Dietary Inclusion of Fermented Parkia in Feeds as an Organic Strategy to Improve Feed Quality and Antioxidant Parameters of African Catfish (Clarias gariepinus) Fingerlings

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Authors' contributions

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

ABSTRACT

Aim: The aim of this study is to assess fermented parkia infusion in feed as organic strategy to improve feed quality and immune system of African catfish (Clarias gariepinus) fingerlings.

Place and Duration of Study: Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria, between 1st January 2017 and 25th March 2017.

Methodology: In the present study, feed ingredients were fermented for 72 hrs with probiotic starter culture from Parkia biglobosa (TF diet) in comparison to a control diet with similar
Ingredients to the experimental diets but totally unfermented (CF diet) and used to make feed pellets. The experimental feeds were fed at 5% of body weight of each dietary treatment to triplicate groups of 20 fish per tank (100 Liter capacity), two times daily for 10 weeks. Proximate composition and mineral content of diets and whole fish, Feed efficiency, growth performance and Biochemical parameters of the fish were evaluated.

**Results:** The result of proximate compositions of experimental diets revealed a significantly higher protein and ash contents in TF compared to the CF. Carbohydrate content was significantly lower in TF diet while crude fibre, moisture and fat contents were not significantly higher in CF compared to TF. The highest values of some minerals such as sodium (Na), potassium (K), phosphorus (P) and magnesium (Mg) were also recorded in the TF while the least was recorded in CF. The result of feed efficiency and growth performance indicated similar feed acceptance and intake between fish fed the fermented diet and the control. The activities of serum antioxidant enzymes activities of superoxide dismutase and catalase were significantly (P < 0.05) higher in serum of fish fed TF compared to that in fish fed CF diet.

**Conclusion:** It can be deduced from this study that the fermented diet improved the immunity of fish and does not adversely affect the growth performance.

**Keywords:** Functional feeds; probiotics; *Clarias gariepinus*.

### 1. INTRODUCTION

Aquaculture according to Ayinla [1] is the fastest growing food producing industry in the world providing sustainable livelihood opportunities and food security for the ever-increasing world population [2]. With the global increase demand for fish over the past two decades, aquaculture production is likely to double in the next fifteen years, as a result of wild fisheries approaching biological limits [3]. Therefore, there is an immense need to intensify fish farming, especially in the developing nations like Nigeria [4]. Aquaculture has greater potential to meet the increasing demand for fish; however, sustainable supply and cost of feed ingredients for aquaculture pose a major challenge. Diets with proper nutrient balance (i.e. functional feed) are important in enhancing fish health and production. Furthermore, the intensification of aquaculture practices applied to maintain high levels of production could result in adverse environmental situations that may acutely stress the fish, altering their biochemical functions and suppressing their immune responses. As a result, diseases, most especially bacterial infections tend to thwart fish production thereby threatening the sustainability of aquaculture [5]. Thus, substantial reliance on vaccines and antibiotics to combat fish diseases is inevitable. Sequential use of antibiotics to control diseases creates adverse effect that result in accumulation of antibiotics residues in the tissue, which consequently leads to immune suppression among others [6]. Suitably, the development of natural growth promoters has become important and urgent alternative for healthier fish production. It is suggested that the growth performance of cultured species could be enhanced through addition of probiotic to diet that will improve functional characteristics of feed and consequently increase feed efficiency [7].

In the era of diminishing feed quality, fermentation of feed ingredients through probiotic could play a role in complementing feed fortification. In addition, the ingestion of the fermented feed along with the live microorganisms could further maintain health-balance of fish by stimulating the immune system, aiding in nutrient digestion and increasing the resistance against enteric opportunistic pathogens [8]. The functionality of enteric microbiota depends on the ability of beneficial microorganisms to interact with the digestive tract, which benefits the host by the influence on the biological functions [9]. Beneficial microorganisms or probiotic activate the digestion process by providing digestive enzymes and vitamins as well as the ability of nutrients to compete the adhesion sites to enhance the feed utilization [10]. Furthermore, the antimicrobial substances produced by intestinal microorganisms enhance the immune response and decrease the pathogenic bacteria [11]. This present study was therefore designed to investigate the effects of dietary inclusion of fermented *iru* infusion as an organic strategy to improve feed quality/utilization, growth performance and biochemical parameters of African catfish (*Clarias gariepinus*) fingerlings.
2. MATERIALS AND METHODS

2.1 Experimental Fish and Husbandry Management

The African Catfish fingerlings were purchased from a commercial hatchery in Bida local government and transported to the fish research laboratory of the department of Biochemistry, IBB University, Lapai, Nigeria. The fish were acclimated to the experimental conditions and fed control diet for two weeks. At the end of the adaptation period, a total of 120 fish (with mean initial weight of 7.4 g) were randomly divided into 2 groups of 3 replicates each. Each replicate contained 20 fish. All cultured tanks contained bore-hole water from the University water supply. Water temperature, pH and dissolved oxygen (DO) were maintained within optimum range for the culture of African catfish. Fish were reared under natural photoperiod and hand-fed assigned experimental diets at 5% body weight twice daily at 9:00 and 16:00 hours for 10 weeks. Approximately half of the water in the system was changed four (4) times a week to reduce the regular accumulation of nitrogenous residue from feed.

Fish were weighed individually at the beginning and end of the experiment, whereas batch weighing per tank was carried out every week to monitor growth performance alongside adjusting feeding rate. At the end of the experiment after 10 weeks, surviving fish were pooled together and randomly grouped into Four (4) per treatment and used to determine serum biochemical parameters and carcass proximate compositions.

2.2 Production of Starter Cultures from Fermentation of Feed

To obtain “Parkia” water, *Parkia biglobosa* (African locust beans) was prepared according to the modified method of Odunfa and Oyewole [12], shown in the flow chart in Fig. 1.

Production of Starter culture involved soaking of dadawa in approximately 3 litres of distilled water in a plastic container, which was covered for 72 hours. Thereafter, five (5) litres of distilled water was used to wash the slimy dadawa. The solid dadawa debris was sieved out while the surface water called *dadawa* or *parkia* water was collected.

![Flowchart showing the production of Dadawa from African locust beans, *Parkia biglobosa*.](chart.png)

**Fig. 1. Production of Dadawa from African locust beans, *Parkia biglobosa***

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control feed (%)</th>
<th>Test feed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Corn starch</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Tapioca</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><em>Parkia</em> water</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Briefly after 72 hours, the surface water (called *parkia* water) from the preparation containing starter cultures was used for the fermentation of the test feed ingredients (called TF diet). No fermentation was carried out for the ingredients for control feed (CF diet). The ratio of liquid to solid feed ingredients used was 2:1 (Volume: Mass). The *parkia* water was mixed with solid feed ingredients in a plastic container, which was covered and allowed to ferment for 72 hours. After 72 hours of fermentation, the excess liquid was pressed out using clean muslin cloth and the moist fermented feed was used to make 2 mm diameter pellets in a manual pelletizer (Jiaozuo Double machinery Co., Ltd, China). Moist feed pellets were dried under ambient temperature, packed separately and stored at 4°C until used during the feeding trial.
2.3 Proximate Analysis

Experimental diets and final fish carcass were analyzed for moisture content, proximate composition of crude protein, crude lipid, crude fiber, ash and carbohydrate (Nitrogen free extract; NFE) contents following standard AOAC methods [13].

2.4 Calculations of Feed Efficiency and Growth Parameters

Feed efficiency and growth parameters were calculated using the following:

- Feed intake (FI) = Total feed intake (g)/number of live fish
- Protein intake (PI) = Feed intake (g) \times \text{percent protein in the diet}
- Feed conversion ratio (FCR) = Feed intake (g)/wet weight gain (g)
- Protein efficiency ratio (PER) = wet weight gain (g)/total protein intake

2.5 Growth Performance Parameters

Fish survival (%) = final number of surviving fish/initial number of fish \times 100

Weight gain (WG) = \frac{(W_f - W_i)}{W_i}

Weight gain (WG %) = \frac{(W_f - W_i)}{W_i} \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 Determination of Antioxidant Enzymes Activities

Comparative study of biochemical profile was investigated by sampling blood from fish. The experimental fish were anesthetized and maintained in water at 5°C. The blood was obtained from ten (10) randomly selected fish in three containers of twenty (20) fish per tank. This was done as described by Argungu et al. [14].

Superoxide dismutase (SOD) and catalase (CAT) activities were determined spectrophotometrically from the serum according to the modified methods of Misra et al. [15] and Beers et al. [16], respectively.

2.7 Statistical Analysis

The Results were presented as mean ± SD of two values. Mean values for all monitored parameters were analyzed by one-way analysis of variance (ANOVA). $P$ values <0.05 were considered significant when compared by Turkey’s test. All statistical analyses were carried out using SPSS software, version 24.0.

3. RESULTS

3.1 Proximate Compositions and Mineral Content of Experimental Diets

The result for proximate compositions and mineral content of experimental diets are presented in Fig. 2. The result indicated that fermented diet (TF) contained significantly higher protein and ash contents compared to the CF. Carbohydrate content was significantly lower in TF diet. Crude fibre, moisture and fat contents were not significantly higher in CF compared to TF. The highest values of analyzed minerals such as sodium (Na), potassium (K), phosphorus (P) and magnesium (Mg) were also recorded in the TF while the least was recorded in CF.

3.2 Proximate Compositions and Mineral Contents of Fish Carcass

The result for proximate compositions and mineral content of experimental fish carcass is presented in Fig. 3. It may be observed that protein, crude fibre and fat contents were numerically but not significantly higher in the carcass of fish fed TF treatment group. Whereas, carcass of fish fed CF diet indicated significantly ($P < 0.05$) higher ash and moisture contents. However, carbohydrate was significantly lower in the carcass of fish fed TF.
3.3 Feed Efficiency of African Catfish Fed Fermented Diet

The result of feed efficiency of African Catfish fed fermented diet is shown in Fig. 4. As indicated, feed acceptance and intake were similar between fish fed the fermented diet and the control.

3.4 Growth Performance of African Catfish Fed Fermented and Non-Fermented Diets

The result for growth performance of African catfish fed experimental diets as shown in Fig. 5 followed a similar trend to that observed for feed efficiency, with no significant (P > 0.05) difference between TF and CF treatment groups.

3.5 Activities of Serum Antioxidant Enzymes in African Catfish Fed Fermented and Non-Fermented Diets for 10 Weeks

The result for activities of serum antioxidant enzymes in African catfish fed fermented and non-fermented diets is presented in Fig. 6. The activities of antioxidant enzymes; superoxide dismutase and catalase were significantly (P < 0.05) higher in serum of fish fed TF compared to that in fish fed CF diet.
Fig. 4. Proximate composition of fish carcass

Fig. 5. Proximate composition and mineral contents of fish carcass

4. DISCUSSION

Functional feeds represent one of the most intensively explored and widely promoted areas in aquaculture industry [17]. According to Aworh et al. [18] the use of probiotics for fermentation serves as a useful strategy in complementing feed fortification. This strategy promotes efficient absorption of nutrients due to the ability of probiotics (such as Bacillus species)
to produce digestive enzymes (amylases, proteases, and lipases, etc.) and provide simpler nutrients (vitamins, fatty acids and amino acids), which could enhance the digestive process and feed utilization. This will result in improved general health condition and growth performance of aquatic animals [19].

Fig. 6. Nutrient utilization of African Catfish fed fermented and non-fermented diets for 10 weeks feeding trial

Fig. 7. Growth parameters of African catfish, *Clarias gariepinus* fed experimental diets for a period of 10 weeks feeding trial
Proximate composition analysis is an important criterion in determining the nutritional quality of feed in animal husbandry [20]. In the present study, there was significant (P< 0.05) increase in protein and ash contents of the formulated diet after fermentation. The increase in crude protein content could be attributed to the ability of the suspected microbes (such as Bacillus species) present in TF diet to carry out different proteolytic enzyme activities, thereby producing free amino acids during fermentation [21].

Protein obtained from the diet provides proper ratio of amino acid necessary for growth performance, which is utilized to synthesize new proteins or maintain existing proteins in tissues while excess protein, is converted to energy [22]. Proteins and their encoding genes in feeds also regulate cellular transport of micro-minerals in fish [23].

The protein content of whole fish in terms of nutritional value depends on the physiological utilization of dietary amino acids after digestion, absorption, and a minimal rate of oxidation [24]. The notable numerical increase in protein content of whole fish in this study indicates adequate utilization and significant impact on nutrient storage of the fermented diet. The increased crude protein content observed in whole fish is in line with the findings of Degani [25], who reported that proteins are the major macromolecules in fish tissue and could make up to 65-75% of the total organic matter on a dry matter basis. Abdel-Tawwab et al. [26] added that changes in protein contents in the fish body could be attributed to changes in deposition rate in muscle and can consequently have effect on growth performance.

The observed decrease in carbohydrate content of fermented (TF) diet may be due to the ability of the microorganisms to utilize carbohydrate as their major energy source from organic matter during microbial protein synthesis [27]. Jonathan et al. [21], Boateng et al. [28] individually reported decrease in carbohydrate content during fermentation as a result of increased production of several digestive enzymes such as amylase, glucosidase and galactanase by fermentative microbes. This observation is also similar to that reported by Nitschke and Pastore [29], Oladele and Oshodi [30], who stated that Bacillus sp produced several enzymes during
fermentation which are capable of degrading carbohydrates into simple sugars that are used by the microorganism as energy source for metabolic activities.

Carbohydrate is one of the dietary ingredients that profoundly affect feed utilization. Feed increase in carbohydrate level above 10% dietary dry matter can result in reduced feed intake and utilization in aquatic animals [31]. The carbohydrate decrease observed in the present study is similar with the findings of Hemre et al. [31] and Erfanullah, [32] who reported improved feed utilization and protein retention in rainbow trout and Atlantic salmon when fed with diets containing low levels of carbohydrate content. Consequently, the decrease in carbohydrate level as a result of fermentation is appropriate for the cultured fish.

Furthermore, the level of carbohydrate content observed in the present study is appropriate for the cultured fish as a preferred oxidative substrate for nervous tissue and blood cell. Consequently, the carbohydrate present in the diet can lower gluconeogenic activity, thus diverting amino acids away from oxidative pathways essential for growth promotion.

The result of proximate composition of TF diet showed decrease in lipid content after fermentation compared to CF diet. This could be as a result of microorganisms producing lipases, which converts the polymeric forms of the lipids to soluble monomers [33]. Furthermore, the loss in lipid content of TF could be attributed to it utilization as modifiers during the fermentation process. This is in line with the argument of Vakili et al. [34] who revealed that lipids (or fatty acids) serve as useful fermentation modifiers.

Different flavors and aroma were observed during the fermentation of diet. This may be as a result of metabolism of lipid components and formation of other microbial metabolites. Modupe et al. [35] reported that the degradation of fat and other available compounds produce aroma during fermentation process. This could also be the cause for the depletion of lipids after the fermentation of feed.

Lipids (fats) are high-energy nutrients that can be utilized to spare protein in aquaculture feeds. Lipids make up about 7-15 percent of fish diets, which provide twice the energy density of proteins and carbohydrates in fish [36]. Other important functions of lipids include supplying fatty acids, improvement in absorption of fat-soluble vitamins, precursors of hormones and prostaglandins and as building blocks of cellular and membrane structures in fish [37]. Dietary lipids play an important role in the production and regulation of active modulators of bone metabolism (such as eicosanoids, cytokines, leucotriens, lipoxigenase). Dietary lipid levels are known to affect bone formation and reabsorption in fish.

The Ash content gives an idea of the inorganic content of the sample from where the mineral content could be obtained [38]. The analysis of mineral compositions of TF diet indicated significantly (P< 0.05) higher contents of sodium, potassium, phosphorus and magnesium than in the control diet. The result obtained were higher than that reported for Berhi cultivar by Kadam et al. [39], who stated that this increase may be due to contribution by fermentation microorganisms in the breakdown of the organic components of the samples during the period of fermentation [40].

The increase in mineral content may also be as a result of the fermentative microorganisms, which provide optimum pH condition for enzymatic degradation of phytates that form complexes with essential minerals [41]. Niba et al. [42] reported an improved bioavailability of minerals in liquid fermented pig feed. Similar trend was also observed on the effect of fermentation on the nutrient status of locust bean where an increase of 30% in ash content was recorded after fermentation [43]. Furthermore, Adams [44] reported an increase in the concentrations minerals after fermentation of feed ingredients.

Fish require the same minerals as terrestrial animals because of their involvement in skeletal formation, as cofactors of many metallo-enzymes, for osmoregulation and other metabolic functions [45].

At the end of the experimental feeding trial, improved nutrient utilization and growth parameters were observed. Fish fed TF diet were relatively better in nutrient utilization and growth performance compared to that obtained in fish fed control diet. The result of the present study agrees with the findings of Lara-Flores et al. [46] who observed improved diet with the addition of live yeast, which explained the better growth performance and feed efficiency seen in the cultured fish. De Schrijver and Ollevier [47] also reported a positive effect on apparent nutrient
utilization when turbot, catla carp and hybrid striped bass feeds were supplemented with bacteria Vibrio proteolyticus [48]. Adamu et al. [49] also reported that, African catfish fed fluted pumpkin leaf (FPL) and parboiled fermented African locust beans (PFALB) had better growth performance and nutrient utilization.

Probiotic inclusion in diet enhances growth performance and nutrient utilization in fish due to the modulatory effect on feeding, metabolism and feed digestibility [7]. The increased feed utilization and growth observed in the whole fish was similar to that of Ai et al. [50], Lamari et al. [51] who reported that Bacillus and Lactobacillus genera seem to be the most related with nutrient utilization and growth improvement, which may have influenced appetite, conversion ratio or reducing myostatin transcription [52], a protein responsible for modifying muscle growth and development in fish [50].

The relatively higher growth performance and nutrient utilization of fish fed TF diet may further be attributed to fermentation process as it has been proven to enhance the quality and palatability of fish feeds [53]. On the other hand, the relatively faster growth rate potential of those fed the fermented feed reveals that the fish accepted and consumed the fermented feed pellets better. Furthermore, due to increased acceptance and consumption of the fermented feed pellets, nutrients such as protein and lipid deposition in the muscle could significantly increase fish growth [26].

The increased growth may also be due to the ability of the suspected microbes in TF diet to adhere to the gastrointestinal tract of the catfish hence producing wide range of relevant digestive enzymes (amylase, lipase and protease) which hydrolyze the indigestible components thus synthesizing biotin and vitamin B12, which enhances feed utilization and digestibility [53].

Similarly, the relatively higher protein efficiency ratio (PER) observed in fish fed TF diet showed improved protein utilization in African catfish. This contributed to optimization of protein used for fish growth. The improvements in the biological value of the fermented diets have shown its efficiency in converting protein to flesh in fish [54].

Oxidative stress refers to the cellular condition that indicates a physiological imbalance between the levels of antioxidants and that for oxidants (i.e. free radicals or reactive species), such that the imbalance favours oxidants. Thus, under normal physiological conditions, aquatic animal cells maintain a balance between generation and removal of reactive oxygen species [55].

The antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are considered the first line of antioxidant defense mechanism against oxidative stress. Superoxide Dismutase is considered the first enzyme responsible for scavenging of free radical and thus protecting cells from been damaged [56]. In addition, SOD converts superoxide anion (O\(^2^-\)) to hydrogen peroxide (H\(_2\)O\(_2\)) while CAT reduces H\(_2\)O\(_2\) to water [57].

Activities of serum antioxidant enzymes revealed that the fish fed fermented (TF) diet suggested increased level of plasma antioxidant enzymes of SOD and CAT compared to the control fish. Similar results were observed when fish fed probiotics (Bacillus coagulans B16 and R. palustris) elicited increased SOD and CAT activities in tilapia [58]. Salinas et al. [59] reported that respiratory burst activity of teleost fish (Sparus aurata L.) increased in vitro by the addition of heat-inactivated Lactobacillus delbrueckii sp.

The significant increase in the antioxidant enzymes activities could be attributed to the microbial production of proteins through proteolytic enzyme activities in the fermented diet, which could serve as precursor in the production of the organic enzymes.

A properly balanced gut microbiota has the ability to positively affect the integrity of the intestinal barrier against colonization by pathogens [60], through its protective and metabolic function and can stimulate the immune system in an anti-inflammatory manner [61].

Furthermore, this elevation in the activities of the antioxidant enzymes is an indication of enhanced scavenging of free radicals in the blood of the TF fed fish group. Therefore, the interplay between free radicals, antioxidants, and diseases in cells, tissues and organisms in the experimental fish is important in maintaining good health [62].

5. CONCLUSION

Fermented diet may be successfully used as an alternative to conventional fish feed for African catfish (Clarias gariepinus) to enhance
antioxidant system as revealed by the results findings.

6. RECOMMENDATIONS

i. Further research should be carried out to ascertain the appropriate amount of microbial cells to be employed in feed fermentation for aquaculture.

ii. The duration of feed fermentation should be prolonged to obtain higher feed microbial pre-digestion for enhanced feed utilization.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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