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Influence of Fermented Maize-meal Infusion on Feed Efficiency, Growth Performance and Antioxidants Status of African Catfish, *Clarias gariepinus* Fingerlings

A. Aliyu-A[1*], M. Aliyu-Paiko[1], J. Abafi[1,2], A. Abdul-Malik[1], K. M. Adamu[3] and M. A. King[2]

[1]Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Nigeria.
[2]Department of Chemical Sciences, Federal Polytechnic, Bida, Nigeria.
[3]Department of Biological sciences, Ibrahim Badamasi Babangida University, Lapai, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author AAA designed the study, carried out the feeding trial and performed the laboratory analyses. Author MAP wrote the protocol and the first draft of the manuscript. Authors JA, AAM and MAK managed the literature searches. Author KMA managed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was conducted to evaluate the influence of fermented maize-meal infusion on feed quality, growth performance and immune status of African catfish, *Clarias gariepinus* fingerlings.
Place and Duration of Study: Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Nigeria, between March 2017 and July 2017.
Methodology: A total of 120 African catfish, *Clarias gariepinus* fingerlings (mean initial weight 7.43±0.24 grams) were randomly distributed into 2 groups of 3 replicates each. Each replicate contained 20 fish. Fermented feed (FF) produced using fermented maize-meal (*ogi*) infusion for 72 h was fed 5% body weight to a group of 60 fish (in 3 replicates) for 10 weeks, compared to fish fed

**Corresponding author: E-mail: aabdulraheem404@gmail.com;*

control pellet containing similar ingredients but was non-fermented (NF). Proximate composition of feeds and whole fish, feed efficiency and growth performance, biochemical and antioxidant parameters in African catfish, *Clarias gariepinus* were evaluated.

Results: The results of proximate composition of FF and NF diets were not significantly (P>0.05) different in crude protein, crude fibre, carbohydrate and moisture contents. However, fat (lipid) was significantly lower in FF diet, while the level of ash was significantly (P<0.05) higher in FF than in the NF control. Proximate composition of whole fish fed NF and FF diets are significantly similar for crude protein, crude fibre, fat and carbohydrate contents. However, ash and moisture contents were significantly higher in fish fed NF than FF. The results for feed efficiency and growth performance of African catfish fed experimental diets indicated similar feed acceptance and intake and consequently similar growth performance. The hematological indices; packed cell volume (PCV), white blood cell (WBC), red blood cell (RBC) and haemoglobin (Hb) were observed to be significantly higher in fish fed the FF diet. The activity of aspartate aminotransferase (AST) was significantly elevated in serum of fish administered the non-fermented diet while alanine aminotransferase (ALT) and alkaline phosphatase (ALP) indicated elevated activities in fish fed fermented diet. The chloride and potassium ions and cholesterol indicated significantly higher concentration in the serum of fish fed fermented diet. Conversely, the inorganic phosphate, calcium, total protein and triglycerides were significantly higher in the serum of fish fed the non-fermented diet. Contrastingly, the concentration of sodium ion and creatinine did not differ significantly between the fish fed the experimental diets. The activities of superoxide dismutase (SOD) and catalase (CAT) were significantly elevated in the serum of fish fed FF diet.

Conclusion: Fermented diet with probiotics from maize-meal infusion improved biochemical and antioxidant parameters of *Clarias gariepinus* without impairing fish performance.

Keywords: African catfish; antibiotic growth promoters; antioxidant; biochemical; fermented feed; haematology; probiotics; microorganisms.

1. INTRODUCTION

Nigeria and Egypt are the largest consumers of fish in Africa and fish remains one of the main products consumed in terms of animal protein [1]. It is particularly an important source of protein and essential fatty acids and a unique source of micronutrients often deficient in diets of vulnerable populations [2].

Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) are among the candidate fish species for aquaculture production in Nigeria. In the last two decades *C. gariepinus* has developed into one of the most successfully cultured species in Africa followed by tilapia [3,4]. The suitability of this species for aquaculture arises from its fast growth rate; tolerance to high stocking density and poor water quality; acceptance of both artificial and non-specialized feeds and high market demand [5].

Globally in recent times, aquaculture remains the fastest growing food-producing sector [6]. However, this rapid growth is confronted by an unbearable cost of feed and outbreak of numerous fish diseases [7] resulting to an outrageous economic burden. In the last decades, antibiotics have been used for fish disease management, feed conversion efficiency and improvement of growth in cultured fish [8]. However, the extensive use of antibiotics has resulted into selective survival of some resistant bacteria species or strains [9,10,11], the inhibition or killing of beneficial microbiota in the gastrointestinal (GI) ecosystem of humans and other farmed animals and also, the bio-accumulation of antibiotic residues in fish products that are harmful for human consumption [12]. This portends significant implications on gut microbial profiles [13] as well as increase competition between gut microflora and the host for available nutrients [14].

Consistently, there has been increasing consideration to develop new dietary strategies, where various health and growth promoting compounds such as probiotics, prebiotics, symbiotic, phytobiotics and other functional dietary supplements will be evaluated [15,8].

Fermented feed through application of probiotics from maize-meal (called *ogi;* in yoruba dialect of Southern Nigeria) infusion could be considered as a biosafe method for replacing antibiotic growth prompters (AGPs) in fish farming [16,17].

Maize-meal is an acid-fermented cereal meal that serves as an important complementary weaning food for infants as well as a dietary staple for adults in Nigeria produced by spontaneous fermentation of maize [18]. Fermentation of maize-meal is by microorganisms from the environment [19]. Microbiological study shows that organisms responsible for fermentation of maize-meal include species of *Cephalosporium*, *Fusarium*, *Aspergillus*, and *Corynebacterium*. Others are *Saccharomyces cerevisiae*, *Candida myocoderma* and *Lactobacillus plantarum* [20].

The present trial highlights the potential of fermented feed on growth performance, biochemical and antioxidant parameters in *C. gariepinus* fingerlings.

2. MATERIALS AND METHODS

2.1 Experimental Diets

In the formulation used, fishmeal and soybean meal served as the principal sources of protein, whereas yellow cornstarch was the energy source for all diets. Tapioca powder was used as binder. Other ingredients used included vegetable oil (including residual oil from fishmeal and soybean meal) as the lipid source, while aqua vitamin and mineral premix (Scepter Agro-Allied Consults, Nigeria) were added as the sources of vitamins and minerals, respectively.

The calculated feed formulation adopted for this trial was such that each 100 g of feed contained approximately 24% fish meal, 27% soybean meal, 34% cornstarch, 8% vegetable oil, 4% vitamin and mineral premix and 3% tapioca [21].

2.2 Production of Starter Cultures from Fermented Maize-meal Infusion for Fermentation of Feed

To obtain fermented maize-meal infusion, white maize (*Zea mays*) (2.90 kg) was purchased from Minna central market and steeped immediately for 24 h using borehole water and milled afterwards [22]. The milled maize was sieved with muslin cloth. The slurry was left for 72 h for the development of starter culture. The surface water (maize-meal infusion) (10 L) as the source of starter culture was collected and used for the fermentation of test feed ingredients (called fermented, FF diet). No fermentation was carried out on the ingredients for the control feed (referred to as non-fermented, NF diet). The ratio of maize-meal infusion to feed ingredients used was 2:1 (Volume: Mass). After fermentation, the excess liquid was pressed out using clean, aseptic muslin cloth and the moist fermented dough was used to make 2 mm diameter pellets using a manual pelletizing machine (Jiaozuo Double Eagle Machinery Co., Ltd, Shanyang District, China). Moist feed pellets were dried under ambient temperature in the laboratory, packed separately and stored at 4ºC in a refrigerator until used during the feeding trial.

2.3 Experimental Design

One hundred and fifty (150) mixed-sex African catfish, *Clarias gariepinus* fingerlings (mean initial weight 7.43±0.24 g) were purchased from a commercial hatchery in Bida, Niger State, Nigeria and transported to the Aquaculture Research Facilities at Biochemistry Department, Ibrahim Badamasi Babangida University, Lapai, Nigeria. The fish species were identified in Fish Laboratory of Biological Sciences Department. A total of 120 fish were randomly selected from the 150 identified fingerlings, and distributed into 2 groups of 3 replicates each. Each replicate contained 20 fish. Fish were acclimated to the experimental facility conditions and fed with control feed for two weeks. After the acclimation period each experimental diet was assigned to their respective group.

The source of water for fish culture was from the University water supply. Water temperature, pH and dissolved oxygen (DO) were observed weekly (according to the method described by American Public Health Association (APHA) [23] and maintained within optimum ranges suitable for the survival of African catfish as stated by Towers [24]. This was achieved by regularly changing approximately two-thirds of the water in the experimental system, to reduce the nitrogenous waste accumulation and optimize fish culture conditions. All tanks were covered with nets throughout the trial, to prevent fish from jumping out. Fish were reared under prevailing photoperiod of light/dark cycle and hand fed the assigned experimental diets at 5% body weight twice daily at 09:00 h and 16:00 h (the feeding rate was 60% morning and 40% evening) for ten weeks.

Fish were weighed individually at the beginning and end of the experiment and batch weighed per tank once weekly, to monitor growth performance, feed consumption and adjust feeding rates. At the end of the ten week experiment, surviving fish were randomly pooled

into five groups per treatment and used to determine growth performance, feed efficiency, haematological and serum biochemical parameters, carcass proximate analysis, microbial and protein analysis.

2.4 Proximate Analysis of Diets and Whole Fish

Experimental diets and whole fish were analyzed for moisture content (dry matter; DM), proximate composition of crude protein, crude lipid, fiber, ash and carbohydrate contents following standard Association of Official Analytical Chemists (AOAC) methods [25].

2.5 Feed Efficiency and Growth Parameters

Feed efficiency and growth parameters were calculated by applying the appropriate formulae where necessary, from the following:

2.5.1 Feed efficiency parameters

Feed intake (FI) = $\frac{total\ feed\ intake}{number\ of\ fish}$

Protein intake (PI) = $feed\ intake\ (g) \times\ percent\ protein\ in\ diet$

Feed conversion ratio (FCR) = $\frac{wet\ weight\ gain\ (g)}{feed\ intake\ (g)}$

Protein efficiency ratio (PER) = $\frac{wet\ weight\ gain\ (g)}{total\ protein\ intake}$

2.5.2 Growth performance parameters

Fish survival (%) =

$$\frac{final\ number\ of\ surviving\ fish}{initial\ number\ of\ fish} \times 100$$

Weight gain (WG) = $\frac{(W_{f-}W_i)}{W_i}$

Weight gain (WG %) = $\left[\frac{(W_{f-}W_i)}{W_i}\right] \times 100$

Specific growth rate (SGR %) = $\left[\frac{\ln W_{f-}\ln W_i}{T}\right] \times 100$

Where W_f refers to the mean final weight, W_i is the mean initial weight and T is the feeding trial period in days.

2.6 Collection of Blood from Experimental Fish

The sampling procedure involved the collection of blood from the test fish. The experimental fish were anesthetized to unconscious in cold water (hypothermia) maintained at 5ºC. The blood was obtained using 1.0 mL plastic syringe from ten (10) randomly selected and pooled fish from three containers of twenty (20) fish per tank. This was done by piercing the ventral side of the fish at about 0.5 cm away from the genital opening. The needle was inserted at the right angle to the vertebral column of the fish where it was gently aspirated during penetration as described by Argungu et al. [26]. Collected blood was transferred into anticoagulant free test tube to clot at room temperature. Serum was obtained by centrifugation using a bench centrifuge for 10 minutes at 3,000 rpm [26], after which the serum was collected by the use of micro pipette and transferred into anticoagulant free test-tube and stored in a refrigerator for subsequent analyses.

2.7 Determination of Haematological Parameters

Haematological components including PCV, Hb, RBC and WBC were determined using the automated haematologic analyzer SYSMEX KX21, SYMEX Corporation, Japan, employing the methods described by Dacie and Lewis, [27].

2.8 Analyses of Liver Function Parameters

The activities of ALT and AST in the fish serum were assayed using enzymatic method of Reitman and Frankel [28] Karmen et al. [29] respectively. ALP was assayed using enzymatic procedure of Klein et al. [30].

2.9 Analysis of Kidney Function Parameters

The serum concentration of sodium was estimated by the method of Maruna [31]. Serum potassium was determined by the method of Terri and Sesin [32]. Serum chloride was evaluated by method of Skeggs and Hochstrasser [33] and inorganic phosphate was determined by colorimetric procedure of Tausskyand and Shorr [34]. While creatinine and total protein concentrations were determined by colorimetric procedure of Bartel and Bohmer [35] and Teitz [36] respectively.

2.10 Analysis of Lipid Profile

The amount of triglycerides in the sample was determined by colorimetric procedure of RANDOX Laboratory Ltd., Antrim, United Kingdom while the quantity of cholesterol in the sample was determined using enzymatic endpoint method of Trinder [37].

2.11 Assay of Antioxidant Enzymes Activities

Superoxide dismutase (SOD) and catalase (CAT) activities were assayed in fish serum spectrophotometrically, according to modified methods of Misra and Fridovich [38] and Beers and Sizer [39] respectively.

2.12 Statistical Analyses

The results are presented as mean ± standard deviation of two value determinations. Mean values for all monitored parameters were analyzed by single factor analysis of variance (ANOVA). $P<0.05$ were considered significant when compared by Turkey's test. All statistical analyses were carried out using SPSS software, version 20, USA.

3. RESULTS

3.1 Proximate Composition of Experimental Diets

Results of proximate composition of FF and NF diets are presented in Table 1. The data indicated that crude protein, crude fibre, carbohydrate and moisture contents were not significantly ($P>0.05$) different for the two diets. However, fat (lipid) was significantly lower in FF diet, while the level of ash was significantly ($P<0.05$) higher in FF than in the NF control.

3.2 Proximate Composition of whole Fish Carcass fed Experimental Diets

Proximate composition of whole fish fed NF and FF diets (as presented in Table 2.) followed a similar pattern to that observed for the experimental diets, with crude protein, crude fibre, fat and carbohydrate contents being similar. It was however, observed that ash and moisture contents were significantly higher in fish fed NF than FF.

3.3 Feed Efficiency in African catfish fed Experimental Diets

The results for feed efficiency of African catfish fed experimental diets are as shown in Table 3. The feed acceptance and intake were similar between fish fed the fermented pellets and the control. There was therefore, no significant ($P \geq 0.05$) difference in the protein intake (PI), protein efficiency ratio (PER) and feed conversion ratio (FCR) between the fermented (FF) and control (NF) treatments.

3.4 Growth Performance of African catfish fed Experimental Diets

The results for growth performance of African catfish fed experimental diets are presented in Table 4. Growth performance followed a trend similar to that observed for feed efficiency, showing no significant ($P \geq 0.05$) difference between FF and NF.

3.5 Haematological parameters in African catfish fed Experimental Diets

The results for various haematological indices of African catfish fed experimental diets are as shown in Table 5. The PCV, WBC, RBC and Hb were observed to be significantly ($P<0.05$) higher in fish fed the FF diet than in fish fed the control NF diet.

3.6 Activities of Liver Function Biomarker Enzymes in African catfish fed Experimental Diets

The results for the activities of liver function biomarker enzymes in African catfish fed

Table 1. Proximate composition of fermented and non-fermented fish diets

Treatments	Moisture	Ash	Protein	Crude Fibre	Fat	NFE
NF	6.30±0.67^a	11.39±0.67^a	29.84±2.46^a	3.10±0.07^a	18.52±1.57^b	30.85±1.56^a
FF	7.60±0.56^a	14.84±0.96^b	32.98±2.09^a	3.20±0.02^a	13.17±1.24^a	28.21±1.67^a

Values are expressed as mean ± SEM of 2 values; Columns with different superscripts, lower case letters are significantly different (P<0.05)

Table 2. Proximate composition of whole fish carcass fed fermented and non-fermented diets

Treatments	Moisture	Ash	Protein	Crude Fibre	Fat	NFE
NF	9.59±1.21^b	14.07±0.02^b	49.00±2.57^a	0.85±0.01^a	21.50±2.01^a	4.99±0.91^a
FF	7.92±1.45^a	13.56±0.13^a	50.75±2.24^a	0.96±0.02^a	23.80±1.57^a	3.01±0.96^a

Values are expressed as mean ± SEM of 2 values; Columns with different superscripts, lower case letters are significantly different (P<0.05)

Table 3. Feed efficiency of African catfish fed fermented and non-fermented diets for 10 weeks

Treatments	Fish survival (%)	Feed intake (g)	Feed conversion ratio	Protein intake (g)	Protein efficiency ratio
NF	92.50±0.50	5.50±0.02	1.30±0.01	1.60±0.02	4.60±0.01
FF	92.50±0.55	5.30±0.01	1.50±0.01	1.70±0.03	4.70±0.03

Values are expressed as mean ± SEM of 2 values

Table 4. Growth performance of African catfish fed fermented and non-fermented diets for 10 weeks

Treatments	Initial weight	Final weight	Weight gain (g)	Weight gain (%)	Specific growth rate	Specific growth rate (%)
NF	7.40±0.03	14.80±0.03	7.40±0.02	100.00±0.04	0.03±0.01	2.90±0.02
FF	7.20±0.02	15.20±0.03	8.00±0.06	111.10±0.03	0.03±0.03	3.00±0.02

Values are expressed as mean ± SEM of 2 values

Table 5. Haematological parameters in African catfish fed fermented and non-fermented diets for 10 weeks

Treatments	Packed cell volume (%)	White blood cell ($x10^6$)	Red blood cell ($x10^{12}$)	Hemoglobin (g/L)
NF	21.75±0.03[a]	3.10±0.02[a]	0.90±0.03[a]	7.94±0.05[a]
FF	27.43±0.12[b]	6.70±0.03[b]	1.80±0.06[b]	13.10±0.07[b]

Values are expressed as mean ± SEM of 2 values
Columns with different superscripts, lower case letters are significantly different (P<0.05)

experimental diets are as shown in Table 6. The activity of AST was significantly elevated in serum of fish fed the NF relative to that in the FF. ALT and ALP indicated elevated activities in fish fed FF diet compared to that in fish fed the NF diet.

3.7 Serum Electrolyte Concentration in African Catfish Fed Experimental Diets

The results for serum electrolyte concentration in African catfish fed experimental diets are presented in Table 7. The results indicated that fish fed FF was significantly (P<0.05) higher in chlorides and potassium ions relative to that in the control. Conversely, the inorganic phosphate and calcium ions were significantly higher in the serum of fish fed the control diet. Contents of sodium ion on the other hand did not differ significantly in the fish fed the fermented diet from that fed the non-fermented control diet. Creatinine content on the other hand did not differ significantly in the fish fed the fermented diet from that fed the non-fermented control diet.

3.8 Serum Total Protein and Lipid Composition in African Catfish Fed Experimental Diets

The concentration of total protein, cholesterol and triglycerides in African catfish fed experimental diets are highlighted in Table 8. The results of metabolites indicated that fish fed NF control diet was significantly (P<0.05) higher in total protein and triglycerides relative to that in the FF diet. Conversely, the cholesterol level was significantly higher in the serum of fish fed the FF diet.

3.9 Activities of Antioxidant Enzymes in African Catfish fed Experimental Diets

The result for the activities of serum antioxidant enzymes in African catfish fed experimental diets are shown in Table 9. The activities of superoxide dismutase and catalase as antioxidant enzymes were significantly (P<0.05) elevated in the serum of fish fed FF diet compared to fish fed the control diet.

4. DISCUSSION

In the present study, there was a relative increase in the crude protein content of formulated diet after fermentation for 72 h. This increase in crude protein in the diet could be attributed to microbial protein synthesis during fermentation process [40,41]. This is similar to results reported by Belewu and Okhawere [42], however, with substantial increase in the crude protein after fermentation. The relative increase in protein content of the FF in this study compared to a substantial increase in the level of

protein reported by other researchers could be attributed to factors such as the length of fermentation, protein utilization by other microorganisms present in the medium or inoculum size. Uaboi-Egbenni et al. [43] reported a maximum value for amino acid concentration of *dadawa* on the 7th day of fermentation. However, some studies have shown optimal microbial protein synthesis within 24-72 h of fermentation [44]. Previous studies have revealed that fermentation may not increase the content of protein and amino acids unless ammonia or urea is added as a nitrogen source to the fermentation media [45,46].

As fermentation involves mixed cultures of various aerobic bacteria, wild yeast, fungi and LAB, competition among these microbes may lead to nutrient depletion. Several studies have reported degradation of free amino acids in the diets during fermentation [47,48]. It has been demonstrated that loss of amino acids during fermentation could be due to the depletion of such amino acids by *E. coli* [49] and *Salmonella* spp [48]. The number of LAB used in fermentation of diet may be insufficient, as suggested in the literature. Niba et al. [48] recommended a high numbers of LAB, approximately 10^9cfu/ml of feed and high concentration of lactic acid of >150 mM with low pH below 4.5, hence eliminating entero pathogens that participate in the depletion of diet nutrients. Uaboi-Egbenni et al. [43] further reported that prolonged period of fermentation beyond three days can increase the level of LAB in the media consequently enhancing microbial protein synthesis and availability and removal of nutrient utilizing pathogenic microbes.

Data from previous studies indicated that optimal levels of protein for catfish are between 25 and 45% [50,51]. Degani et al. [52] reported that African catfish can grow on a lower crude protein diet of 30% at the growth rate of 1.2-0.8% per day. It may be inferred that the crude protein content of diet used in the present study was still appropriate for the growth of the cultured fish.

Carbohydrate is the major energy source from organic matter for microbial protein synthesis [53]. Some researchers have suggested that it would be more appropriate if the efficiency of microbial protein synthesis is expressed as a function of carbohydrate digested rather than organic matter digested [54,53]. The microbial protein yield can be estimated on the basis of fermentable metabolizable energy, digestible carbohydrates or fermentable organic matter [55,53].

The process of submerged fermentation adopted for diet in this study resulted in the depletion of carbohydrate (nitrogen free extract; NFE) and lipid content. This loss in the content of carbohydrate and lipid during the fermentation process was probably utilized as sources of energy by the fermentative microbes [40]. The primary function of the microbial carbohydrate metabolism is to release the adenosine triphosphate (ATP) required for microbial growth [53]. Therefore, patterns and rate of microbial protein metabolism are dependent upon the rates of carbohydrate fermentation [56,53]. A typical catfish feed contains 25% or more soluble (digestible) carbohydrates [51]. This indicates that the level of carbohydrates in the FF diet is still optimum to supply energy to the cultured fish and not at the expense of protein. The result of proximate composition of FF diet displayed a decrease in the lipid content after fermentation. The effects and mechanism of action of essential oils on microbial fermentation has been studied [53]. Vakili et al. [57] revealed that essential oils serve as useful fermentation modifiers. Therefore, the decrease in lipid content of FF diet was likely used to favor the overall fermentation process.

Similar result of proximate composition of whole fish was obtained for crude protein and carbohydrate with a slight increase in the content of crude lipid (fat) in the present study.

The protein content of fish carcass fed FF diet was better compared to that of the whole fish fed NF diet. The relative increase in the protein level

Table 6. Activities of liver function enzyme biomarker enzymes in African catfish fed fermented and non-fermented diets for 10 weeks

Treatments	Aspartate amino transferase	Alanine amino transferasee	Alkaline phosphatase
NF	26.52±0.00^b	48.20±4.36^a	9.214±0.78^a
FF	5.30±0.01^a	62.40±3.46^b	11.09±0.78^b

Values are expressed as mean ± SEM of 2 values.
Columns with different superscripts, lower case letters are significantly different (P<0.05)

Table 7. Serum electrolytes concentration in African catfish fed fermented and non-fermented diets for 10 weeks

Treatments	Chlorides	Inorganic phosphates	Calcium	Sodium	Potassium	Creatinine
NF	91.60±4.57[a]	5.70±1.24[b]	30.60±2.35[b]	46.90±4.56[a]	0.90±0.01[a]	0.40±0.00[a]
FF	104.80±4.56[b]	3.90±0.35[a]	8.30±0.35[a]	46.60±4.56[a]	1.30±0.04[b]	0.30±0.01[a]

Values are expressed as mean ± SEM of 2 values
Columns with different superscripts, lower case letters are significantly different (P<0.05)

Table 8. Serum total protein and lipid composition in African catfish fed fermented and non-fermented diets for 10 weeks

Treatments	Total Protein	Cholesterol	Triglycerides
NF	3.50±0.19[b]	6.85±2.42[a]	200.00±12.50[b]
FF	1.75±0.21[a]	30.85±2.35[b]	146.66±19.38[a]

Values are expressed as mean ± SEM of 2 values
Columns with different superscripts, lower case letters are significantly different (P<0.05)

Table 9. Activities of antioxidant enzymes in African catfish fed fermented and non-fermented diets for 10 weeks

Treatments	Superoxide dismutase (U/L)	Catalase (U/L)
NF	0.03±0.00[a]	2.61±0.09[a]
FF	0.05±0.00[b]	3.87±0.03[b]

Values are expressed as mean ± SEM of 2 values
Columns with different superscripts, lower case letters are significantly different (P<0.05)

of whole fish fed FF diet could be the reflective effect of its relative abundance in the diet. The 50% protein content in fish fed FF diet is higher than protein content reported for wild *S. sardinella, C. reba, G. mullya, R. daniconius* and *P. conchonius* by Pawar et al. [58]. This result indicates that fermented diet can be used to enhance muscle protein level of cultured African catfish.

Fish contains very low level of carbohydrates [59]. Carbohydrate is readily utilized as prime energy source and does not take part in tissue formation in fish. It is therefore practically possible to measure a low carbohydrate level in whole fish. Fish is a good source of all nutrients except carbohydrates and vitamin C [59].

In this trial, the observation of nutrient efficiency and growth performance in the fish fed fermented feed pellets being numerically but not significantly higher than in fish fed the control dietary treatment indicates a potential of fermentation as a process that enhances feed palatability and quality, as already established [60]. The relatively faster growth rate potential of those fed the fermented feed likely indicates that fish accepted and consumed the fermented feed pellets better. This is possibly due to enhanced feed quality in protein and palatability, thus improving fish appetite, thereby displaying potentials for accelerating fish growth performance. The feed could therefore be deemed suitable for optimal growth of African catfish (*Clarias gariepinus*).

This observation is similar to findings of other studies where Nile Tilapia (*O. Niloticus*) indicated optimized nutrient utilization and growth performance when fed diets supplemented with fermented fish Offal meal and fermented Mango seed meal respectively [61,62]. Sultan et al. [63] however, reported a significant reduction in growth and nutrient utilization in African catfish when fish meal was replaced with higher level (75-100%) of fermented fish silage in feed. The differences of results from that of the current study are likely to be related to factors other than fermentation, such as higher fiber and/or ash contents and acceptability of the fish silage [63].

It is also worthy to note that probiotics helps to modulate intestinal microbial-flora of fish when ingested along with the fermented diet leading to significantly higher PER and hence improved growth [64]. Lara-Flores et al. [65] reported better SGR and PER in *Oreochromis niloticus* treated with *L. acidophilus* and *S. faecium*. This improvement in probiotic-treated fish could be as a result of their increased potential to tolerate harmful conditions that fish may be exposed to as affirmed by Rollo et al. [66] in *Sparus aurata* (Sea bam). Al-Dohail et al. [67] reported a significant (P<0.05) growth performance in Catfish cultured on *Lactobacillus acidophilus* supplemented diet relative to that fed probiotic-free diet. Carnevali et al. [68] also reported a significant (P<0.05) growth in sea bass juvenile fed with *Lactobacillus delbrueckii* supplemented diet.

Furthermore, improvement in growth performance and nutrient utilization could be as a result of lower level of stressors in fish fed with probiotics enhanced diets. Carnevali et al. [68] reported that when fish was fed a diet supplemented with *L. delbrueckii,* there was a decrease in cortisol levels which affects the transcription of insulin-like growth factor (IGF-1) and myostatin (MSTN), which are both known to

be a regulator of growth performance and in effect leads to an appreciable increase in body weight of the fish when compared with the control diet [64].

The similarity in the previous studies and this study in relatively higher growth performance and feed efficiency with the use of probiotic fermented diet could be attributed to higher nutrient digestibility, better absorption and enhanced enzyme activities which could further be attributed to proper intestinal micro flora balance or exo-enzyme secretion [69,64].

In the present study, at 10 weeks post feeding, the monitored blood parameters, PCV, Hb, RBC, WBC, ALT, ALP, cholesterol, chloride and potassium ion values were significantly higher (P<0.05) in the fish maintained on the fermented diet than in those fed non-fermented diet.

Higher RBC count in the FF fish group was indicative of higher oxygen absorption and transportation capacity [70]. This may also be due to lower swelling and hence reduced RBC damage [71]. This is also applicable to hematocrit and haemoglobin. The observed reduction in PCV, Hb and RBC in fish fed non-fermented diet could generally be as a result of nutritionally deficient diet and thus increases in anti-nutritional factors [72,73,74]. PCV and Hb are also major and reliable indicators of various sources of stress leading to decreased fish activeness [75,76]. Low level of PVC is also used as an indicator of aneamic condition in fishes [77]. The activeness of fish may also be associated with higher Hb value [78].

White blood cells play a major role in the defense mechanism in fish [79]. WBC in the fish fed the fermented diet was significantly higher than that in the fish maintained on the non-fermented diet. This could be because the fish fed fermented diets had better capacity and lower stressor levels to resist infection compared with the fish fed non-fermented diet [71,74]. Dhabhar et al. [80] and Akinwande et al. [81] opined that a measurable increase in WBC count of fish or any animal is a function of immunity and animals' resistance to some vulnerable illness or disease. This increase could indicate that the fish under study had high immunity or resistance to disease and therefore little or no stress was placed on the health of the fish fed fermented diet [82]. The reduction in white blood cells may result from bioaccumulation of the anti-nutrients consumed by the fish fed non-fermented diet [79].

AST and ALT are biomarkers of hepatic integrity and to a greater extent can be used to assess the extent of hepatocellular damage. The ALT activities however, give more valuable information relevant to the integrity of the hepatocyte than AST [83]. ALP is often used to assess the integrity of plasma membrane and endoplasmic reticulum [84].

The effect of probiotic treatment on specific activities of ALT and ALP were significant. This could be attributed to the fact that the fish fed fermented diet had higher diet protein and capacity to utilize it. According to Hansen et al. [85], ALT and AST are two major enzymes which are quantitatively important in transamination of amino acids in the liver and kidney.

Liver enzyme activities affect various chemical and biological reactions in the body of the fish. According to Gabriel and George [86], transamination is one principal pathway for synthesis and deamination of aminoacids, enabling carbohydrate and protein metabolism during fluctuating energy demands of the organism under various adaptive conditions.

Both AST and ALT function as a link between carbohydrate and protein metabolism by the interconversion of strategic compounds like α-ketoglutarate and alanine to pyruvic acid and glutamic acid, a process referred to as transamination [87,88]. On the contrary, elevation of ALP, AST and ALT reflect hepatic disease, some inflammatory disease or injury to the liver [89,90,91].

Concentrations of individual ions and total osmolarity in blood plasma are physiological variables that have been used as indicators of assessing the wellbeing of fish [92]. Ions are very important for any organism because they are involved in most biological processes [93], and responsible for the maintenance of osmotic pressure in blood [94].

Sodium, chloride, potassium and phosphorus are major anions important in the maintenance of cation/anion balance between intra- and extra-cellular fluids. These electrolytes are therefore essential to the maintenance of proper hydration, osmotic pressure, acid/base equilibrium, bone formation and integrity [95].

The significant concentration observed in chloride in fish fed FF diet is an indication of normal function of kidneys [95]. Conversely,

phosphorus, calcium, triglyceride and total protein were significantly higher in the fish fed the non-fermented diet than in those maintained on the fermented diet. The lower concentrations in some of the electrolytes and biochemical molecules observed in fish fed FF diet as compared to fish fed NF diet could be attributed to the biochemical requirements of organic and inorganic compounds for growth and reproduction or loss of electrolytes due to the permeability of renal tubules [96,97].

Fish also mobilizes triglycerides and protein to meet an increased demand for energy resulting from increased physical activity, biotransformation and excretion [91,98]. The decrease in total protein level could also be as a result of protein synthesis of an organism for the production of enzymes, hormones, and antibodies [99].

Cholesterol is the most important sterol occurring in animal fats. Change in the blood cholesterol and triglyceride concentration could be due their involvement in the synthesis of steroid hormones. The serum sodium and creatinine of the fish fed fermented and non-fermented diets did not differ significantly. Creatinine plays important roles in determining the synthetic and excretory roles of the kidney and liver [100]. Creatinine leaves the muscle and enters blood, where it is removed by filtration through the glomeruli of kidneys and excreted into urine. The creatinine clearance test has become one of the most sensitive tests for measuring the glomerular filtration rate [101,102]. This effect is used as an indicator of renal function. According to Kaptan and Szabo [103], about 50% of kidney function must be lost before a rise in the serum creatinine can be detected. The similarity in the creatinine level observed in fish fed fermented and non-fermented diet may be an indication of a healthy renal function as regards to this metabolite.

The antioxidant enzymes Superoxide Dismutase, Catalase and Glutathione Peroxidase are considered the first line of antioxidant defense mechanism against oxidative stress by reactive oxygen species, to protect cells from oxidative damage by the free radical process [104]. In the present study, fish fed with fermented feed expressed higher level of plasma antioxidant enzymes compared to the control fish. The significant increase could be attributed to the effects of probiotic production of proteins in the fermented feed which can serve as precursor to the production of the organic enzymes. Furthermore, elevation in the activities of the antioxidant enzymes is a sign of enhanced scavenging of free radicals in the blood. The interplay between free radicals, antioxidants, and diseases in cells, tissues and organisms is important in maintaining health, aging and age-related diseases [105].

The cellular condition or phenomenon called oxidative stress is one which occurs due to physiological imbalance between levels of antioxidants and that for oxidants (i.e. free radicals or reactive species), such that the imbalance favours oxidants. Consequently, oxidative stress through free radicals or reactive oxygen species, have evidently been implicated in the incidence and progression of several health conditions such as atherosclerosis, diabetes, cancer, neurodegenerative disorders, cardiovascular disorders and other chronic disease conditions in humans [106].

A very common antioxidant enzyme, CAT is present in almost all living cells and tissues (including the blood) that utilize oxygen. CAT utilizes iron or manganese as a cofactor and catalyzes the degradation of hydrogen peroxide (H_2O_2) to water and molecular oxygen, thus completing the detoxification process initiated by SOD. The abundance of CAT in cells enables it to continuously scout for hydrogen peroxide molecules. It is therefore, highly efficient, as it is capable of breaking down millions of hydrogen peroxide molecules per second [107].

Therefore, the increase in the activities of SOD, CAT, which constitute the first line of antioxidant defense system plays a fundamental role in the total defense mechanisms and strategies in biological systems, to protect against oxidative damage and mitigate organisms against infection by microorganisms [104]. Based on the results of the present study, feeding fish with fermented feed positively influenced growth performance and elevated oxidative stress response parameters such as SOD and CAT.

5. CONCLUSION

Fermented diet with probiotics from maize-meal infusion improved biochemical and antioxidant parameters of *Clarias gariepinus* without impairing fish performance.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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