus* may not pose any toxic impasse on the fish. In comparison to our report, Iheanacho et al. (2018) reported non-significant difference in leucocyte differentials in *C. gariepinus* fed diet supplements of *Z. officinale*.

Increase in levels of SGPT/ALT, SGOT/AST and ALP in fish have been related to liver dysfunction or swelling which results in releasing these enzymes into the bloodstream through leakages (Akra et al., 2015). In line with our findings, Shalaby et al. (2006) documented that the reactions of SGPT/ALT and SGOT/AST was reduced significantly with increment in the level of *Allium sativum* in *Oreochromis niloticus* also in *M. amblycephala* that were fed diets supplemented with curcumin (Xia et al., 2015). Curcumin can improve the liver functions through stimulation and regeneration of liver cells as well as protecting the liver cells from the toxic compounds (Dewi et al., 2018).

Cholesterol plays a vital role in assimilation of fatty acids from the intestine and in their subsequent transport in the blood or haemolymph. Reduction in level of cholesterol in our study is in line with Abdel-Tawwab et al. (2018) who reported decreased level of serum cholesterol levels with elevated *Ocimum gratissimum* leaf extract in the feed of *C. gariepinus*. Hypcholesterolaemia has also been reported in Japanese flounder (*Paralichthys olivaceus*) fed diet *Chlorella ellipsoidea* supplementation (Kim et al., 2002), in *Huso huso* fed diet containing varying levels of *Allium cepa* (Akrami et al., 2015) and also in *C. gariepinus* fed diet supplemented with pawpaw seed and onion peel powder (Fawole et al., 2020).

Hyperglycaemic differences found in this study could be a change in metabolism of carbohydrate which elevated glucose-6-phosphatase reaction in the liver or
triggered the rate of glycogen breakdown to meet energy requirement of the fish (Ajima et al., 2015). Increment in level of glucose in dietary supplementation with plant extract/powder has been reported (Saedi et al., 2017; Abdel-Tawwab, 2015). Also, increased level of glucose was found in Carassius auratus fed diet supplemented with sugar beet leaf extracts (Inanan and Acar, 2019).

The no significant increase in the level of serum protein found in this study could be attributed to the elevation of non-specific immune responses in fish (Abdel-Tawwab et al., 2018). Increase in serum protein has been reported in rainbow trout fed diet incorporated with stinging nettle (Urtica dioica) (Saedi et al., 2017) and in C. auratus fed dietary Ferula elaeochytris root powder (Inanan et al., 2021). The result of both creatinine and urea levels in the fish indicated no significant difference between the control and those fed dietary supplement of turmeric powder indicating that inclusion of turmeric powder in the diet has no effect in the kidney function of the fish. Decrease in the level of creatinine in C. gariepinus fed diet incorporated with Z. officinalis has been reported (Olaniyi et al., 2020).

Conclusion

The study demonstrated that inclusion of turmeric powder in the diet of C. gariepinus improved the haematological parameters by increasing the white blood cell of the fish; reduced the activities of some liver function enzymes including SGPT/ALT, SGOT/AST and ALP, improved the immune response of the fish by increasing the level of serum protein. The result further showed that turmeric powder can be used as a feed additive in the diet of C. gariepinus at 1% inclusion without any adverse effect on the physiological function of the fish because of the better results compared with other inclusions levels.

Ethical Statement

This research was conducted in accordance with the recommendations in the Guide for the Care and use of Laboratory Animals of Abia state University, Umuahia campus, Abia state, Nigeria. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University.

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No funding was received for the study.

Author Contribution

All the authors contributed equally in carrying out the study and drafting the manuscript.

Conflict of Interest

No conflict of interest.

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