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Fermenting rice bran as a carbon source for biofloc technology improved the water quality, growth, feeding efficiencies, and biochemical composition of African catfish *Clarias gariepinus* juveniles

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Abstract

In this study, African catfish (*Clarias gariepinus*) (9.68 ± 0.16 g, mean ± *SE*) were reared with biofloc technology (BFT) with three different carbon sources; raw rice bran (RRB) or when incubated (24 hr) with *Bacillus species* under aeration (cellular respiration, ResRB), or without aeration (fermentation, FerRB). The proximate composition, water solubility, and total soluble sugars of RRB, ResRB, and FerRB were measured. A control included fish cultured in a recirculating system. Water quality, biofloc production/proximate composition and subsequent effects to growth, feeding efficiencies, body proximate composition, and selected plasma biochemical parameters to triplicate groups of *C. gariepinus* were measured after 42 days. FerRB had the lowest crude fibre and higher total soluble sugars and water solubility compared to ResRB or RRB. Biofloc produced with FerRB had significantly higher ($p < 0.05$) crude protein. Ammonia-N was significantly lower ($p < 0.05$) in the first 3 weeks in both control and FerRB treatments. Using FerRB significantly improved ($p < 0.05$) growth, feeding efficiencies, and whole-body crude protein in African catfish compared to the control and ResRB. Therefore, using FerRB with BFT can be a highly effective strategy to create a zero-exchange culture system while also significantly improving growth and feeding efficiencies of African catfish juveniles.

KEYWORDS
African catfish, *Bacillus*, biofloc technology, fermentation, rice bran, water solubility

1 | INTRODUCTION

Biofloc technology (BFT) system can be an environmentally friendly strategy to establish a near to zero water exchange culture system while providing potentially consumable biomass to the cultured animal (Avnimelech, 2012; Bossier & Ekasari, 2017). This operates on the principle of increasing carbon to nitrogen ratios, through the addition of an exogenous carbon source that consequently stimulates natural heterotrophic bacterial growth in the system (De Schryver, Crab, Defoirdt, Boon, & Verstraete, 2008; Hargreaves, 2006). These bacteria will then convert otherwise toxic nitrogenous metabolites into microbial biomass known as bioflocs (Avnimelech, 2012; Ebeling, Timmons, & Bisogni, 2006). Bioflocs consist of various organic materials such as bacteria, microalgae, uneaten food, and zooplankton, which can serve as a constant supply of additional nutrients for aquatic animals that are capable of collecting and consuming these small particles (Bossier & Ekasari, 2017; Emerenciano, Gaxiola, & Cuzon, 2013). The use of BFT has successfully improved the growth and feeding efficiencies in various aquatic species such

as Pacific white shrimp *Litopenaeus vannamei* (Khanjani, Sajjadi, Alizadeh, & Sourinejad, 2017; Serra, Gaona, Furtado, Poersch, & Wasielesky, 2015), tilapia *Oreochromis niloticus* (Long, Yang, Li, Guan, & Wu, 2015), and the carps *Cyprinus carpio* and *Hypophthalmichthys molitrix* (Zhao et al., 2014). Additional benefits may also include an improved immune response and/or resistance to diseases as observed in *L. vannamei* (Ekasari et al., 2014), rohu *Labeo rohita* (Ahmad et al., 2016) and African catfish *Clarias gariepinus* (Dauda, Romano, Ebrahimi, et al., 2018).

The choice of carbon sources in a biofloc-based system, however, can substantially affect the biofloc productivity/nutritive value, water quality as well as performance of the animal. For example, the use of complex carbohydrates over simple sugars led to a better growth performance to *L. vannamei* and the black tiger shrimp *Penaeus monodon*, which was believed to be due to a higher nutritional value of the bioflocs (Kumar et al., ; Serra et al., 2015). On the other hand, simple sugars or glycerol is more effective at removing ammonia-N in biofloc-based systems compared to complex carbohydrates (Dauda et al., 2017; Khanjani et al., 2017; Serra et al., 2015). In the case of *C. gariepinus*, the slower utilization of rice bran subsequently led to excessive ammonia-N levels, which was believed to substantially reduce survival in a biofloc-based system (Dauda et al., 2017). It has been suggested that water quality management could be achieved by enhancing the water solubility of rice bran through pre-treatments with microorganisms, enzymes and/or fermentation (Dauda et al., 2017; Ekasari et al., 2014).

Microorganisms, such as some fungi or bacteria, are known to produce various hydrolytic enzymes that can reduce the fibre content of complex carbohydrates and thus improve their water solubility (Karaki, Aljawish, Humeau, Munigila, & Jasniewski, 2016; Maisonnier-Grenier, Clavurier, Saulnier, Bonnin, & Geraert, 2006; Oduguwa, Edema, & Ayeni, 2008; Supriyati Haryati, Susanti, & Susana, 2015). For example, fermenting rice bran with the bacterium *Bacillus amyloliquefaciens* decreased and increased the crude fibre and protein content respectively (Supriyati, et al., 2015). Similarly, fermenting pearl millet, *Pennisetum glaucum* or *Moringa oleifera* seed powder decreased their fibre content along with increasing water solubility (Oloyede, James, Ochenne, Chinma, & Akpa, 2015; Osman, 2011). Therefore, it appears possible that pre-treating complex carbohydrates with microorganisms may improve water solubility by decreasing the fibre content and thus enhance their potential use as a carbon source in a biofloc-based system. If successful, this could allow more flexibility in the choice of carbon sources, which in the case of rice bran, is a low-cost agricultural by-product that is readily available in various Asian countries (Oladosu et al., 2016).

The aim of this study was to compare the use of raw rice bran (RRB) with those that were treated with *Bacillus* spp., with or without aeration, in a biofloc-based culture system with *C. gariepinus* over 42 days. Various water quality parameters, biofloc production/proximate composition and subsequent effects to the growth, feeding efficiencies, and biochemical composition of *C. gariepinus* juveniles were determined.

2 | MATERIALS AND METHODS

2.1 | Rice bran preparation and analysis

Raw rice bran was obtained from a livestock feed ingredient store in Serdang, Selangor, then hammer milled to a fine powder and passed through a sieve (425 μm). For pre-treatments, the rice bran was then incubated with commercial *Bacillus megaterium* and *B. licheniformis* with aeration (cellular respiration, ResRB) or without aeration (fermentation, FerRB). The reason for using aeration was based on the methodology used for an Aquamimicry protocol that was developed in Thailand and later described by Romano (2017). For both ResRB and FerRB, a solid-state treatment was performed each day with a commercial synbiotic product, White cap™ (Engest® produced by Baxel Co, Ltd., Thailand) according to the manufacturer's recommendations. This was done by adding 1 ml of this concentrate in 10 L of water, followed by adding 5 kg of rice bran. After mixing thoroughly, this was covered with a black polythene nylon, with or without aeration, for approximately 24 hr, with a temperature range of 28–30°C during the incubation period. White cap™ (synbiotic product) contained a combination of pre- and probiotics, *B. megaterium* and *B. licheniformis* at a total colony forming unit of 1×10^9 and the hydrolytic enzymes included amylase and protease (CAS No 68920-42-3).

The proximate composition of RRB as well as ResRB and FerRB were measured according to standard methods (AOAC, 2012). The estimated organic carbon content was determined using the formula of Hart, Lovis, Schulenberg, and Urquhart (2007) as follows,

$$\text{Organic carbon} = 0.80 \times \text{lipid} + 0.53 \times \text{protein} + 0.42 \times \text{fibre} + 0.42 \times \text{Nitrogen free extract}$$

The total soluble sugars of these rice bran samples were determined according to Irigoyen, Emerich, and Sanchez-Diaz (1992). Briefly, 0.2 g of freshly prepared RRB, ResRB, and FerRB were homogenized in 10 ml of 96% (v/v) ethanol and washed with 5 ml of ethanol 70% (v/v) and then centrifuged at 1,055 *g* for 10 min. The supernatant was gently decanted, briefly kept at 4°C and 0.1 ml was added to 3 ml of an anthrone reagent (150 mg anthrone with 100 ml of 72% (v/v) sulphuric acid). This mixture was heated in a boiling water bath (100°C) for 10 min, then after cooling to room temperature, the absorbance was read at 625 nm. A standard curve was produced using increasing glucose concentrations and the slope (*K*) was used to determine the total soluble sugars in the samples using the following equation according to Bano et al. (2013).

$$\text{Total soluble sugars (mg/g)} = [\text{Absorbance of sample} \times K \text{ value} \times \text{dilution factor} \times 1000]/\text{weight of sample}$$

Meanwhile, the water solubility (WSI) and water absorption (WAI) indices of the RRB, ResRB, and FerRB were determined following the methods described by Romano et al. (2018) with slight modifications. Briefly, the rice bran samples were oven-dried at 60°C and then sieved (200 μm mesh size). Next, 2.5 g (Wds) of each sample was added to a 50 ml centrifuge tube filled with 30 ml of distilled water, which was kept at 30°C for 30 min, shaken gently

intermittently, and then centrifuged at 905 *g* for 10 min. The supernatant was added to a previously weighed (Wai) aluminium dish and after drying at 135°C for 2 hr, the aluminium dish with the recovered solid was weighed (Waf). The weight of the leftover gel in the centrifuge tube was recorded as Wg. The WSI and WAI were evaluated using the following equations,

$$\mathrm{WSI}\,(\%) = [(\mathrm{Waf} - \mathrm{Wai}) \times 100]/\mathrm{Wds}$$

$$\mathrm{WAI} = \mathrm{Wg}/\mathrm{Wds}$$

2.2 | Source of experimental animal, acclimation, and tank preparation

A total of 600 *C. gariepinus* juveniles with an average size of 8.5 g (±*SE* of 0.28) were purchased from a commercial farm in Serdang, Malaysia and transferred in oxygenated polythene bags to the aquaculture experimental research station at Universiti Putra Malaysia (UPM) (Puchong). The fish were acclimated in two previously prepared 1,000 L fibreglass tanks filled with 600 L of water that were gently aerated with two aeration stones. The fish were fed twice daily at approximately 3% body weight with a commercial fish feed designed for groupers (Star Feedmills (M) Sdn. Bhd, with 43% crude protein and 6% lipid). The acclimation period was 10 days and during this time a 50% water change was carried out every 3 days.

During the acclimation period, an inoculation tank was prepared to produce bioflocs. This was done by collecting 200 L of wastewater from a reservoir tank (2000 L capacity) that housed 20 African catfish broodstock (approximately 2 kg each), which were fed to apparent satiation once daily with a commercial feed of 6 mm size and 32% crude protein (Star Feedmills (M) Sdn. Bhd). The collected wastewater (200 L) was equally added into two 180 L capacity glass inoculation aquaria. Each tank was vigorously aerated with four aeration stones and glycerol was added every other day at a ratio of 6:1 of the ammonia-N in order to promote faster biofloc production.

Meanwhile, 12 glass aquaria (180 L capacity; 90 cm × 45 cm × 45 cm) were prepared for the experiment by covering the tops with nylon netting to prevent any potential escapees. Allocation of treatments was then done randomly. Afterwards, four aeration stones that were connected to a central blower system were set up to provide vigorous aeration in the nine BFT tanks. Three tanks were used as control treatment, and had two airstones each and were equipped with a biofilter consisting of fine meshed transparent plastic material (34 cm × 6 cm), and a bio-sponge mat (polyethylene terephthalate) within a plastic box (42 cm × 15 cm). A submersible pump (17 Watts, model P-708) was attached to the box to circulate the water at a rate of 0.08 l/s (or 273.6 l/day).

One day prior to stocking of the fish, the bioflocs produced from the inoculation aquaria were sieved with a 100 µm mesh size sieve. A total of 10 L of this biofloc filtrate was added to each of the nine glass aquaria used for the BFT treatments. Afterwards, 90 L of freshwater was added to create a total volume of 100 L. Therefore, all aquaria (control and BFT treatments) had an initial water volume of 100 L. The photoperiod was ambient at 13 light:11 dark. This study was conducted in accordance with the Animal Ethics Committee of UPM (IACUC 101 for Research).

2.3 | Experimental design

All 12 tanks were stocked with 25 juveniles of African catfish (9.68 ± 0.16 g, mean ± *SE*) and each of the four treatments (control, RRB, ResRB and FerRB) was triplicated. The fish were fed at 3% body weight with the same commercial diet used for acclimation and the fish were sampled weekly to adjust for the amount of feed supplied to the new weight. In the BFT treatments, rice bran was added at a carbon to nitrogen ratio of 15, following the methods of De Schryver et al. (2008).

The control (RAS) treatment received a weekly 50% water exchange to prevent the accumulation of nitrate-N, whereas the BFT treatments were meant to be a zero-exchange culture system. However, a 50% water exchange in all the BFT tanks was performed on day 20 due to high amounts of ammonia-N in the RRB and ResRB treatments. The water exchange performed in FerRB was not out of necessity, as the ammonia-N levels were low, and only done to keep conditions consistent. Meanwhile, there was also a daily addition of 0.1 to 0.3 g of agricultural hydrated lime to each tank as a buffer to prevent the pH from decreasing below 7 because the treated rice bran had pH of between 4.8 and 5.6. The water temperature ranged between 25.81°C and 26.78°C during the cultivation period.

2.4 | Measurements of biofloc formation and proximate composition

The biofloc volume was measured weekly by filling an Imhoff cone with 1 L of water and, after allowing the bioflocs to settle for 30 min, the volume was recorded. The settled floc was then collected with a plankton net of 60 µm mesh size, excess water was drained and the wet weights were measured. Samples were then oven-dried at 60°C overnight and the biofloc dry weights were recorded.

The bioflocs collected from each of the BFT treatment tanks were taken on day 42, stored at −20°C and analyzed for the proximate composition within 1 week according to the standard methods of AOAC (2012).

2.5 | Water quality

The pH, dissolved oxygen (DO), and total dissolved solids (TDS) were measured twice a week using a digital multiparameter probe (YSI 556, MPS) and the weekly mean was reported. Ammonia-N, nitrite-N, and total suspended solids (TSS) were measured twice each week and the weekly mean was presented. Ammonia-N and nitrite-N were measured using a portable spectrophotometer (HACH DREL/2400) while the TSS was measured gravimetrically following standard methods of AOAC (2012).

2.6 | Measurements of fish growth, survival, body indices, and production parameters

On week 3 and 6, a total of three fish in each replicate were randomly chosen and removed, euthanized in clove oil, and the livers were dissected out for later histopathological analysis (Section 10).

One day prior to final sampling (week 6) of all remaining fish, no additional feeds were provided. The following day, all fish in each tank removed, euthanized with clove oil, and then individually counted and weighed. Five fish from each tank were randomly selected for blood collection and analyzed for plasma biochemistry (Section 9), and then dissected to remove and weigh the viscera and liver to calculate the viscerosomatic index (VSI) and hepatosomatic index (HSI) respectively. From the remaining fish (n = 12–15/replicate), these were measured for the whole-body proximate composition according to standard AOAC methods (AOAC, 2012).

Various parameters for production performance were calculated from the obtained data using the following equations:

$$\text{Weight gain}(\%) = [(\text{mean final weight}(\text{g}) - \text{mean initial weight}(\text{g})) / \text{mean initial weight}(\text{g})] \times 100$$

$$\text{Specific growth rate (SGR)}(\%/day) = [(\ln \text{mean final weight} - \ln \text{mean initial weight}). / \text{number of culture days}] \times 100$$

$$\text{Feed intake}\ (\text{g}) = \text{total sum of feed consumed}\ (\text{g})\ \text{per fish after 42 days}$$

$$\text{Food conversion ratio (FCR)} = \text{Total feed intake per fish}\ (\text{g}) / \text{wet weight gain per fish}(\text{g})$$

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

$$\text{Input/output ratio} = (\text{total feed intake} + \text{total carbon sources supplied})(\text{g}) / \text{weight gain}(\text{g})$$

$$\text{Viscerosomatic index (VSI)}\ (\%) = (\text{weight of visceral}(\text{g}) / \text{weight of fish}(\text{g})) \times 100$$

$$\text{Hepatosomatic index (HSI)}\ (\%) = (\text{weight of the liver}(\text{g}) / \text{weight of the fish}(\text{g})) \times 100$$

2.7 | Plasma biochemical composition

The blood was collected with needle (GT 27) attached to a syringe (1 ml), which was inserted into the caudal vein. The blood from all fish in each replicate was pooled and then centrifuged at 603 *g* for 10 min. The plasma was then stored at −20°C and the glucose, triglycerides, bilirubin, and cholesterol were measured the following day on a Hitachi 902 automatic analyzer (Boehringer Mannheim Diagnostics, Indianapolis).

2.8 | Liver histopathological examination

After 24 hr in 10% (v/v) phosphate buffered formalin, the livers were transferred to ethanol 70% (v/v) until being further processed, embedded, and sectioned (6 μm). Sections were stained with hematoxylin and eosin or Periodic-acid Schiff according to Romano et al. (2018).

2.9 | Statistical analysis

All data are presented as mean ± standard error. Prior to statistical analysis, the results were tested for normality and heterogeneity of variances, where necessary data transformation was done. All the parameters were analyzed using a one-way ANOVA. If significant differences were detected ($p < 0.05$), a Duncan's multiple range test was used to identify differences among the treatments. All analyses were performed using SPSS version 23.

3 | RESULTS

3.1 | Proximate composition, water solubility/water absorption indices of raw or pre-treated rice bran

The proximate composition, total soluble sugars, WSI, WAI, and organic carbon content of the RRB, ResRB, and FerRB are shown in Table 1. Pre-treating increased the crude protein and lipid contents in the rice bran, but higher increases were observed for ResRB. The ash content and nitrogen free content decreased and increased respectively in FerRB while the opposite was observed in ResRB. Meanwhile, pre-treating rice bran decreased both the fibre content

TABLE 1 Proximate composition (% dry weight), total soluble sugars, water solubility index, and water absorption index (n = 3) of raw rice bran (RRB), and rice bran treated with *Bacillus* spp. with (ResRB) or without aeration (FerRB)

	RRB	ResRB (%Δ)	FerRB (%Δ)
Crude protein	16.66	18.51 (+11.12)	17.51 (+5.07)
Crude lipid	10.38	11.92 (+14.79)	11.48 (+10.54)
Crude ash	10.98	11.01 (+0.27)	9.90 (−9.84)
Crude fibre	14.40	14.27 (−0.90)	13.07 (−9.24)
NFE*	47.58	44.29 (−6.92)	48.04 (+0.97)
Carbohydrate**	61.98	58.56 (−5.52)	61.11 (−1.40)
Total soluble sugar (mg/l)	3.69	3.77 (+2.02)	5.50 (+48.99)
Water solubility index (%)	12.23	19.07 (+55.93)	21.52 (+96.00)
Water absorption index	2.84	2.86 (+0.70)	2.63 (−7.40)
Organic carbon content (%)	43.17	43.94 (+1.78)	44.13 (+2.22)

Note. Percent change (%Δ) = (value of treated rice bran − value of raw rice bran) × 100/value of raw rice bran.

*Nitrogen free extract (NFE) = 100 − (% crude protein + % crude lipid + % crude ash + % crude fibre).

**Carbohydrate (CHO) = 100 − (% crude protein + % crude lipid + % crude ash).

and estimated carbohydrate, but a greater reduction in fibre content occurred in FerRB. There was a similar increase in the organic carbon content in the pre-treated rice bran, along with the total soluble sugars, but this was higher in the FerRB. The WAI slightly increased and decreased in ResRB and FerRB respectively. The WSI was increased in both ResRB and FerRB, but this was substantially higher in FerRB.

3.2 | Selected water quality parameters

The overall and weekly results of the water quality parameters are shown in Table 2 and Figure 1a,b respectively. There was no significant difference ($p > 0.05$) in DO or pH among the treatments. The total dissolved and TSS were both significantly higher ($p < 0.05$) in BFT groups than the control, but were not significantly different ($p > 0.05$) among the BFT groups. Overall, there were no significant differences ($p > 0.05$) in ammonia-N or nitrite-N among the treatments, but weekly analysis showed some differences. This included ammonia-N being significantly higher ($p < 0.05$) in RRB and ResRB treatments than either the control or FerRB during the first 3 weeks (Figure 1a). Weekly results of nitrite-N (Figure 1b) also showed some significant differences ($p < 0.05$), which were significantly higher in the control in week 1 than all others and, then during week 4, nitrite-N was significantly higher in the RRB treatment than the other treatments.

3.3 | Biofloc formation and proximate composition

There was no significant difference ($p > 0.05$) in the overall results of any of the biofloc formation parameters, but the weekly performance showed some significant differences (Figure 2a–c). The biofloc volume was higher significantly ($p < 0.05$) in RRB than FerRB in week 1, while in week 4, RRB and FerRB had significantly higher biofloc volume compared to ResRB. During week 6, the biofloc volume was significantly higher in FerRB compared to ResRB (Figure 2a).

The biofloc wet weight was significantly higher ($p < 0.05$) in RRB than other treatments in weeks 2 and 3 (Figure 2b). For the biofloc dry weight, RRB was significantly higher ($p < 0.05$) than others in weeks 1 and 2, while both RRB and FerRB were significantly higher ($p < 0.05$) than ResRB in weeks 3 and 4 and finally during week 6, the biofloc dry weight was significantly higher in FerRB than either RRB or ResRB.

The proximate composition of the bioflocs is shown in Table 3. The crude protein was significantly different ($p < 0.05$) among the treatments in the order of FerRB > ResRB > RRB. The estimated carbohydrate was also higher significantly ($p > 0.05$) in RRB and ResRB than FerRB but there were no significant differences ($p > 0.05$) in moisture, lipid, or ash content among the treatments.

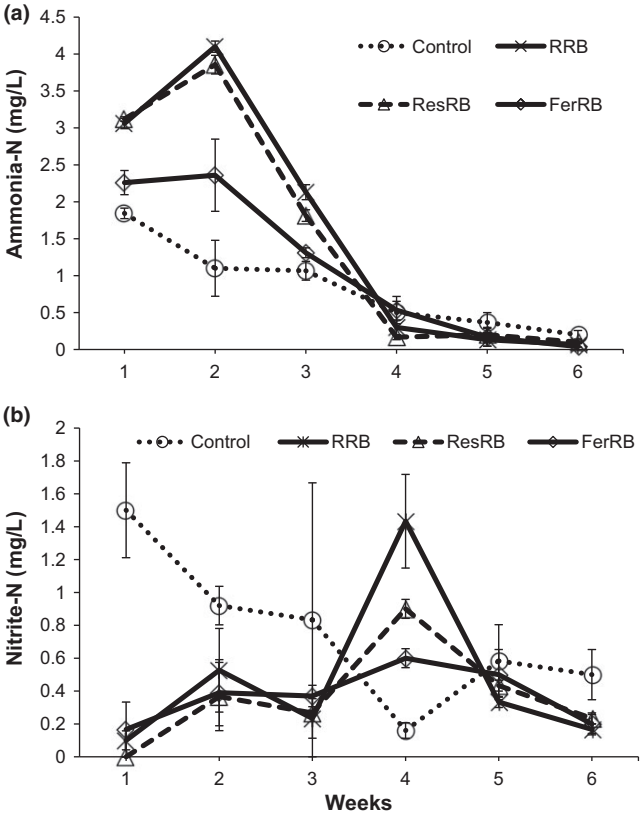

FIGURE 1 Mean (±*SE*) weekly ammonia-N (a) and nitrite-N (b) levels (mg/l) in the control or biofloc-based systems that used raw rice bran (RRB) or rice bran treated with *Bacillus* spp. with (ResRB) or without aeration (FerRB) as the carbon source for rearing African catfish juveniles over 6 weeks

TABLE 2 Mean (±*SE*) selected water quality parameters ($n = 3$) when culturing African catfish juveniles over 6 weeks in the control or with biofloc technology (BFT) when using raw rice bran (RRB) or rice bran treated with *Bacillus* spp. with (ResRB) or without aeration (FerRB) over 6 weeks

Parameters	Control	RRB	ResRB	FerRB	*p*-Value
Dissolved oxygen (mg/l)	6.83 ± 0.21	6.88 ± 0.23	6.97 ± 0.21	6.79 ± 0.21	0.942
pH	7.90 ± 0.07	7.89 ± 0.07	8.00 ± 0.09	7.99 ± 0.06	0.417
Total dissolved solid (mg/l)	207.11 ± 4.16^{b}	245.17 ± 8.16^{a}	260.06 ± 8.01^{a}	255.39 ± 6.88^{a}	0.001
Total suspended solid (mg/l)	182.50 ± 19.42^{b}	578.06 ± 52.43^{a}	508.89 ± 43.16^{a}	570.28 ± 59.48^{a}	0.001
Ammonia-N (mg/l)	0.85 ± 0.15	1.63 ± 0.38	1.54 ± 0.37	1.11 ± 0.24	0.220
Nitrite-N (mg/l)	0.75 ± 0.16	0.47 ± 0.12	0.37 ± 0.07	0.37 ± 0.06	0.064

Note. Different letters as superscript in each row indicate significant differences ($p < 0.05$).

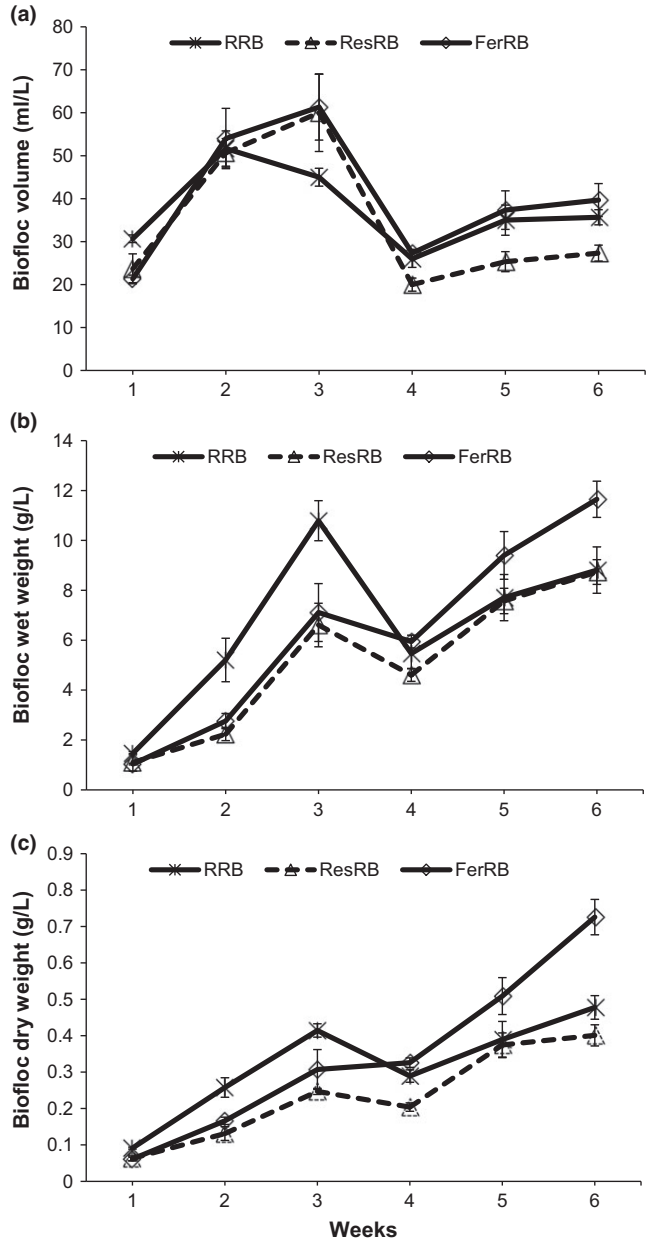

FIGURE 2 Mean (±*SE*) weekly biofloc volume (ml/l) (a), biofloc wet weight (g/l) (b), and biofloc dry weight (g/l) (c) in biofloc-based system that used raw rice bran or rice bran treated with *Bacillus* spp. with (ResRB) or without aeration (FerRB) as carbon source for rearing African catfish juveniles over 6 weeks

3.4 | Growth, feed efficiencies, production parameters, and whole-body proximate composition

The growth parameters, feeding efficiencies, survival, and body indices of African catfish in the control or with BFT using differently processed rice bran are shown in Table 4. The highest weight gain and specific growth rates were observed in FerRB, which were significantly higher ($p < 0.05$) than the control or ResRB treatments. Meanwhile, the FCR in African catfish juveniles was significantly lower ($p < 0.05$) in FerRB compared to ResRB and an opposite trend was observed with the PER. The lowest input/output ratio was observed in the control, which was significantly lower ($p < 0.05$) than all other groups. Among the BFT groups, the input/output ratio was significantly lower ($p < 0.05$) in FerRB compared to ResRB. The highest survival was recorded in FerRB, which was different significantly ($p < 0.05$) from that of the ResRB, but not from the control or RRB groups. Both the VSI and HSI values were not significantly different ($p > 0.05$) among the treatments.

TABLE 3 Mean (±*SE*) proximate composition of biofloc (% dry weight) ($n = 3$) collected from African catfish-based biofloc technology (BFT) system after 6 weeks of the experimental duration when using raw rice bran (RRB) or rice bran treated with *Bacillus* spp. with (ResRB) or without aeration (FerRB)

Parameter	RRB	ResRB	FerRB	*p*-Value
Moisture	91.27 ± 0.25	92.28 ± 0.16	92.30 ± 0.47	0.102
Protein	27.06 ± 0.85^{c}	29.79 ± 0.69^{b}	36.35 ± 0.64^{a}	0.001
Lipid	3.01 ± 0.25	3.19 ± 0.17	2.84 ± 0.25	0.592
Ash	10.31 ± 0.61	11.03 ± 0.79	11.34 ± 0.10	0.478
CHO*	59.62 ± 1.48^{a}	55.99 ± 1.13^{a}	49.47 ± 0.44^{b}	0.002

Note. Different letters as superscript in each row indicate significant differences ($p < 0.05$).
*Carbohydrate (CHO) = 100 − (% crude protein + % lipid + % ash).

The whole-body proximate composition of the fish in the different treatments is shown in Table 5. The crude protein was significantly higher ($p < 0.05$) in African catfish juveniles from FerRB than all other treatments. Crude lipid was significantly ($p < 0.05$) higher in all the BFT treatments than the control, and among the BFT treatments, crude lipid was significantly higher ($p < 0.05$) in RRB than all other groups. The ash and moisture content were not significantly different ($p > 0.05$) among the treatments. Meanwhile, the estimated carbohydrate content in African catfish juveniles was significantly higher ($p < 0.05$) in both the control and ResRB compared to the FerRB group.

3.5 | Plasma biochemistry and liver histology

The plasma biochemistry in African catfish in the different treatments is shown in Table 6. Plasma glucose was significantly higher ($p < 0.05$) in fish from the control and RRB than either ResRB or FerRB groups, while triglycerides were significantly higher ($p < 0.05$) in RRB than all other treatments. No significant differences were observed ($p > 0.05$) for bilirubin or cholesterol levels.

Liver sections of African catfish after 3 and 6 weeks in the control group (Figure 3a,b) showed normal structure but those cultured after 3 weeks in the RRB (Figure 3c) and ResRB (Figure 3e) showed some instances of necrosis, pyknotic nuclei, and cellular degeneration. Some instances of inflammatory responses, which included the presence of white blood cells and lipofuscin-like material were observed after 6 weeks (Figure 3d). The liver of catfish in the FerRB treatments showed no pathology after either week 3 (Figure 3g) or 6 (Figure 3h). In addition, those in the FerRB treatments had

TABLE 4 Mean (±*SE*) growth production parameters (n = 3) of African catfish juveniles reared in the control or biofloc technology (BFT) when using raw rice bran (RRB) or rice bran treated with *Bacillus* spp. with (ResRB) or without aeration (FerRB) after 6 weeks

Parameter	Control	RRB	ResRB	FerRB	*p*-Value
Weight gain (%)	163.23 ± 10.73^{b}	181.25 ± 5.88^{ab}	149.81 ± 19.04^{b}	213.56 ± 10.32^{a}	0.032
SGR %/day	2.42 ± 0.10^{b}	2.58 ± 0.05^{ab}	2.27 ± 0.20^{b}	2.85 ± 0.08^{a}	0.047
Feed intake (g)	15.47 ± 0.45	16.54 ± 0.36	15.41 ± 0.43	16.49 ± 0.22	0.101
FCR	0.99 ± 0.05^{ab}	0.92 ± 0.07^{ab}	1.10 ± 0.13^{a}	0.82 ± 0.03^{b}	0.049
PER	2.36 ± 0.12^{b}	2.53 ± 0.04^{ab}	2.17 ± 0.23^{b}	2.85 ± 0.10^{a}	0.045
Input/output ratio	0.99 ± 0.05^{c}	2.55 ± 0.05^{ab}	3.05 ± 1.49^{a}	2.30 ± 0.11^{b}	0.001
Survival (%)	96.00 ± 2.31^{ab}	94.67 ± 1.33^{ab}	90.67 ± 2.67^{b}	100.00 ± 0.00^{a}	0.047
VSI (%)	5.17 ± 0.26	6.05 ± 0.38	6.20 ± 0.26	5.85 ± 0.34	0.117
HSI (%)	1.27 ± 0.13	1.53 ± 0.13	1.51 ± 0.12	1.23 ± 0.07	0.154

Note. Different letters as superscript in each row indicate significant differences ($p < 0.05$) among the treatments. FCR: food conversion ratio; HIS: hepatosomatic index; PER: protein efficiency ratio; VSI: viscerosomatic index.

TABLE 5 Mean (±*SE*) whole-body proximate composition (% wet weight) (n = 3) of African catfish juveniles reared in the control or biofloc technology (BFT) system with raw rice bran (RRB) or rice bran treated with *Bacillus* spp. with (ResRB) or without aeration (FerRB) after 6 weeks

Parameter	Control	RRB	ResRB	FerRB	*p*-Value
Moisture	76.45 ± 0.23	75.77 ± 0.34	75.67 ± 0.20	75.57 ± 0.04	0.090
Protein	13.96 ± 0.18^{b}	14.13 ± 0.06^{b}	14.62 ± 0.28^{b}	15.37 ± 0.23^{a}	0.005
Lipid	4.31 ± 0.13^{c}	5.53 ± 0.13^{a}	4.95 ± 0.03^{b}	4.94 ± 0.09^{b}	0.001
Ash	3.13 ± 0.01	2.89 ± 0.06	2.97 ± 0.06	2.99 ± 0.12	0.226
CHO[a]	2.16 ± 0.22^{a}	1.68 ± 0.13^{ab}	1.79 ± 0.20^{a}	1.12 ± 0.17^{b}	0.024

Note. Different letters as superscript in each row indicate significant differences ($p < 0.05$).
[a]Carbohydrate (CHO) = 100 − (% moisture + % crude protein + % lipid + % ash).

TABLE 6 Selected mean (±*SE*) plasma parameters (mmol/l) (n = 3) in African catfish juveniles reared in the control or biofloc technology system with raw rice bran (RRB) or rice bran treated with *Bacillus* spp. with (ResRB) or without aeration (FerRB) after 6 weeks

Parameter	Control	RRB	ResRB	FerRB	*p*-Value
Glucose	4.40 ± 0.11^{a}	4.63 ± 0.18^{a}	4.00 ± 0.06^{b}	3.77 ± 0.03^{b}	0.002
Bilirubin (conjugated)	0.27 ± 0.07	0.57 ± 0.23	0.23 ± 0.09	0.43 ± 0.03	0.308
Bilirubin (total)	1.10 ± 0.21	1.27 ± 0.32	1.17 ± 0.23	1.27 ± 0.28	0.961
Cholesterol	4.33 ± 0.15	4.10 ± 0.12	3.23 ± 0.69	3.90 ± 0.47	0.366
Triglyceride	1.24 ± 0.06^{b}	1.55 ± 0.09^{a}	1.25 ± 0.05^{b}	1.25 ± 0.02^{b}	0.015

noticeably more Periodic-acid Schiff positive material in their livers at both week 3 (Figure 4g) and 6 (Figure 4h) compared to all other treatments (Figure 4a–f).

4 | DISCUSSION

There are various carbon sources to choose from when implementing BFT, but ideally, this should be both cheap and readily available. Rice bran fulfils these criteria in most Asian and African countries that farm *C. gariepinus* (FAO, 2014; Oladosu et al., 2016). However, the sole use of rice bran in biofloc-based systems can delay the removal of ammonia-N (Khanjani et al., 2017; Serra et al., 2015) and, in the case of *C. gariepinus*, was believed to be the cause for excessive mortalities (Dauda et al., 2017). It has been suggested that improving the water solubility of complex carbohydrates could facilitate the conversion of dissolved nitrogenous waste by heterotrophic bacteria into microbial biomass (Dauda et al., 2017; Ekasari et al., 2014).

In the present study, it was indeed found that incubating rice bran with *Bacillus* spp. enhanced water solubility and increased total soluble sugars, likely due to the degradation of crude fibre. However, fermentation led to the greatest change in these parameters, compared to treating rice bran with aeration (ResRB). It is known that the amount and type of enzyme production in microbes, including *Bacillus* spp., greatly depend on the amount of available oxygen (Nadeem, Qazi, & Baig, 2009). Production of starch degrading enzymes such as α-amylase and xylanase was enhanced in *Bacillus* spp. under anaerobic conditions (Divakaran, Chandran, & Pratap,

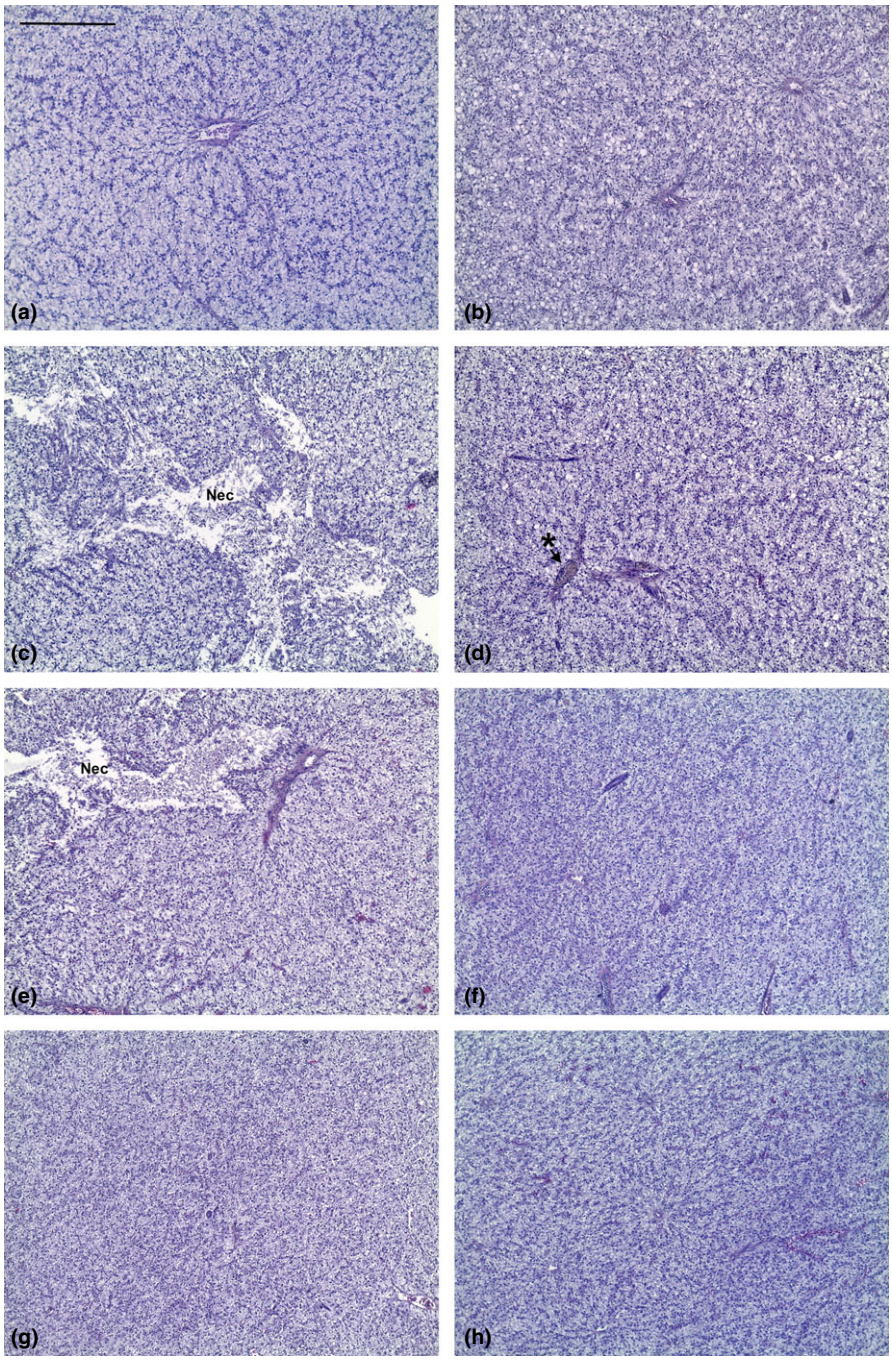

FIGURE 3 Sections of the liver in African catfish juveniles after 3 and 6 weeks of culture in the control (a, b) or in biofloc-based systems with raw rice bran (RRB) (c, d), or rice bran treated with *Bacillus* spp. with (ResRB) (e, f) or without aeration (FerRB) (g, h). There were some instances of necrosis (Nec) in the liver of fish in the RRB and ResRB treatments after 3 weeks, as well as occasional inflammatory response (asterisk) in ResRB treatments after 6 weeks. H&E staining. Arrow bar = 200 μm

2011; Irfan, Asghar, Nadeem, Nelofer, & Syed, 2016). This seems to support the lower crude fibre content in the FerRB. On the other hand, aerobic conditions can stimulate higher protease production in *B. licheniformis* (Nadeem et al., 2009), and in the current study, may have broken down the polypeptides in the rice bran into amino acids leading to higher crude protein in ResRB.

When using RRB, ResRB, or FerRB in a biofloc-based system housing *C. gariepinus* there was no significant difference to the overall biofloc production. Nevertheless, it became necessary to perform a partial water exchange after 20 days in the RRB and ResRB treatments due to ammonia-N approaching dangerously high levels. This likely explained the observed liver damage that included some instances of hemorrhaging and necrotic tissue in the RRB and ResRB treatments after 3 weeks. These lesions were occasional, and in the future more liver samples will be obtained to provide greater confidence in these observations. However, after performing a water exchange, the overall water quality became more stable and no abnormal liver histopathology was observed after another 3 weeks of culture. Moreover, the plasma bilirubin levels, which are indicators of liver function (Ebrahimi et al., 2017), were not significantly different by the end of the study. This indicated that bioflocs produced with either RRB or ResRB became better established. In contrast, the FerRB was more effective at removing ammonia-N from the system and a water exchange was

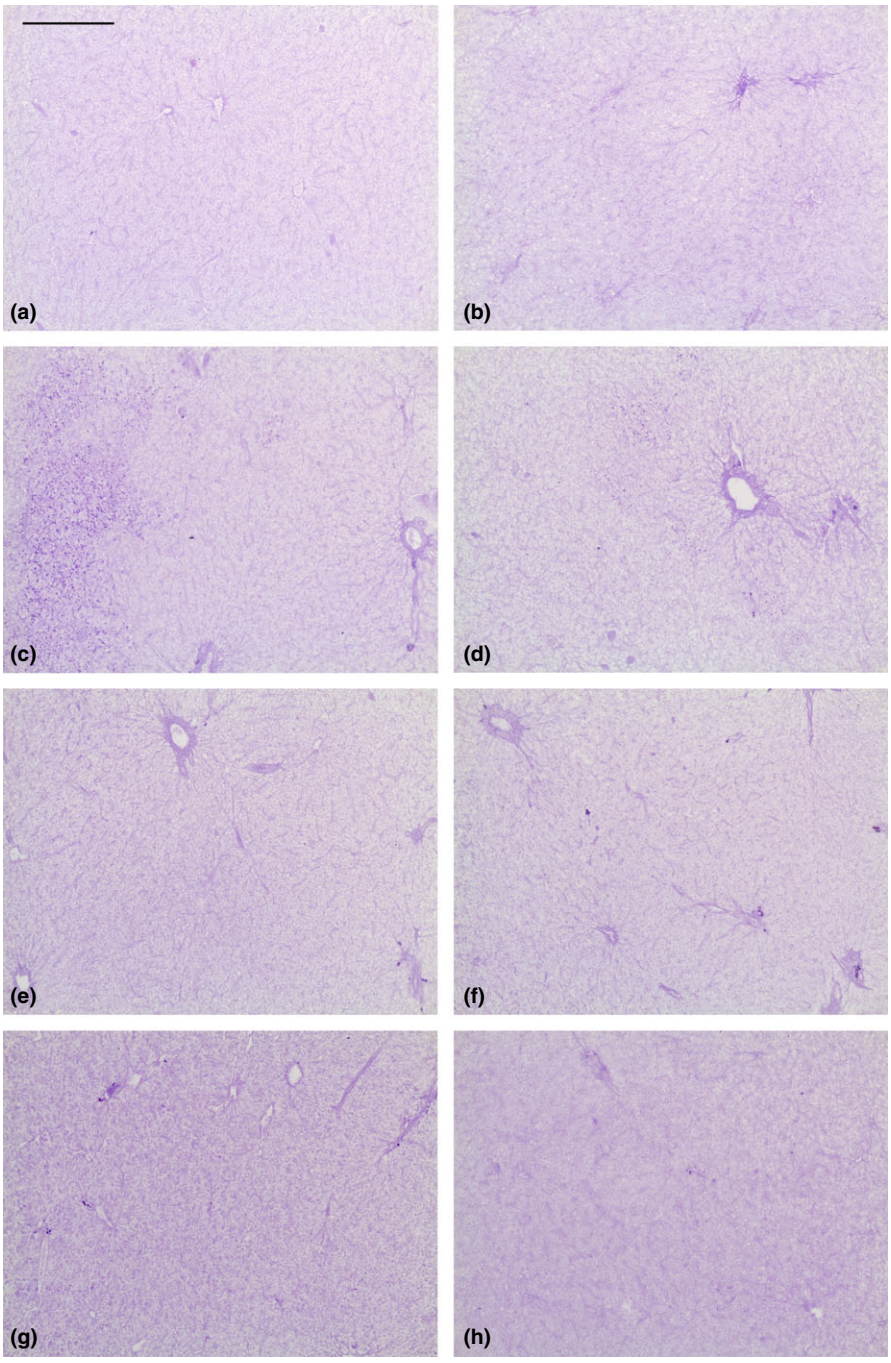

FIGURE 4 Periodic-acid Schiff (PAS) staining of the liver in African catfish after 3 and 6 weeks of culture in the control (a, b) or in biofloc-based systems with raw rice bran (RRB) (c, d), or rice bran treated with *Bacillus* spp. with (ResRB) (e, f) or without aeration (FerRB) (g, h). There was noticeably more PAS-positive material in the liver of fish in the FerRB treatments compared to the others, while in the RRB treatment after 3 weeks, the PAS-positive had a patchier distribution. Arrow bar = 200 μm

only performed to maintain similar conditions with respect to biofloc productivity and other water quality parameters. This was also reflected in the liver histology appearing normal and healthy at both time frames (i.e., 3 and 6 weeks). These findings, along with the highest crude protein of the bioflocs produced from FerRB, likely indicate that heterotrophic bacteria could better convert this more soluble source into microbial protein. It is important to note that the biofloc protein level varies among studies, which can be as low as 23% (Bauer, Prentice-Hernandez, Tesser, Wasielesky, & Poersch, 2012; López-Elías et al., 2015) or as high as 58% (Crab, Chielens, Wille, Bossier, & Verstraete, 2010). However, Crab et al. (2010) found that the combined use of glycerol and *Bacillus* spp. substantially increased the crude protein content of bioflocs compared to only using glycerol. This finding, along with those of this study, indicates that *Bacillus* spp. can elevate the biofloc crude protein and thus potentially improve the feasibility to replace traditional dietary protein sources with bioflocs (Crab et al., 2010; Kuhn, Lawrence, Crockett, & Taylor, 2016) and/or provide direct nutrition to the animal for those that can effectively consume these particles.

Previous studies have described *C. gariepinus* juveniles as being inefficient collectors and consumers of bioflocs and the reason for no growth improvement when cultured under BFT conditions, regardless of the carbon source that included rice bran, molasses,

glycerol, or sucrose (Dauda, Romano, Chen, Ikhsan, & Kamarudin, 2018; Dauda, Romano, Ebrahimi, et al., 2018; Ekasari et al., 2014). In this study, however, the use of FerRB significantly improved both the growth and feeding efficiencies of *C. gariepinus* juveniles. Moreover, *C. gariepinus* cultured with FerRB also had a higher whole-body crude protein and liver glycogen storage than the other treatments. It has been shown that additions of *Bacillus* spp. to the water can improve the growth of *C. gariepinus* (El-Haroun, 2007). However, considering the same probiotics were used to create ResRB, which led to the lowest growth performance, may not fully explain this discrepancy. Another factor may include the consumption of bioflocs to a certain extent by *C. gariepinus* considering the bioflocs produced by FerRB had a significantly higher crude protein level than the others.

In contrast, RRB significantly increased the whole-body lipid content of *C. gariepinus* as well as elevating their plasma glucose and triglyceride levels. While this could be viewed as indications of more available energy due to better nutrition, this was not supported by the liver glycogen or growth data. In fact, this seemingly contradictory response was previously observed in *C. gariepinus* when using RRB as a carbon source compared to sucrose or glycerol in biofloc-based systems (Dauda et al., 2017). Research regarding biochemical changes in animals cultured in biofloc-based systems with different carbon sources are still limited, however, one study showed that the use of complex carbohydrates with BFT significantly decreased the crude lipid content in *L. vannamei* compared to the use of sugarcane molasses or control (Rajkumar et al., 2016). Considering the influence of different carbon sources have on the biochemical composition of animals and thus product quality, more research should be performed. Some of these research directions could include different carbon sources on biofloc ingestion rates by aquatic animals as well as biofloc size and composition.

5 | CONCLUSIONS

The use of FerRB as a carbon source in a biofloc-based system effectively removed ammonia-N, produced bioflocs with a higher crude protein content and significantly enhanced the growth and feeding efficiencies of *C. gariepinus*. These findings should have a particular significance based on previous research showing no improvement to the productivity of *C. gariepinus* when other carbon sources were tested that were linked to their more carnivorous feeding habits. Therefore, the feasibility of BFT when using fermented complex carbohydrates on other poor filter-feeding species should be explored. If also successful, this would broaden the applications of BFT to more species and thus could further expand the aquaculture industry in a more sustainable way.

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CONFLICTS OF INTEREST

The authors declared that there is no conflict of interest.

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