*Original Research Article*

Evaluating Fermented and Hydrolyzed Rice Bran for Improved Biofloc Performance in African Catfish (*Clarias gariepinus)* Rearing

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ABSTRACT

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| --- |
| **Background**: Biofloc system uses the presence of microorganisms in the culture system to generate flocs from nitrogen waste, thus permitting continued water usage. Factors like carbon source, carbon to nitrogen ratio, and stocking density, affect the quality and density of microorganism and the productivity of the biofloc system. This study aims to determine the growth, feed conversion ratio (FCR), and proximate composition of catfish reared in a biofloc system using rice bran (RBB), fermented rice bran (FRB), and hydrolyzed rice bran (HRB) as carbon sources. **Methods**: Fingerling catfish of an initial mean weight of 10.55 ± 2.60g were stocked in outdoor 200-liter plastic tanks in a randomized design with the three treatments in two replications. A biomass (g) to volume (l) ratio of 1:2 was maintained throughout the experiment. The carbon-nitrogen contents in the feed, water, and treatment carbon were used to ensure an overall 15:1 C-N content in the system. **Results**: The weight parameters and FCR at the end of the 8-week rearing trials were significantly higher (P˂0.05) for the catfish cultured in FRB and HRB treatment, with no significant difference between the two. The highest weight gain (46.55g) was recorded in FRB treatment while the lowest value of weight gain (40.50g) was in RBB. The crude protein (65.44%) was significantly higher (P˂0.05) in the FRB and lowest in the RBB, with values of (61.85%). The crude protein and lipid of the biofloc produced in the system were not significantly different (P<0.05) between FRB and HRB treatment. The moisture content was highest in FRB while the ash was lowest in HRB. **Conclusions**: This experiment showed that acid-hydrolysis and solid-phase fermentation of rice bran could boost its performance as a biofloc carbon source, even though latter has been well reported. |

*Keywords: Acid-hydrolyzed rice bran, Biofloc, Catfish, Carbon sources, Fermented rice bran.*

1. INTRODUCTION

“Aquaculture has become an important component of global food security. As the world's population projected to reach 9.7 billion by 2050” (Gichuki et al., 2024), the demand for fish protein is expected to increase significantly. Traditional capture fisheries are unable to meet this growing demand due to overfishing, habitat destruction, and climate change. As a result, aquaculture has emerged as a sustainable alternative to provide the necessary fish protein to support human population growth (Tacon and Shumway, 2024; Babatunde et al., 2019). One of the challenges facing aquaculture is its sustainability and environmental impact. Conventional fish farming methods often rely on high inputs of feed, water, and energy, leading to issues such as water pollution, resource depletion, and greenhouse gas emissions. To address these challenges, innovative fish culture technologies like aquaponics and biofloc have been developed (Nair et al., 2025; Chauhan and Mishra, 2022). Biofloc technology (BFT) is an innovative approach to sustainable aquaculture. The biofloc system relies on the cultivation of microbial communities, known as bioflocs, which consist of bacteria, fungi, algae, and other microorganisms. These bioflocs convert nitrogenous waste, such as ammonia and nitrite, into microbial biomass that can be consumed by the fish as a supplementary food source (Crab et al., 2012). This process not only improves water quality but also reduces the need for external feed inputs, making the biofloc system more sustainable and cost-effective. The biofloc system results in a greater growth rate and production of fish compared to other production approaches because the floc acts as a food supplement for the fish in the system (Haridas et al., 2017; Minabi et al., 2020). Both the quantity and quality of flocs created are significantly impacted by the type of carbon sources used (Li et al., 2023). Grain, sugarcane bagasse, chopped hay, and tapioca are just a few of the many materials that have found application as carbon sources in the biofloc system (Ahmad et al., 2016; Bakhshi et al., 2018; Dauda et al., 2017). Materials made of simple and complex sugars typically perform better than less expensive cellulose and lignified counterparts. Because of this, it is desirable to treat cellulose and lignin in a way that increases their carbon release for use in biofloc. In the biotech industry, acid hydrolysis is utilized to create reducing sugar from rice bran (Sutanto et al., 2017), and fermentation is employed to boost the nutritional value (Christ-Ribeiro et al., 2021). Nutritionally quality assessment of African catfish reared in biofloc system using untreated, yeast fermented, and acid hydrolyzed rice bran as carbon sources was conducted in this study.

2. material and methods

**Experimental setup**

The experiment was carried out in the biological garden, Umaru Musa Yar’adua University from the period of September to November, 2023. Fingerlings of African catfish, *Clarias* *gariepinus*, of 10.55 ± 2.60g, initial weight were obtained from hatchery-reared breeding and kept in 200-liter plastic tank in aerated water for 7 days acclimation period. The fish were fed diet at 5% biomass twice daily (8 - 9.00 hrs) and (17 – 18.00 hrs). The fish were randomly distributed to the three experimental treatments of untreated, fermented and hydrolyzed rice bran biofloc in a triplicate of 20 fishes per tank at 2:1 biomass (g) to volume (l) ratio. Each experimental setup was seeded with 2 litres of water from a pre-fertilized earthen fish pond containing abundant phytoplankton.

**Fermentation and acid hydrolysis of rice bran**

The milled rice bran was sieved through a 0.50 mm sieve and sterilized in an autoclave. The fermentation procedure was adapted from Chinma et al. (2014) with slight modifications. 50g of rice bran was mixed with 45ml of distilled water, and 5g of baker's yeast dissolved in 5ml of water was added to achieve a 1:1 weight-to-volume ratio of rice bran to water. The mixture was incubated in a beaker at 27ºC for 24 hours. The fermented product was then oven-dried at 45ºC for 6 hours, powdered, sieved through a 100 µm mesh, and subsequently used as a carbon source. Acid hydrolysis of the rice bran was performed using 50ml of 2% sulfuric acid mixed with 50g of rice bran, and the mixture was incubated at 90ºC for 3.5 hours, as modified from Sutanto et al. (2017). The hydrolysate product was oven-dried at 45ºC for 6 hours, powdered, and sieved through a 100 µm mesh. The fermented rice bran (FRB) and hydrolyzed rice bran

**Water quality and floc monitoring**

Water parameters were measured every two weeks. Temperature (° C) was determined using a mercury in glass thermometer while the pH was measured using a Metrohm Herisau E520 pH meter. Dissolved oxygen concentration was determined through the Winkler-Azide method (APHA, 1995). Chemical oxygen demand (COD) was determined titrimetrically, while biological oxygen demand (BOD) was determined using the incubation method at 20 oC for five days (APHA, 1995). The total ammonium nitrate concentration was determined using the spectrophotometric method (APHA, 1995). By assuming 16% of protein is nitrogen (Craig et al., 2017) and 46% carbon in rice bran (Choi, 2020), the amount of carbon to be added was calculated following (Avnimelech, 1999). The total feed consumed per day was estimated and the C:N were adjusted daily to 15:1 by adding treatments C of untreated, fermented, or hydrolyzed rice bran. The treatment carbon was mixed with 1 liters of water from the treatment before added to the experimental setup. Biofloc volume ((mL/L) was measure every 14-days for each experimental treatments using Imhoff cone. The floc solution was allowed to settle down for one hour before the reading was taken.

**Growth analysis**

At the end of the experiment, all fishes in the tank were counted and the survival rate was determined. Growth performance in each treatment was estimated by weighing 10 randomly selected fishes from each treatments and replicates. The following parameter were estimated:

Weight gain = [Mean final weight − Mean initial weight],

Specific growth rate (%∕day) = (ln mean final weight − ln mean initial weight)/ no. of culture

 days ×100,

Survival (%) = (Number of harvested fish/ number of stocked fish) × 100,

FCR = Total Feed fed (g)/Total wet weight gain (g)

**Proximate analysis**

At the end of the 8-week experiment, biofloc and fish samples were obtained from each treatment for proximate analysis. 100g samples floc and fish muscle were dried separately in an oven at 55 °C until constant weight and stored in desiccators for further analyses. The proximate composition of biofloc and catfish was analyzed in duplicated according to (AOAC, 2000). Moisture content was determined by oven drying 10g fresh sample at 105º C for 2 hrs and cooled in desiccators. Ash was determined by incineration of dried sample at 600 °C for 6 h. Crude protein was determined by Kjedal method. Solvent extraction method was employed for the determination of crude fat by using 5g dried sample in 200 ml petroleum ether B.P 40–60C for 3h.

**Statistical analysis**

Statistical analysis was done using SPSS version 23 for Windows. One-way analysis of variance (ANOVA) was used to determine the significant differences among treatments. The significant level was set at 5% (P < 0.05); Duncan’s multiple range tests were used for post hoc comparison of mean between different groups.

3. results

**Water quality parameters**

In the present study, temperature did not differ significantly among all treatments (Table 1). However, a significant reduction (p < 0.05) in the level of dissolved oxygen (DO) was observed in FRB (6.05 ± 0.50 mg/L) compared to RBB (6.55 ± 0.80 mg/L) and HRB (6.50 ± 0.10 mg/L). The highest DO and COD level was recorded in the RBB treatment (Table 1). Average value for biological oxygen demand (BOD), total dissolved solid (TDS), and pH were highest in RBB treatment, while TDS and BOD had highest average value in FRB treatment. HRB treatment recorded the highest average value for nitrite.

**Table 1. Physicochemical parameters of water in Catfish biofloc system where rice bran (RBB), fermented rice bran (FRB) and hydrolyzed rice bran (HRB) were used as carbon sources.**

|  | **Substrate types** |  |
| --- | --- | --- |
| Parameters | Rice bran (RBB) | Fermented rice bran (FRB) | Hydrolyzed rice bran (HRB) | SignificantLevel |
| Temp 0C | 25.80 ± 0.50a | 26.50 ± 0.50 a | 26.50 ± 0.50 a | P > 0.05 |
| pH | 7.55 ± 0.10a | 7.45 ± 0.45b | 6.90 ± 0.50c | P < 0.05 |
| DO (mg/l) | 6.55 ± 0.80a | 6.05 ± 0.50ab | 6.50 ± 0.10b | P < 0.05 |
| TDS (mg/l) | 205.00 ± 15.00a | 273.00 ± 25.60b | 255.00 ± 21.80c | P < 0.05 |
| COD (mg/l) | 145.50 ± 10.20a | 125.10 ± 12.50b | 105.00 ± 15.50ab | P < 0.05 |
| BOD (mg/l) | 55.10 ± 10.00a | 61.50 ± 15.50a | 45.50 ± 21.50b | P < 0.05 |
| Nitrate (mg/l) | 3.30 ± 0.55a | 2.50 ± 0.20b | 2.50 ± 0.35b | P < 0.05 |

*Means with a different superscript in the same row are significantly different (P<0.05)*

**Growth performance**

The growth parameters and survival of the catfish in biofloc system after 8 weeks rearing (Table 2) showed that treatment of rice bran through solid phase fermentation and acid hydrolysis enhanced its utilization as carbon sources in biofloc as weight gain and specific growth rates were higher compared with untreated rice bran. The highest weight gain (46.55g) was recorded in FRB treatment while the lowest value of weight gain (40.50g) was in RBB. The feed conversion ratio was also highest in HRB treatment. The survival was however lowest in HRB and this was significant (P ˂ 0.05).

**Table 2. Growth parameters of Catfish biofloc system where rice bran (RBB), fermented rice bran (FRB) and hydrolyzed rice bran (HRB) were used as carbon sources.**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters | Rice bran (RBB)  | Fermented rice bran(FRB) | Hydrolyzed rice bran(HRB) |
| Initial weight (g) | 10.55 ± 2.60a | 10.55 ± 2.60a | 10.55 ± 2.60a |
| Final weight (g) | 51.15 ± 2.10a | 57.50 ± 2.7b | 55.20 ± 2.5b |
| Weight gain (g/fish) | 40.50 ± 2.80a | 46.55 ± 1.35b | 44.95 ± 1.90c |
| Specific growth rate | 1.22 ± 0.12 | 1.31 ± 0.19 | 1.28 ± 0.11 |
| Feed conversion ratio | 1.66 ± 0.05a | 1.49 ± 0.07b | 1.53 ± 0.04b |
| Survival% | 90 ± 0.5a | 95 ± .14 a | 86 ± 1.52 b |

*Means with a different superscript in the same row are significantly different (P<0.05)*

**Proximate composition**

The results of the proximate composition showed that catfish utilizing fermented rice bran had significant higher (P<0.05) crude protein (65.44%) and moisture content (75.03%), followed by the HRB treatment with crude protein (63.10%) and moisture (73.17% (Table 3). The crude lipid was lowest (P < 0.05) in RBB treatment, while, the catfish crude lipid was not significantly different between FRB and HRM treatment. For the biofloc, the crude protein and lipid were not significantly different (p < 0.05) between FRB and HRB treatment. The moisture content was highest in FRB while the ash was lowest in HRB.

**Table 3. Proximate composition of floc and Catfish reared in biofloc system where rice bran (RBB), fermented rice bran (FRB) and hydrolyzed rice bran (HRB) were used as carbon sources.**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters (%) | Rice bran (RBB)  | Fermented rice bran(FRB) | Hydrolyzed rice bran(HRB) |
| **Catfish** |  |  |  |
| Crude protein | 61.85 ± 1.5b | 65.44 ± 1.30a | 63.10 ± 0.80b |
| Crude lipid | 7.1 ± 0.10b | 8.80 ± 0.7a | 8.72 ± 0.5a |
| Moisture | 71.26 ± 0.80c | 75.03 ± 0.35a | 73.17 ± 0.16b |
| Ash |  3.51±0.40c  |  2.20±0.0b  |  4.80±1.10a |
| **Floc** |  |  |  |
| Crude protein | 36.55±1.55b | 38.22±1.26a | 37.67±1.10a |
| Crude lipid | 1.34±0.57b | 1.65±0.75ab | 1.82±0.55a |
| Moisture | 81.26±2.55b | 87.15±3.20a | 82.94±3.10b |
| Ash | 15.55±1.56a | 14.72±1.15a | 12.90±1.42b |

*Means with a different superscript in the same row are significantly different (P<0.05*

**Discussion**

“The use of fermented rice bran in biofloc systems has been well-studied. Our findings suggest that acid hydrolyzed rice bran can also be effective. Given rice bran’s affordability, research has focused on enhancing its carbon release capabilities” (Mansour et al., 2022; Babatunde, 2021). Acid hydrolysis converts the starch and cellulose components of rice bran into reducing sugars (Sirohi et al., 2019), making hydrolyzed bran a promising addition to biofloc, as investigated in this study. “Our results demonstrated that all water quality parameters in the system were within the recommended range for aquaculture and catfish production” (Huynh et al., 2016). Temperature is a critical ecological factor that significantly affects fitness, growth, and metabolism in aquatic organisms (Green and Fisher, 2004). “The temperature variations in our study were not significant, indicating no external influence on the treatments used. Dissolved oxygen (DO) is a key abiotic factor influencing the growth and survival of fish” (Taylor and Miller, 2001). “A notable reduction in DO levels was observed in the FRB treatment compared to others, possibly due to higher microorganism activity in this system” (Adineh et al., 2022; Ajamhasani et al., 2024). “All treatments maintained the pH of catfish biofloc within the recommended range of 7-8.5” (Pérez-Rostro et al., 2014; Azim and Little, 2008; De Schryver et al., 2008). Recovering acid after hydrolysis is a current challenge, which may explain the significantly lower (p<0.05) pH in the HRB treatment. Survival rates were significantly higher (p<0.05) for the RBB and FRB treatments compared to the HRB treatment, potentially due to the reduced pH in HRB. Our study also observed a lower concentration of total ammonium nitrate in the HRB treatment. This aligns with findings by Abiri et al. (2022) and Ajamhasani et al. (2023), which reported that complex carbon sources like brans reduce ammonia concentration in biofloc compared to simpler carbon sources like sugars. Growth parameters for catfish in the biofloc system after 8 weeks showed that treatment of rice bran via solid-phase fermentation and acid hydrolysis enhanced its efficacy as a carbon source in biofloc, resulting in higher weight gain and specific growth rates compared to untreated rice bran. Similar results have been reported, indicating that the use of probiotics to ferment organic materials improves food efficiency and digestibility, leading to a lower feed conversion ratio (FCR) and increased specific growth rate (SGR) (Abiri et al., 2022). This study found no statistically significant difference in the crude protein and lipid content of the floc at the end of the experiment. Therefore, the performance of hydrolyzed rice bran as a carbon source for catfish production in biofloc is comparable to that of fermented rice bran. The significantly higher crude protein content observed in catfish fed with FRB (65.44%) suggests that fermentation of rice bran enhances its utilization in biofloc. This aligns with previous studies demonstrating that fermentation can improve the nutritional quality of feed ingredients by breaking down anti-nutritional factors and increasing protein digestibility (Abasubong et al., 2024). The moisture content was also significantly higher in catfish fed with FRB (75.03%), indicating that the fermentation process may increase the water-holding capacity of the feed. In contrast, the HRB treatment resulted in significantly higher ash content, indicating the presence of essential minerals (Rahman et al., 2018); it may also imply the presence of indigestible components (Atkinson et al., 1984), which could affect nutrient absorption and utilization. This requires further investigation to determine the optimum digestion of bran for biofloc utilization. The crude protein and lipid content in the biofloc (FRB and HRB) treatments were not significantly different, indicating that both treatments can effectively utilize the biofloc system without negatively impacting the nutritional composition. This finding supports the feasibility of acid hydrolyzed treatment of bran as biofloc carbon.

4. Conclusion

This study has found that treatment of rice bran through fermentation and hydrolysis could improve its performance as biofloc carbon sources for the production of *C. gariepinus* with enhanced nutritional quality of the fish. Acid hydrolyzed rice bran has potential for use in biofloc as carbon source without compromising the efficacy of the system.

Ethical approval

Fish were handled according to standard procedure and guideline on the use of animals contained in the Veterinary Surgeon Act Cap V3 LFN 2004 of the Federal Republic of Nigeria, Criminal Code Act. Cap C38 LFN 2004.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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