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Evaluation of Haematological and Biochemical Changes in Catfish (*Clarias Gariepinus*) Fed Ginger Additive Meal Diets

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Abstract

Clarias gariepinus adults were fed varying levels of ginger additive meal diets (0 mg/g, 25 mg/g, 50 mg/g, 75 mg/g and 100 mg/g), respectively, in order to evaluate their effects on the biochemical and haematological parameters of fish. Blood biochemical analysis was performed at the end of 84 days by taking the portion of the collected blood and was dispensed into non-heparinised tubes. There were significant increases ($P < 0.05$) in glucose, aspertate transferase, alanine transferase, and sodium concentrations respectively. The activity of potassium, creatinine and serum protein changed in a non-significant ($P > 0.05$) manner with increasing concentrations of additive diets compared to the control. Although no definite trend in the values of computed biochemical indices was observed. However, glucose, aspertate transferase (AST), alanine transferase (ALT), urea, creatinine, sodium, and potassium increased with varying additive inclusion concentration. Also, Glucose, ALT, AST, Urea, creatinine, sodium and potassium activities of all the fish, which have been fed ginger additive meal diets were significantly higher ($p<0.05$) in comparison to their respective control. Results also showed that there were increase in the blood parameters of *Clarias gariepinus* fed ginger additive meal diets compared to the control diet except in mean cell haematocrit (MCV) but were statistically different ($p<0.05$). Highest Packed cell volume (PCV) of 31.00% were recorded in diet (GD3), White Blood Cell (WBC), 8.00 x 103 in GD2, and red blood cell (RBC) 8.70 x 106; while highest Haemoglobin (Hb), 11.00 g/dl were recorded in fish fed diet GD3. All blood parameters obtained were between the range of recommended fish blood. It is concluded that using ginger as additive feed on *Clarias gariepinus* showed a slight increase in the haematological parameters compared with the control diet (GD1) except in MCV but it has no negative effect on the health status of the studied fish.

Keywords: Catfish; Biochemical; Ginger; Additive

Introduction

Aquaculture is the farming of aquatic organisms, which include fish, crustaceans, molluscs, aquatic plants in freshwater, brackiculture and mariculture environments. The use of immunostimulant, as dietary supplements, can improve the innate defense of animals providing resistance to pathogens during period of high stress, such as grading, reproduction, sea transfer and vaccination. The application of immunostimulants is increasing currently because of the frequent outbreaks of fish diseases in culture systems and hatcheries. The African catfish (Clarias gariepnus) [1], is a species of catfish of the family Clariidae and is an important fish species in both aquaculture and capture fisheries [2]. It contributes 22% of animal protein in sub-saharan Africa and 40% of animal protein for consumption in Nigeria [3].

Currently, natural materials (medicinal plants) such as bitter leaf, scent leaf, bitter kola have been widely accepted as feed additives to enhance feed utilization and aquaculture productive, performance and sustainability [4].

Phytogenic feed additives, also known as phytobiotics products are plant derived products, used in animal feeding to improve performance through amelioration of feed properties, promotion of production performance and improving the quality of animal origin food/feed [5]. Also, ginger being a natural and readily available additive could be used as fertility booster in fish which might also be a possible solution to the inadequacies of fingerlings supply, hence increasing the African catfish market in Nigeria. In fish, ginger has been successfully used to control an Aeromonas hydrophila infection in rainbow trout [6]. In addition, ginger has been used as immune-modulatory agent in animals and fish to reduce the losses caused by diseases in aquaculture. Therefore, this research study, evaluate the effect of ginger additive meal diets on the biochemical profile of African catfish (*Clarias gariepinus*).

Materials and Methods

Collection and Processing of Feed Additives

Ginger (*Zingiber officinale*) rhizomes were purchased from Gombe main market. Washed with distilled water, sun-dried, and cleaned of its dirts by hand picking. The rhizomes size were reduced with pestle and mortar first, then air dried at ambient temperature before milling with hammer machine after which it was sieved using a sieving material (house hold siever 0.2mm) and kept in polythene bag until when needed.

Experimental Fish

A total of six hundred and fifty (650) catfish (*Clarias gariepinus*) of average wet weight 15.0 ± 0.2 g were obtained from Dr Yahaya Commercial Fish Farm in Gombe, and kept in aerated semi flow-through fresh water.

Formulation and Composition of Experimental Diet

A 40% iso-nitrogenous feed was formulated from the feed ingredients fish meal, soybean meal, yellow maize, Vitamin, Minerals, Palm oil, dicalcium phosphate, starch, lysine and methionine purchased from Gombe feedstuff market, Gombe. The dry ingredients were milled with grinding machine to very fine particle size and sieved. The ingredients were weighed according to the formulation and mixed to homogeneity. Each of the feeds was used to process the five different experimental diets in four groups. Each of the experimental diet group contained ginger and the inclusions with the experimental diet codes areas shown in Table 1. The mixed ingredients were pelleted with a pelleting machine at 0.2mm diameter size. The pellets were air dried at room temperature, packed in polythene bags, labeled and stored at room temperature in the laboratory until when needed.

Experimental System and Stocking Density

Sixty 50L plastic tanks were used for the experiment indoor. The water level in each tank was maintained at volume of 35 litres throughout the study period. Water in each tank was replaced every three (3) days throughout the period to maintain relatively uniform physiochemical parameters. The source of water was from de-chlorinated water and each tank was well aerated using aerators. In order to avoid overcrowding the fingerlings were stocked at a rate of ten fish per plastic bowl of 50 litres each for experimental purpose [7].

Water Quality Parameters

Water quality parameters of the experimental fish showed little variation throughout the duration of the experiment (Table 2). Temperature ranges from 26.80 - 27.80°c, Dissolved Oxygen from 4.50 - 5.50mg/l, pH from 6.01 - 6.51 and Ammonia from 0.53 - 0.71.

Haematological Examination

Collection of Blood Sample: Haematological analysis of the fish was carried out after the experiment at the Department of Biochemistry, Gombe State University, Gombe, within 60 minutes of sampling. Four samples of each experimental fish from each treatment were removed randomly. The caudal fin of each was cut using sharp surgical blade. Before the experiment, 3ml of blood was gently obtained from the bleeding caudal peduncle of the juveniles. At the end of the experiment 10ml of blood were also collected using disposable heparinised sterile syringe and needle.

Haematological Analysis

Haematocrit: The blood taken was transferred in to heparinised capillary tube by suction pressure and one end sealed with cha-seal tube sealing compound (Medline). The tube was centrifuged for 5 minutes using a micro-haematerit centrifuge. Haematolcrit values were obtained using the micro-haemacrit tube reader or the packed cell volume was read by the use of rami haematocrit reader. The result was expressed in percentage. As described by the methods [8].
Plasma Volume: This was determined according to the method of Lamb [9] after determining the haematicrit, the

sample was used for the plasma volume determination (per 100ml of blood) which is the difference between the total volume of the blood centrifuged and the packed cell volume.

Haemaglobin Concentration (Hb): The cyanmthaemoglobin method of schalm, et al. [10] was used for this analysis. 0.02ml of well mixed blood was added to 4ml to modify Drabkins solution (a mixture of 250mg potassium ferricynanide, 200mg potassium cyanide and 50mg of potassium dihydrogenophosphate) and the volume was diluted to 1 litre with distilled water. The mixture was allowed to stand for 35 minutes and the haemoglobin concentration (GDL-1) was read photometrically by comparing with cynamethaemoglobin standard with yellow-green filler at 625mm.

Red and White Cells Counts: The blood cells counts were carried out by the use of new baurer haemocytometer as described by Kelly, et al. [11]. For red blood cell, blood sample collected was diluted, 1:200 with dacies fluid (a mixture of 99ml of 3% aqueous solution of sodium citrate and 1ml of 40% formaldehyde) which keeps and preserve the shape of the red blood cell. For white blood cell, the dilution was 1.20 using 3% aqueous solution of acetic acid to which gentia violet was added. 1ml of the sample was dropped on a microscope slide and labelled according to the diet treatments. Counting was done using binocular light microscope. Red blood cell and white blood cell counts was from x 106 DL-L respectively.

Mean cells volume (MCV)

The mean volume of red blood cells is termed the mean cells volume (um-3). This was determined according to the method used by Oyelese, et al. [12] as.

$$\mathrm{MCV}(\text{um-3}) = \frac{\text{haematocrit}}{\text{number of red blood cells per 100 ml blood}} \times 10$$

Mean cells haemoglobin (MCH)

This was calculated following the method used by Schalm, et al. [10] as:

$$\mathrm{MCV}(\mu\text{g-3}) = \frac{\text{haematocrit}}{\text{number of red blood cells per 100 ml blood}} \times 10$$

Blood Biochemical Analyses: Blood biochemical analysis was performed taking the portion of the collected blood and were dispensed into non-heparinised tubes. This was then centrifuged at 1,006 xg for five minutes to obtain the serum. Serum glucose concentrations were estimated based on glucose oxidase method as described by Schalm, et al. [10]. Serum total triglyceride concentrations were estimated based on enzymatic method as described by Schalm, et al. [10] using a commercially available kit (Randox Laboratory Limited, United Kingdom). Serum total protein concentrations were estimated based on Biuret method as described by Schalm, et al. [10] using an auto-analyzer (Bayer Express Plus, Model 15950, Germany). Aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were estimated as described in the Reference method by the International Federation of Clinical Chemistry as described by Schalm, et al. [10] using the same auto-analyzer as above. Alkaline phosphatase concentrations were estimated based on the enzymatic hydrolysis method as described by Schalm, et al. [10]. All the indices determined were measured using standard units: Creatinine in mg/dL, Sodium in mmol/L, Potassium in mmol/L, Chloride in mmol/L, Alanine Transferese (ALT) in U/L, Aspartate Transferese (AST) in U/L, Urea in mg/dL and Alkaline Phosphotase (ALP) in IU/Litre .

Results

Table 1 indicates the ingredient composition of experimental diet at 35% CP which was use in feeding the Juveniles and Brood stock. Maize was used as the source of carbohydrate in the feed and 3 ingredients were used as source of protein. Groundnut cake, Soybeans and Fish meal are the source of protein. The quantities were calculated using Pearson square method. Premix, Salt, Lysine, Methionine and Vitamin C were added respectively in the feed. Feed in T1 was used as control without addition of Cyprus escalentus while T2, T3, T4 and T5 feed were added a quantity of Cyprus escalentus 5g/kg, 10g/kg, 15g/kg and 20g/kg. The water quality parameter during the experimental period is shown in Table 2 and it indicates temperature, 24.50–27.80 o C; Dissolved Oxygen, 6.34–6.54 mg/l; pH, 6.20–7.40.

Ingredients (%)	GD1 (Control)
Fish Meal	30
Soybean Meal	33
Yellow Maize	30
Ginger	**0**
Dicalcium Phosphate	1
Vitamin / Premix	2
Methionine	0.5
Lysine	0.5
Palm Oil	1.5
Salt	0.5
Starch	1
Total	100

Table 1: Percentage Composition of Ingredients (g/100g diets) with Ginger Meal for the Feeding Trials Ingredients (%).

- **Keys**: GD1= Control (0g/100g of diet), GD2= 0.10g/100g of diet, GD3= 0.20g/100g of diet, GD4=0.30g/100g of diet, GD5= 0.40g/100g of diet

- **Vitamin and mineral mixture (product of HEPOMIX):** 12.000.000 IU Vitamin A; 2.000.000 IU Vitamin D3; 10g Vitamin E; 2g Vitamin K3; 1g Vitamin B1; 5g Vitamin B2; 1.5 g Vitamin B6; 10g Vitamin B12; 30g Nicotinic acid; 10g Pantothenic acid; 1g Folic acid; 50g Biotin; 250g Choline chloride 50%; 30g Iron; 10g copper; 50g Zinc; 60g Manganese; 1g Iodine; 0.1g Selenium and Cobalt 0.1g.

Haematological Parameters of *Clarias Gariepinus* Fed Ginger Additive Meal Diet

Table 3 presents the blood parameters of fish fed control diet (0%) and ginger additive meal diets (GD1(25%), GD2(50%), GD3(75%), and GD5(100%). The packed cell volume (PCV) ranged between 27% and 31%; white blood cell (WBC) ranged from 8.10 x 103 to 8.90 x 103; red blood cell (RBC) ranged between 3.00 x 106 and 8.70 x 106; Haemoglobin (Hb) has values range from 9.10g/dL to 11.00g/dL; mean corpuscular volume (MCV) values ranged from 32.10fL to 96.76fL; mean corpuscular haemoglobin (MCH) ranged between 11.10pg and 32.34pg and mean corpuscular haemoglobin concentration (MCHC) ranged from 33.46g/dL to 35.51g/dL respectively.

Biochemical Values of *Clarias gariepinus* Fed Ginger Diets

Table 4 presented the results of the biochemical analysis of the fish fed ginger diets. The highest value of glucose and the lowest value was recorded in GD2 (90.97) and GD1 (59.13) diet. The urea contents of samples ranges from 9.30 to 12.39. Creatine values were highest in GD2 with the value of 0.85 while the lowest value was in GD1 (0.44). Fish fed on GD3 had rather significantly (P<0.05) highest value of 179 while the fish fed diet GD1 was the lowest AST (93). Similarly, sodium, Na was recorded to be highest in GD5 (149) and lowest value in GD1 (72), the control diet. Significantly higher (P<0.05) chloride (Cl) was observed in fish fed on GD5 (113.00) compared to the control diet (GD1) (64.30) which is the lowest.

Parameter	Min
Temp ^{0}C	24.50+0.23
Dissolved Oxygen(mg/L)	6.34+0.22
Water pH	6.20+0.52

Table 2: Water Quality.

Parameter	GD1	GD2	GD3	GD4	GD5	SEM
Red Blood Cell	3.00± 0.100C	8.70± 100	3.36±. 15b	3.50 ±. 10b	3.40 ±.10b	0.07
White Blood Cell	8.50 ± 0.1a	8.90 ± .10a	8.30 ±. 10b	8.10 ±. 10c	8.10 ±. 10c	1.83
Haemoglobin	9.50 ± 0.10c	9.73 ±. 152c	11.00 ±. 10a	10.10 ±. 10b	9.10 ±. 10d	0.65
Packed Cell Volume	29.00± 1.00b	28.00 ± 1.00b	31.00 ±.1.00a	29.00 ±. 1.00bc	27.00 ± 00c	0.58
Mean Cell Volume	96.76 ± 5.60a	32.10 ± 0.80c	91.13 ±. 25a	82.90 ± 5.25bc	77.36 ± 3.49b	2.19
Mean Cell Haematocrit	32.30 ± 1.27a	11.10 ± 00d	32.34 ± 1.26a	28.83 ± 1.15b	26.70 ± 0.50	0.57
Mean Cell Haematocrit Concentration	33.46 ± .81ab	34.64 ± .88ab	35.51 ± 1.47ab	34.84 ± .85ab	33.72 ± .88ab	0.58

Mean ± Std on the same row with different superscripts are significantly different (P<0.05).
Table 3: Some Haematological Parameters of *Clarias gariepinus* fed Ginger Additive Meal Diets.

Parameters	Gd1	Gd2	Gd3	Gd4	Gd5
Serum Protein	2.60 ±0.00^b	3.43 ±0.43^a	2.38 ±0.55^b	3.04 ±0.13^{ab}	2.35 ±0.38^b
Glucose	59.13 ±5.71^e	90.97 ±0.48^a	64.04 ±0.44^d	76.45 ±1.30^b	69.32 ±0.26^c
Trigyceride	0.18 ±0.01^a	0.03 ±0.01^c	0.08 ±0.01^b	0.05 ±0.01^c	0.07 ±0.02^b
Aspertate Transferase	93.00 ±1.00^e	126.00 ±1.00^d	179.00 ±1.00^a	164.00 ±1.00^c	169.00 ±1.00^b
Alanine Transferase	72.00 ±1.00^d	79.00 ±1.00^b	74.00 ±1.00^c	90.00 ±1.00^a	79.00 ±1.00^b

Alkaline Phosphatase	174.00 ±1.00^{a}	21.00 ±1.00^{d}	25.43 ±4.31^{c}	30.53 ±1.20^{b}	23.43 ±1.05^{cd}
Urea	9.30 ±0.90^{b}	12.39 ±0.93^{a}	9.36 ±0.90^{b}	12.24 ±0.57^{a}	11.22 ±0.77^{a}
Creatinine	0.44 ±0.07^{c}	0.85 ±0.08^{a}	0.66 ±0.10^{b}	0.72 ±0.07^{ab}	0.62 ±0.11^{b}
Sodium	72.00 ±1.00^{e}	109.00 ±1.00^{d}	111.00 ±1.00^{c}	140.00 ±1.00^{b}	149.00 ±1.00^{a}
Potassium	2.00 ±0.10^{c}	2.52 ±0.46^{bc}	3.78 ±0.84^{a}	3.19 ±0.21^{ab}	3.00 ±0.01^{ab}
Chlorine	64.30 ±2.26^{d}	81.60 ±0.95^{c}	9.68 ±0.88^{e}	104.00 ±1.00^{b}	113.00 ±1.00^{a}

Mean ± Std on the same row with different superscripts are significantly different (P<0.05).
Table 4: Blood Biochemical values of *Clarias gariepinus* fed Ginger Additive Diets.

Discussion

Water Quality

The water quality parameters of the experimental tanks, temperature, dissolved oxygen and pH were closely related. The highest temperature was recorded in 75% inclusion while the least value was recorded in control. The temperature, dissolved oxygen and pH measured during the experiment were within recommended limits for warm water fishes (Hogendoorn et al reported that the optimum temperature for the growth of small C. gariepinus between (0.5 – 5 g) was 30oC and for large (25g) was 25˚C. Olaifa, et al. [13] reported 25˚C, 6.4mg/l and 7.10 for temperature, dissolved oxygen and pH respectively for C. gariepinus juveniles. Also, Olaifa, et al. [14] reported 25˚C -26˚C, 6 -7mg/l and 7.0 for temperature, dissolved oxygen and pH respectively for C. gariepinus juveniles. From the result obtained ginger, garlic, onion bulb and black cumin could be used in aquaculture as they did not significantly alter the water quality.

In this present study PVC values increased from initial value of 29.00% in fish fed the control diet (T1) to final value of 31.00% in fish fed diet 3 (T3) which agrees with Korzhuev et al who stated that fish haematocrit values ranged between 20% and 35%. The results obtained falls within the range of 20% and 50% in agreement with Etim, et al. [15] who also stated that PCV values above 50% are rarely reported. Increased PCV results to an increased primary and secondary polycythemia however, Etim, et al. [15] 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. The red blood cell (RBCs) increased from the initial value of 3.00% in the fish fed control diet (T1) to the final value of 8.70% in fish fed 25% ginger additive diet (T2). These values were higher than 1.9x1012L-1 reported for *Clarias gariepinus* juveniles [16]. The final white blood cell counts (WBC) of C.gariepinus fed ginger 25% additive diets is 8.90 in fish fed 100% ginger based diet (T5) and were generally higher and significantly different from the initial WBC count (8.50;T1). Ayoola, et al. [17] stated that increasing or decreasing numbers of WBCs are normal physiological reactions to treatments and these shows the response of the immune system under toxic conditions.

However, Soetan, et al. [16] and Alkahem, et al. [18], 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 [19]. 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 control diet (T1), gave the highest volume (96.76Fl) while the fish fed 25% ginger additive diet 2 (T2) had the least (32.10Fl) MCV value. These values are lower when compared to 79.20-105.32µg/ml reported for Heteroclarias [20]. This decrease may be attributed to the non-swelling of the RBCs as a result of high oxygen condition and impaired water balance in fishes exposed to metal pollution [21]. Mean corpuscular haemoglobin (MCH) values were significantly different. The highest value (32.34) was recorded in fish fed 50% ginger additive diet (T3) while the least (11.10) was recorded in fish fed 25% ginger additive diet (T2). These values agree with Olasunkanmi, et al. [22] 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) (33.46g/dl, 33.72g/dl, 34.64g/dl, 34.84g/ dl and 35.51g/ dl) compares fairly well with 33.97% recorded by Adeyemo, et al. [23] and values ranging between 28.75 and 37.62% recorded for fish fed M. oleifera leaf meal-based diet) [24].

The biochemical values of the additive treated fish was relatively higher in additives compared to control diet which strengthen the study of Onu and Aja et al who found that natural additives inclusion slightly increase total protein and albumin concentration in animals. This result was in agreement with Onu, et al. who found higher serum protein concentration in ginger and mixture of

ginger supplementation group compared to control in an experiment. Inclusion of additives significantly increase the creatinine level and increase in blood urea level in additives treated diets group; and this come to the agreement with the study of Onu and Aja, who found increased creatinine and urea in additive inclusion diet meal.

Conclusion

Research study were conducted for 12 weeks to examine the biochemical and haematological performances of *Clarias gariepinus* fed inclusion levels of (25%, 50%, 75%, and 100%) of ginger (Zingiber officinale). The biochemical contents of the ginger additives meal diets were sigificantly high compared to the control diets. The additives showed good haematological and biochemical profiles. There were significant difference ($p<0.05$) in fish fed the control and ginger additive diets. And this work has revealed that ginger additive diets has nutritive values, which can be compared with other natural additives, they have good nutrient, haematological and biochemical properties; therefore *Clarias gariepinus* fingerlings can be fed on ginger additive diet without any negative effects to the fish's growth, nutrient utilization and health. This study has also shown the possibility of inclusion of ginger additive meal diets in the formulation of fish feed and that this meal can be use as additive in fish feed.

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