

Growth, Hematology and Immuno-Modulatory Potential of Ginger (*Zingiber officinale*) Supplemented Diets in *Clarias gariepinus* Juvenile (Burchell, 1822)

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Abstract

A-70 day study was conducted to investigate the growth, hematology and immune-modulatory effect of ginger supplemented diets in *Clarias gariepinus*. Ginger was added to the diet of fish at 0% (control), 0.5% (T1), 1.0% (T2), 1.5% (T3) and 2.0% (T4). Fish fed 1.0% (T2) ginger had best growth performance with final weight (75.07±2.97g), weight gain (49.98±2.96g), Specific growth rate (SGR) (5.58±0.08g) and protein efficiency ratio (PER) (1.31±0.08) which was significantly different ($P<0.05$) from others while T4 (2.0%) showed the least growth performance; final weight (37.47±5.52g), weight gain (12.39±5.54g), SGR (3.22±0.78g) and PER (0.32±0.14). Study on HSI showed that all treated fish groups had stable liver conditions and did not differ significantly ($P>0.05$) when compared to the control. T2 (1.0% ginger) had best haematological profile. Result on red cell indices revealed that Mean cell haemoglobin concentration and mean cell hemoglobin were not significantly different ($P>0.05$) among fish groups while mean cell volume differed significantly ($P<0.05$). Result on immunological study (leukocyte differentials) revealed insignificant changes ($P>0.05$) in the values of all parameters examined with reference to the control. Phytochemical screening of ginger showed that it contains good amount of minerals (sodium, potassium, magnesium, calcium, phosphorus, iron, zinc, copper and manganese) and anti-nutrients (tannin, oxalate, phytate and saponin). *C. gariepinus* fed 1.0% dietary inclusion level of ginger had better growth and haematological profile.

Introduction

Previous report has it that about one billion people world-wide rely on fish as their primary source of animal protein (Iheanacho *et al.*, 2017a). The consumption and demand for fish as a cheap source of animal protein is increasing in Africa. In most countries, vast majority of the fish supply comes from the rivers as captured fisheries (FAO, 2014), and may not be able to meet the growing global demand for aquatic foods. Hence, there is a need for a viable alternative fish production system that can sufficiently meet this demand, and aquaculture is the solution (Iheanacho *et al.*, 2017b). As aquaculture

production becomes more intensive in Nigeria, fish feed will be a significant factor in increasing the productivity and profitability of aquaculture (Akinrotimi, Gabriel, Owhonda, Onukwo, & Opara, 2007).

Nutrition plays an important role in intensive fish production (aquaculture) depending on the type of feed fed, its availability and cost. Nutritional status has been increasingly acknowledged as a crucial factor in host defence against pathogens (Iheanacho *et al.*, 2017a). As such, use of feed supplements aiming to improve not only the growth but also the health of aquaculture species has gained widespread interest and acceptance (Iheanacho *et al.*, 2017a).

Extensive use of antibiotics and biocides in aquaculture leads to the emergence of antibiotic-resistant bacteria and generation of toxicants which may cause risks to the environment (Reveter, Bontemps, Lecchini, Banaigs, & Sasal, 2014). To alleviate these problems, attention is being paid to the use of herbal plant such as ginger for disease-control strategies in aquaculture, due to its efficacy in combating infectious diseases by increasing the non-specific and specific immune mechanisms (Harikrishnan, Balasundaram, & Heo, 2011; Iheanacho *et al.*, 2017c), thus contain natural organic materials which does not pose any threat to fish health, the environment or to human health (Maqsood, Singh, Samoon, & Munir, 2011). Ginger (*Zingiber officinalis*, Roscoe), is a medicinal herbal plant and considered safe for consumption (Weidner & Sigwart, 2000). Ginger contains alkaloids, flavonoids, polyphenols, saponin, steroids, tannin, fibre, carbohydrate, vitamins, carotenoids and minerals (Otunola, Oloyede, Oladiji, & Afolayan, 2010; Shirin & Prakash, 2010). Chari, Manasa, Srinivas, and Sowbhagya (2013) report that ginger also contains natural antioxidants as gingerols, shogaols and zingerone. Ginger were reported by several authors to be effective as an immune-modulatory agent in animals and fish and help to reduce the losses caused by disease in aquaculture (Ali, Blunden, Tanira, & Nemmar, 2008; Nya & Austin, 2009; Bellik, 2014). Kumar, Chelladurai, Veni, Peeran, and Mohanij (2014) report significant increase in growth indices (final weight, specific growth rate and weight gain), haematology (erythrocyte count, haematocrit and haemoglobin) and some immunological parameters in Indian catfish (*Mystus montanus*) fed ginger supplemented diet against *Aeromonas hydrophila* infection. Harikrishnan, *et al.* (2010) assessed the efficacy of herb enhanced diet on *A. hydrophila* infected fish (*Carassius auratus*) and reported that herb enhanced diet triggered innate immune system response against *A. hydrophila* infection and restored normal haematological stability in fish.

The present study investigated the effect of ginger supplemented diet on growth performance, haematology and immune-modulatory potentials in *Clarias gariepinus* juvenile.

Materials and Methods

Experimental Fish

One hundred and fifty (150) juveniles of *Clarias gariepinus* were procured from Regina Pacies fish farm in Abakaliki and transported in 50 litre gallon half filled with water from the farm to laboratory for acclimatization. They were acclimatized for two

weeks in a concrete pond (4m x 3m x 1.5m) and were fed with commercial feed (Coppens 4mm, Netherland) throughout the acclimation period. The fish were starved for 24 hours prior to the commencement of experiment.

Collection and Processing of Ginger (*Zingiber officinale*)

Fresh ginger rhizomes (yellow) were purchased from the Abakpa main market Abakaliki, Ebonyi state. They were washed properly to remove mud on the surface and neatly peeled. They were cut into small sizes (to aid drying) and sun-dried for one week. They were milled at the Abakpa main market and sieved with a hand sieve and stored in an airtight container. Phytochemical analysis of ginger was done at the International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State Nigeria.

Experimental Diets

Five (5) iso-nitrogenous diets were formulated to contain 37% crude protein. Ginger in powdered form was added to the formulated fish diets at 0% (T0) as control diet, 0.5% (T1), 1.0% (T2), 1.5% (T3) and 2.0% (T4) (Table 1). Pearson square method was used in the formulation of fish diet (Pearson, 1976). Feed ingredients used for the experimental diet include: ginger, soya bean meal (SBM), maize meal (MM), fish meal (FM), vitamin/mineral premix, oil, starch (binder) and salt. The formulation was based on the percentage composition of the ingredient (Table 1). The ingredients for each diet were weighed and mixed thoroughly in a bowl, water was then added to the mixture and stirred until it gelatinized and it was pelletized using fabricated electric pelleting machine in the feed mill unit of Ebonyi state university Abakaliki. The moist pellets were sun-dried on a black surface, packaged into air-tight containers and stored at room temperature. Formulated diet samples (10g) were analyzed following the procedures of A.O.A.C (2005).

Experimental Design

A total of 150 *Clarias gariepinus* juveniles (average weight 25.08±0.02) were randomly assigned to five test diets containing ginger at the following percentage inclusion levels ; 0% coded as T1, 0.5 % (T2), 1.0% (T3), 1.5% (T4) and 2.0% (T5). Fish specimens were distributed into fifteen (15) wooden vat tanks (1m x 1m x 1m) at ten fish per tank in three replicates in a completely randomized design (CRD). Fish were fed with test diets twice daily (9.00am and 4.00pm) at 5% body weight each day for 70 days. The

Table 1. Gross composition of experimental diets (g/2000g) containing ginger fed to *Clarias gariepinus* juvenile

Ingredient	T0 (0%)	T1 (0.5%)	T2 (1.0%)	T3 (1.5%)	T4 (2.0%)
FM	745	745	745	745	745
SBM	455	455	455	455	455
MM	600	590	580	570	560
Ginger	-	10	20	30	40
BM	40	40	40	40	40
Vit. Premix	50	50	50	50	50
Methionine	15	15	15	15	15
Lysine	15	15	15	15	15
Starch	50	50	50	50	50
Vegetable Oil	20	20	20	20	20
Salt	10	10	10	10	10

FM- Fish Meal, SBM- Soybean Meal, MM- Maize Meal, BM- Bone Meal, Vit-premix- Vitamin Premix.

Fish were monitored daily for abnormal behaviours and mortality. Total fish weight in each tank was determined fourth nightly and the amount of diet was adjusted according to the new weight.

Estimation of Growth Parameters

At the end of the experiments, the final weight of the fish was measured using a sensitive weighing scale (S. smettler, China) and length using a plastic meter rule to determine the growth response of the fish, the following parameters were calculated (Iheanacho *et al.*, 2017b):

Mean Weight Gain (g) (MWG)

$$MWG = \frac{WT_2 - WT_1}{N} \quad (1)$$

Where

Wt₁ = initial mean weight of fish at time T1

Wt₂ = final mean weight of fish at time T2

N = Number of days

Protein Efficiency Ratio (PER)

$$PER = \frac{\text{fish Weight Gain (g)}}{\text{Protein intake (g)}} \quad (2)$$

Where;

$$\text{Protein intake} = \frac{\% \text{ protein in feed} \times \text{total diet consumed}}{100}$$

Specific Growth Rate (SGR)

$$SGR = \frac{100 (\text{Log}_e W_f - \text{Log}_e W_i)}{\text{Time (days)}} \quad (3)$$

Wf = Final average weight at the end of the

experiment

Wi = Initial average weight at the beginning of the experiment

Loge = Natural Logarithm reading

Survival Rate (%)

$$\text{Survival Rate (\%)} = \frac{\text{Number of fish that survived} \times 100}{\text{Total number of fish stocked}} \quad (4)$$

Condition Factor (k)

$$\frac{100W}{L^3} \quad (5)$$

W = final mean body weight (g)

L = standard length (cm)

Hepato-Somatic Index

$$\frac{\text{Liver weight (g)} \times 100}{\text{Total fish weight (g)}^{-1}} \quad (6) \quad (\text{Ogunji } et al., 2008)$$

Water Quality Analysis (Water Parameters)

Physico-chemical characteristics of experimental tank water were closely monitored and measured daily throughout the duration of the study. Water in experimental tanks was siphoned out and replaced with fresh water every three days to remove faecal waste and maintain stable water quality. The following parameters were tested for; water temperature using a thermometer, hydrogen ion concentration (pH), ammonia, nitrate, nitrite and dissolved oxygen (DO) using a water testing kit (PRO KIT™, Florida) following the manufacturer's instructions.

Haematology

Three fish per replicate were sampled for blood collection for haematological analysis. Haematological analysis was carried out at the Department of Federal Teaching Hospital Abakaliki, Ebonyi state. Blood samples were collected at the end of the experiment from the caudal vein into an EDTA lithium tubes. According to Houston (1990), red blood cell (RBC) and white blood cell (WBC) counts were counted manually by haemocytometry using Neubauer haemocytometer after diluting blood samples by adding of Hayem solution for RBC and Turk solution for WBC, respectively. Haematocrit was measured with microcentrifuge method, using standard heparinied microhaematocrit capillary tubes (75 mm at 7000 g for 15 min). Haemoglobin content (%) was measured spectrophotometrically at 540 nm with cyanmethenoglobin method (Dacie & Lewis, 2011). Red cell indices (mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration) was calculated according to the method described by (Dacie & Lewis, 2011).

Statistical Analysis

Values obtained from each parameter after the experiment were subjected to one-way analysis of variance (ANOVA), using SPSS (Statistical package for

Social Science 2006, version 22). Duncan multiple range test (DMRT) was used to compare the differences between means at ($P < 0.05$). Data were presented as mean \pm SE.

Results

Phytochemical Constituents of Ginger (*Zingiber officinale*)

From the results below (Table 2) yellow ginger was found to contain some macro and micro minerals such as Na, P, Mg, Ca, P, Fe, Zn, Cu and Mn with their respective values. It also contain some anti-nutrients such as phytate, oxalate, tannin and saponin

Proximate Composition of Ginger and Diets.

The results for proximate composition of the experimental diet (Table 3) shows that ginger contains 8.93% crude protein, 3.13% crude fat, 7.60% crude fibre, 5.81% ash and 10.65% moisture. T1 had the least crude protein of 37.93 ± 0.03 and the highest was recorded in T0 40.13 ± 0.03 .

Physico-Chemical Characteristics

Physico-chemical parameters for water such as temperature, dissolved oxygen, pH, ammonia, nitrate

Table 2. Phytochemical constituents of powdered yellow ginger

Minerals	Values (%)	Minerals	Values (mg/kg)	Phytochemical compounds	Values (%)
Sodium (Na)	0.06	Iron (Fe)	25.02	Phytate	0.180
Potassium (K)	1.70	Zinc (Zn)	36.30	Oxalate	0.150
Magnesium (Mg)	0.19	Copper (Cu)	5.10	Tannin	0.003
Calcium (Ca)	0.08	Manganese (Mn)	12.70	Saponin	0.250
Phosphorus (P)	0.12				

Table 3. Proximate Composition (%) of ginger and experimental diets

Parameters	Ginger	Control	T1(0.5%)	T2(1.0%)	T3(1.5%)	T4(2.0%)
Crude protein	8.93	40.13 ± 0.03^a	37.93 ± 0.03^d	38.13 ± 0.03^c	38.02 ± 0.03^d	38.34 ± 0.03^b
Crude fat	3.13	3.48 ± 0.01^a	3.37 ± 0.01^c	3.44 ± 0.12^b	3.38 ± 0.01^c	3.41 ± 0.01^b
Crude fibre	7.60	6.98 ± 0.08^{ab}	7.12 ± 0.09^a	7.03 ± 0.04^{ab}	7.00 ± 0.03^{ab}	6.90 ± 0.01^b
Ash	5.81	2.67 ± 0.03^{ab}	2.72 ± 0.02^{ab}	2.74 ± 0.01^a	2.70 ± 0.04^{ab}	2.63 ± 0.02^b
Moisture	10.65	9.19 ± 0.02^a	9.23 ± 0.03^a	9.27 ± 0.02^a	9.24 ± 0.12^a	9.16 ± 0.02^a
NFE	63.88	37.55 ± 0.12^b	39.63 ± 0.06^a	39.39 ± 0.09^a	39.65 ± 0.12^a	39.56 ± 0.04^a
Dry matter	89.35	90.80 ± 0.02^a	90.77 ± 0.04^a	90.72 ± 0.02^a	90.76 ± 0.06^a	90.84 ± 0.03^a
Gross energy (KJ)	14.60	17.56 ± 0.02^a	17.36 ± 0.02^c	17.39 ± 0.01^c	17.38 ± 0.03^c	17.46 ± 0.11^b

Means among rows with different superscripts are significantly different. NFE-Nitrogen free extract, NFE=100- (%crude protein + %fat + %crude fibre + % ash). Dry matter (DM) = $100 - (\%Crude\ protein + \%Fat + \%Crude\ fibre + \%Ash + NFE)$. Calculated by; crude protein = $23.9Kj/g$, crude fat = $39.8Kj/g$, NFE = $17.6Kj/g$ (Schulz, Kloas, Wirth, & Rennert, 2005).

and nitrite were measured (Table 4). Apart from ammonia, there was no significant difference in all the parameters measured at ($P>0.05$).

Growth Parameters and Feed Utilization

The mean weight gain amongst the various treatments showed that there was significant difference at ($P<0.05$). The values for SGR and PER recorded showed that there was significant difference among the different treatments. There was no significant difference ($P>0.05$) for condition factor and survival rates among the various treatments. The liver weight of T2 was significantly different at ($P<0.05$) from that of T1 (control) but was not different significantly ($P>0.05$) from other treatment groups. There was no significant difference ($P>0.05$) from the values recorded for hepatosomatic index of the various fish from all the treatments (Table 5) below.

Haematology

Data on haematological response of *C. gariepinus* fed ginger supplemented diets are presented in Table 6. Insignificant changes ($P>0.05$) in RBC, Hb, WBC and PLT values were observed among ginger treated groups compared to the control. Result on red cell indices revealed insignificant changes in MCHC and MCH values among ginger treated groups compared to the control while significant difference

was observed in MCV in treated fish compared to the control.

Leukocyte Differentials

Table 7 contains result on leukocyte differentials of *C. gariepinus* fed ginger supplemented diets. There were no significant differences ($P>0.05$) in all the parameters examined in treated fish groups compared to the control.

Discussion

Plant extracts have been reported to favour various activities like antistress, growth promotion, appetite stimulation, enhancement of tonicity and immune stimulation, maturation of culture species and antipathogen properties in fish and shrimp aquaculture (Reveter *et al.*, 2014). Bioactive compounds present in ginger (gingerol, shogaols and 6-paradol) (Chari, *et al.*, 2013) may be attributed to be the reason why ginger is so effective against microbial infections (Romero, Carmen, & Paola, 2012). The values for mineral elements such as phosphorus (0.12mg), sodium (0.06mg), potassium (1.70mg) and calcium (0.08mg) are lower than the values reported by Iheanacho *et al.* (2017d) for phosphorus (0.27mg), sodium (1.24 mg), potassium (1.98 mg) and calcium (1.50 m). Adanlawo and Dairo (2007) reported the following values; phosphorus

Table 4. Physico-chemical parameters of water

Parameters	Control	T1(0.5%)	T2(1.0%)	T3(1.5%)	T4 (2.0%)
Temperature ($^{\circ}$ C)	26.53 \pm 0.09 ^a	26.70 \pm 0.15 ^a	26.63 \pm 0.09 ^a	26.63 \pm 0.07 ^a	26.53 \pm 0.03 ^a
DO (mg/l)	6.63 \pm 0.78 ^a	6.50 \pm 0.87 ^a	6.50 \pm 0.87 ^a	6.40 \pm 0.92 ^a	6.40 \pm 0.92 ^a
Ph	7.50 \pm 0.00 ^a	7.50 \pm 0.00 ^a	7.57 \pm 0.03 ^a	7.57 \pm 0.03 ^a	7.57 \pm 0.03 ^a
Ammonia(mg/l)	0.02 \pm 0.01 ^b	0.03 \pm 0.01 ^b	0.03 \pm 0.01 ^b	0.07 \pm 0.01 ^a	0.07 \pm 0.01 ^a
Nitrate(mg/l)	0.01 \pm 0.01 ^a	0.03 \pm 0.01 ^a	0.05 \pm 0.02 ^a	0.07 \pm 0.02 ^a	0.07 \pm 0.02 ^a
Nitrite(mg/l)	0.03 \pm 0.01 ^a	0.04 \pm 0.02 ^a	0.30 \pm 0.23 ^a	0.10 \pm 0.03 ^a	0.10 \pm 0.03 ^a

Means among rows with different superscripts are significantly different at ($P<0.05$).

Table 5. Growth performance of *C. gariepinus* juvenile fed ginger supplemented diets

Parameters	Control	T1 (0.5%)	T2 (1.0%)	T3 (1.5%)	T4 (2.0%)
Initial weight (g)	25.08 \pm 0.02 ^a	25.06 \pm 0.03 ^a	25.09 \pm 0.01 ^a	25.11 \pm 0.01 ^a	25.08 \pm 0.02 ^a
Final Weight (g)	61.77 \pm 1.13 ^{ab}	46.77 \pm 4.97 ^{bc}	75.07 \pm 2.97 ^a	47.27 \pm 7.20 ^{bc}	37.47 \pm 5.52 ^c
Weight gain (g)	36.69 \pm 1.14 ^{ab}	21.71 \pm 4.97 ^{bc}	49.98 \pm 2.96 ^a	22.16 \pm 7.21 ^{bc}	12.39 \pm 5.54 ^c
SGR (%/week)	5.15 \pm 0.04 ^a	4.32 \pm 0.35 ^{ab}	5.58 \pm 0.08 ^a	4.19 \pm 0.63 ^{ab}	3.22 \pm 0.78 ^b
PER (%)	0.91 \pm 0.03 ^{ab}	0.57 \pm 0.13 ^{bc}	1.31 \pm 0.08 ^a	0.58 \pm 0.19 ^{bc}	0.32 \pm 0.14 ^c
Condition factor (K)	0.62 \pm 0.03 ^a	0.45 \pm 0.06 ^a	0.72 \pm 0.08 ^a	0.64 \pm 0.09 ^a	0.54 \pm 0.13 ^a
Liver weight (g)	0.30 \pm 0.03 ^{ab}	0.23 \pm 0.01 ^b	0.37 \pm 0.07 ^a	0.26 \pm 0.03 ^{ab}	0.26 \pm 0.04 ^{ab}
Hepatosomatic Index (%)	0.49 \pm 0.06 ^a	0.49 \pm 0.03 ^a	0.61 \pm 0.05 ^a	0.58 \pm 0.09 ^a	0.70 \pm 0.07 ^a
Survival rate (%)	70.00 \pm 10.00 ^a	86.67 \pm 3.33 ^a	76.67 \pm 6.67 ^a	63.33 \pm 12.02 ^a	70.00 \pm 5.77 ^a

Means within rows with different superscripts are significantly different ($P<0.05$)

SGR= Specific growth rate, PER=Protein efficiency ratio

Table 6. Hematological profile of *C. gariepinus* juvenile fed ginger supplemented diets

Parameters	Control	T1 (0.5%)	T2 (1.00%)	T3 (1.50%)	T4 (2.00%)
RBC ($\times 10^{12/L}$)	3.65 \pm 0.03 ^a	3.75 \pm 0.18 ^a	3.82 \pm 0.21 ^a	3.59 \pm 0.04 ^a	3.60 \pm 0.01 ^a
WBC ($\times 10^9/L$)	5.13 \pm 0.087 ^a	6.33 \pm 0.52 ^a	6.17 \pm 0.58 ^a	6.47 \pm 0.41 ^a	6.27 \pm 0.22 ^a
Hb (g-dL ⁻¹)	9.63 \pm 0.32 ^a	10.67 \pm 0.72 ^a	11.03 \pm 0.67 ^a	10.27 \pm 0.47 ^a	9.63 \pm 0.29 ^a
HcT (%)	30.00 \pm 1.15 ^{ab}	32.00 \pm 1.53 ^{ab}	34.67 \pm 2.40 ^a	30.00 \pm 1.15 ^{ab}	29.00 \pm 0.58 ^b
PLT (%)	140.67 \pm 0.88 ^a	135.67 \pm 10.65 ^a	170.67 \pm 27.64 ^a	156.00 \pm 24.00 ^a	148.33 \pm 22.38 ^a
MCV (fL)	83.03 \pm 1.91 ^{ab}	82.87 \pm 1.49 ^{ab}	85.43 \pm 1.31 ^a	81.53 \pm 2.95 ^{ab}	78.87 \pm 0.72 ^b
MCH (pg)	26.43 \pm 0.61 ^a	27.60 \pm 1.21 ^a	28.50 \pm 0.95 ^a	28.00 \pm 1.07 ^a	26.47 \pm 0.54 ^a
MCHC (%)	32.17 \pm 0.15 ^a	33.17 \pm 1.02 ^a	33.37 \pm 0.72 ^a	33.47 \pm 0.58 ^a	32.87 \pm 0.27 ^a

Means within rows with different superscripts are significantly different (P<0.05).

Table 7. Leucocyte differentials (%) of *C. gariepinus* fed ginger supplemented diets

Parameters	Control	T1(0.5%)	T2 (1.0%)	T3 (1.5%)	T4 (2.0%)
Neutrophils	40.33 \pm 0.88 ^a	39.67 \pm 2.03 ^a	38.00 \pm 3.10 ^a	40.67 \pm 2.03 ^a	38.67 \pm 5.24 ^a
Lymphocytes	53.33 \pm 1.45 ^a	55.00 \pm 3.51 ^a	61.33 \pm 1.76 ^a	54.00 \pm 3.21 ^a	59.33 \pm 0.88 ^a
Basophils	0.00 \pm 0.00 ^a	0.33 \pm 0.33 ^a			
Monocytes	1.00 \pm 0.60 ^a	1.33 \pm 0.33 ^a			
Eosinophils	3.00 \pm 0.60 ^a	2.67 \pm 0.33 ^a	3.67 \pm 0.88 ^a	3.33 \pm 1.45 ^a	2.67 \pm 0.88 ^a

Means within rows with different superscripts are significantly different (P<0.05).

(25.70mg), sodium (40.96 mg), potassium (37.34mg), calcium (35.66mg). The present study also revealed that ginger contains magnesium (0.19mg), iron (25.02mg), copper (5.1mg), zinc (36.3mg) and manganese (12.3mg). The current findings differs from the report of Iheanacho *et al.* (2017d) who reported higher values for magnesium (0.47mg) and iron (45.01mg) but reported a lower value for zinc (5.55mg). The reasons for these differences may however be due to changes in agronomic conditions, curing methods, preparation and storage methods as reported by Iheanacho *et al.* (2017d).

The physico-chemical parameters of experimental tank water were observed to be within the range recommended for fresh water fish culture (Iheanacho *et al.*, 2017b). Bhatnagar *et al.* (2004) recommended 25-30°C for culture of *C. gariepinus*. Dissolve Oxygen level greater than 5mg/l is essential to support good fish production. Bhatnagar *et al.* (2004) also suggested that 1-3mg/l has sub lethal effect on growth and feed utilization. Authors further reported 0.3-0.8mg/l to be lethal to fishes and oxygen concentration above 14mg/l is lethal to fish fry, and gas bubble disease may occur. Santhosh and Singh (2007) reported that the ideal pH level is between 7.5 and 8.5 and any value above and below this could be stressful to the fishes.

The findings of the present study on growth performance revealed that *C. gariepinus* juvenile fed with diets which had maize replaced with graded levels of ginger accepted and utilized the nutrients present in the treated diets when compared to the

control (Table 6). Highest values for final weight and weight gain were observed in T2 (1.0%) ginger which was higher than that of control. These values were higher than that of T4 (2.0%) where the least growth values in terms of final weight and weight gain was observed. The same trend was observed for SGR and PER (Table 6). Iheanacho *et al.* (2017a) reported significant increases in weight gain, specific growth rate and final weight when *C. gariepinus* juvenile were exposed to varying concentrations (0.25, 0.50, 0.75 and 1.0 g/35L) of ginger as compared to the control. Adewole (2014) also reported significant increases in growth parameters (final weight, weight gain, specific rate and relative growth rate) in *C. gariepinus* fed roselle supplemented diets when compared with the control. The positive response to growth in treated fish especially at 1.0 g inclusion level of ginger could be attributed to the high proximate content of ginger (Table 3). Ginger is a good source of carbohydrate, mineral elements, vitamins and contains good number of phytochemical constituent that enhance growth and health of animals (Iheanacho *et al.*, 2017a).

Condition factor is an index which compares the health of a fish and is based on the hypothesis that heavier fish of a given length are in better condition (Bagenal & Tesch, 1978). HSI is associated with the liver energetic reserve. High HSI value implies large amount of food availability and favourable conditions (Ogunji, Toor, Schulz, & Kloas, 2008). Highest values for condition factor and hepatosomatic index was observed in T2 (1.0%) and T4 (2.0%) respectively

(Table 5). The HSI values (Table 5) reported in this study did not differ significantly between treatment groups compared to the control. This signifies that the liver conditions were stable; however it also implies that there was less fat deposition in the liver. Ogunji *et al.* (2008) reported HSI values ranging from 3.08g – 3.14% when they used housefly maggot meal to replace fish meal in diets fed to *Oreochromis niloticus*, but no significant difference between treated groups and the control. Afuang, Siddhuraju and Becker (2003) fed *O. niloticus* (initial weights of 15.5g–17.0 g) with varying amounts and extracts of Moringa (*Moringa oleifera*) leaf meals to replace fish meal, they reported HSI values ranging from 1.5g to 2.7%. They attributed this to correlation with body lipid incorporation which was influenced by dietary nutrient intake and availability. Highest HSI value in the present study was observed in fish fed 2.0% ginger (T4) and was closely followed by fish fed diet T2 (1.0% ginger).

Haematological parameters are important health indicators whose study reveals the health conditions of fish regarding diseases and immune system conditions before and after an experiment (Iheanacho *et al.*, 2017b; Ogueji *et al.*, 2017). In feeding of *C. gariepinus* juvenile with ginger, Iheanacho *et al.* (2017b) reported significant difference for blood parameters among treatment groups. In the present study, highest values for RBC, Hb, PLT and HcT were seen in fish group that was fed 1.0% ginger treatment compared to other treatments and the control (Table 6). Increases in the aforementioned blood parameters may be attributed to ginger that was added to the fish diet. Ginger contains good amount of phytochemical constituents (Table 2) and aromatic compounds (Shirin & Prakash, 2010; Bellik, 2014) capable of stimulating antioxidant activities and immune functions in fish. Jeorg and Lee (1998) state that elevations in some haematological indices indicate immune system stimulation and efficient function of organs (thymus, spleen and bone marrow) responsible for blood cell formation. The reason for the decrease in HcT level observed in T4 is not known. Khalil and El-houseny (2013) reported enhanced immunity in fish, cultured in water with 20 mg/l of ginger for 15 days. Antache *et al.* (2014) also observed an increase in pack cell volume, haemoglobin and erythrocyte count when ginger supplemented diet and other phytobiotics were fed to *Oreochromis niloticus*. This study is in agreement with findings of Haghighi and Mostafa (2013), who reported significant difference ($p < 0.05$) for immune-stimulatory effect and increased RBC, HcT and WBC values above in fish fed ginger based diets compared to the control.

Red cell indices are vital blood parameters as

they help in diagnosis of some blood related diseases such as anaemia in most animals including fish (Dacie & Lewis, 2011). Macrocytic and microcytic anaemia may be resultant effect of significant increase or decrease in red cell indices (Dacie & Lewis, 2011). This study shows that MCV differed significantly, with the highest value observed in T2 and the least value in T4 (Table 6). MCH and MCHC showed no significance amongst the treatment groups and control. Findings of the present study suggest normocytic type of anaemia in cultured fish and suggest that ginger had no adverse effect on fish. Zomraw, Abdel, Dousaaa and Mahala (2012) fed broiler chicks with ginger supplemented diet levels (0.5, 1.0 and 1.5%). They reported no significant difference ($P > 0.05$) for MCV and MCH.

Leukocyte differentials (Lymphocytes, neutrophils, monocytes, basophils and Eosinophils) are sensitive biomarkers of stress which reveal information on the immune condition of organisms (Jeney, 2017). Insignificant changes observed in all the parameters (leukocyte differentials) examined across fish groups indicate that ginger had no toxic effect or oxidative stress tendency in fish. The findings of the present study validate the reports of previous studies on the immune-modulatory potentials of ginger in rainbow trout (*Oncorhynchus mykiss*) (Nya & Austin, 2009; Haghighi & Mostafa, 2013), shellfish (Harikrishnan *et al.*, 2011) and mice (Zhou, Deng, & Xie, 2006)

Conclusion

The results obtained from the present study indicate that powdered ginger rhizome supported the growth and hematology of *Clarias gariepinus* juvenile especially at 1.0 % dietary inclusion level. HSI index from this study showed that the cultured fish had stable liver conditions in relation to the growth observed. Data obtained from immunological study revealed that ginger did not provoke phagocytosis, thus proves that ginger is an immune-modulatory agent.

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