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Effect of Distillery Spent Wash as Carbon Source in Biofloc System on Nutrient Profile of GIFT Tilapia

P. Yuvarajan[1], Antony Cheryl[1], A. Gopalakannan[2], N. Mahadevi[1]

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ABSTRACT

Background: Biofloc technology (BFT) is an ecofriendly aquaculture production system. In this, various carbon sources being used for floc production. However, the cheapest carbon source would make it economically feasible. In this context, the present study used purified distillery spent wash (DSW) as a carbon source to evaluate its viability on biofloc composition and carcass quality of GIFT strain.

Methods: Nursery trial was conducted in high density poly ethylene (HDPE) outdoor lined pond (0.01 ha) for period of 30 days (8th January to 7th February 2018). In both BFT and control system, proximate composition and fatty acid profile of GIFT tilapia were analyzed at the end of the trial by following the standard method.

Result: Dried microbial floc encompassed 16.61 ± 1.2% of crude protein and 15.3 ± 0.01% of linoleic acid. The Whole-body composition of GIFT was not significantly (P>0.05) varied in between biofloc and control. Significantly (P<0.05) rich fatty acids found in biofloc fed GIFT whole body except stearic acid, behenic acid and docosahexaenoic acid. In the present study, DSW did not show much effect on carcass quality but showed better growth performance of GIFT strain in the BFT system.

Key words: Distillery spent wash, Eicosapentaenoic acid, GIFT tilapia, Low protein diet, Nursery biofloc system, Outdoor lined pond.

INTRODUCTION

The world population is continuously rising with food demand, meanwhile, more than one in nine people are suffering from hunger which leads to food insecurity and malnutrition (WHO, 2018). Hence, aquaculture is one kind of possible food production sector that can supply a nutritious diet to fulfil the food necessity (FAO, 2014). This is fast growing and profit based industry whilst fish price varies according to its production cost and demand. But, tilapia would provide healthy nutrition at an affordable cost (Dey, 2000; Chavan *et al*., 2011). It, is hardy nature, can tolerate a wide range of environmental condition (El-Sayed, 2006), have prolific breeding characteristics so its somatic growth hampered (Gupta, 2004; Mahadevi *et al*., 2019). Considering this issue, the Genetically Improved Farmed Tilapia (GIFT) program was achieved by World Fish Centre through selective breeding of Nile tilapia. GIFT strain has a fast growth rate compared to normal tilapia that driven the rapid progression of tilapia farming (Eknath and Acousta, 1998). Though tilapia intensive farming paves high production cost particularly, feed input cost (Chavan *et al*., 2011) whilst uneaten feed and faecal matters affect the water quality through high nutrient discharge, that prone to cause disease to culture species (Machimbirike *et al*., 2019). To tide over that, advanced technologies could be adopted to increase food production within minimal feed input and without annoying the environment (Emerenciano *et al*., 2017), in this sense, biofloc technology (BFT) could serve as the additional protein source and reduce feed cost (Avnimelech, 1999; Da Silva *et al*., 2018).

Biofloc technology (BFT), is an ecofriendly and limited water exchange system, generates more heterotrophic bacteria than algae (Avnimelech, 2007), act as live food and enhance the water quality. Intensive aeration and addition of carbon source are responsible for biofloc production which was maintained by the carbon-nitrogen ratio (C: N) of 10 to 20:1, which converts waste into microbial protein (Asaduzzaman *et al*., 2008). Cost-effective and locally available carbon source should be selected (Avnimelech, 1999). Molasses is a good source for biofloc production, but it has legal constraints in Tamil Nadu, India due to misusing this source for alcohol production and more cost (Antonyraj, 2016). In this situation, cost-effective distillery spent wash (DSW) was used as a carbon source in the present study. During alcohol production from molasses, DSW was removed as waste material. Raw DSW pollutes the environment when discharged directly. After anaerobic and aerobic treatment of DSW would not harm

[1]Tamil Nadu Jayalalithaa Fisheries University, M.G.R Fisheries College and Research Institute, Ponneri-60 1204, Tamil Nadu, India.
[2]ICAR-Krishi Vigyan Kendra, Tamil Nadu Jayalalithaa Fisheries University, Sikkal-611 108, Tamil Nadu, India.
Email: yuvarajandono@gmail.com

Corresponding Author: P. Yuvarajan, Tamil Nadu Jayalalithaa Fisheries University, M.G.R Fisheries College and Research Institute, Ponneri-60 1204, Tamil Nadu, India.

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the environment (Mohana *et al*., 2009). Hence, treated DSW act as a good carbon source (26%) in biofloc production (Antonyraj, 2016; Liu *et al*., 2017; Yuvarajan *et al*., 2018; Menaga *et al*., 2020).

Biofloc system reduced 20% of commercial feed input (Avnimelech., 2012; Perez – Fuentes *et al*., 2018) due to it serves protein so fishes grew better within a minimal protein diet (Avinimelech, 1999). Hence, the feasibility of protein reduction in BFT tilapia was performed by various authors with ranged from 17 to 35% of crude protein in glass aquaria, fibre reinforced tanks, greenhouse shed farm and circular polyethylene tanks with different carbon sources and the C/N ratio (Azim and Little, 2008; Da Silva *et al.* 2018; Hisano *et al.,* 2019; Sgnaulin *et al*., 2020) and finalized as 28% CP (Da Silva *et al*. 2018; Hisano *et al*., 2019) for tilapia culture in BFT. Usually, nutrient profile of GIFT tilapia is depends on natural food and supplement feed which are responsible for growth and survival of fish. On other hand, biochemical composition differs based on carbon sources and the C/N ratio. Since DSW serve as the cheapest carbon source, the efficacy of BFT microbial protein has to be determined over the nutrient profile of culture species in the outdoor biofloc system. Thus, the present study was aimed to evaluate the efficacy of distillery spent wash in biofloc production and its efficiency on the nutrient profile of GIFT strain.

MATERIALS AND METHODS

Experimental design

Thirty days nursery experiment was conducted in the outdoor lined pond ($10 \times 10 \times 1$ m) at advanced research farm facility (ARFF), Chennai-51, off campus facility of TNJFU - Dr. M.G.R Fisheries College and Research Institute, Ponneri, Tamil Nadu. The duplicate ponds with and without floc were termed as biofloc and control respectively. Biofloc was developed one week before stocking as per the standard protocol of Taw (2006) and Avnimelech (1999) with minor modification (The following materials has been used for floc production (kg/pond): They are fertile soil - 10, urea - 0.11, triple super phosphate - 0.014, commercial feed - 0.8, dolomite - 1.4, corn flour - 0.4, wheat flour - 0.4 and DSW - 3). Purified DSW (26% of organic carbon) procured from M/s. Rajshree Biosolutions Company, Tamil Nadu.

Experiment animal and diet

GIFT tilapia seeds were obtained from the State Fisheries Department, Krishnagiri, Tamil Nadu, India. Seeds were acclimatized and conditioned to the pond environmental condition for one week and fed a commercial diet (17% crude protein). Before the experiment, healthy fishes were stocked at 50 numbers (0.2054±0.02 g/seed) /m^2 in both biofloc and control ponds.

Proximate analysis of biofloc and GIFT tilapia

Proximate composition of biofloc and the whole body of fishes (dry weight basis) were analyzed at the end of the experiment by following the standard method (AOAC, 1995). Crude protein (CP) was calculated by multiplying the total nitrogen with a factor of 6.25. Total nitrogen was estimated by the kjeldahl method (KEL Plus - Classic DX VA). The ether extract was analyzed by soxhlet extractor (SOCS PLUS-SCS 08 AS) using petroleum ether (Boiling point 60-80°C) as a solvent which indicated crude lipid (CL) content. Crude fibre (CF) was analyzed by an automatic fibre analysis system (FIBRA PLUS, FES04ASDLS and FIBRO flow). Fat and moisture free sample (1 g) used for crude fibre extraction. Total ash content was estimated by taking a known weight of dry sample in silica crucible and placing it in a muffle furnace at 550°C for 6 hours, weight difference indicated the ash content. Proximate values were expressed in %.

Fatty acid profiling of biofloc and GIFT tilapia whole body

Fatty acid profiling was done by following standard procedure (AOAC, 1995). Methyl esters prepared and methylated fatty acids were separated using GC-MS (QP2010, Shimadzu, USA) equipped with DB Wax (30m x 0.25 mm internal diameter x 0.25 µm film thickness) capillary column. Helium used as carrier gas. Injector and detector temperatures were set at 250°C. Injection performed in split mode (1:15) with an injection volume of 1 µl FAME. The initial column temperature maintained at 50°c for 2 minutes. Then allow the temperature to reach 230°c and kept for 35 minutes. Fatty acid methyl esters separated at a constant pressure of 82.5 Kpa. After completion of the process, peaks have been identified by comparing mass spectra with mass spectral data to produce fatty acid values, it was expressed in %.

Statistical analysis

The nutrient profile of floc and GIFT tilapia (whole-body) data were analyzed by Independent sample't' test using SPSS Version 24 software.

RESULTS AND DISCUSSION

The nutrient profile of biofloc

Proximate and fatty acid profile results of biofloc have been given in Table 1. In the present study, dried biomass of biofloc contained low crude protein (16.61 ± 0.02%) due to the major influence of diet (17% crude protein) and DSW as carbon source (Da Silva *et al.,* 2018). This result was falling with the report of Da Silva *et al*. (2018) who analyzed biofloc crude protein ranged from 15.09 to 20.31%. On the other hand, GIFT fry growth performance was enhanced in outdoor lined pond biofloc system, which is indicated by significantly ($P<0.05$) better average body weight, food conversion ratio, protein efficiency ratio and 37% more total weight gain compared to control system (Yuvarajan *et al*., 2018). These results were coherent with the findings of Luo *et al*. (2014) and Long *et al.* (2015) in indoor biofloc system based GIFT culture. The availability of balanced protein diet (biofloc) and stress free environment (lower nitrite and ammonia) may be the major reason for the growth performance of GIFT compared to a high CP supplementary diet in the biofloc system. According to Hisano *et al*. (2020), tilapia have been feeding with 36, 32 and 36% of CP diet in nursery BFT (sugar cane molasses used as carbon source) and no significant difference was observed in terms of growth performance among treatments, but, the author suggested that 28% of CP reduced the feed cost and environmental impact. Floc encompassed low crude lipid and high crude fibre. The fibre content may condense the lipid level, the low level of lipid results was similarly reported by Long *et al*. (2015) in GIFT tilapia culture and higher fibre content might influence the other nutrients of floc, similar findings were obtained by Mahanand *et al*. (2013) in the biofloc based rohu culture pond. Total ash content found to be higher in dried biofloc, this result was parallel with Soares *et al.* (2004) report (22-46%) in biofloc system. Out of twelve fatty acids (FAs), palmitic acid (16:0), palmitoleic acid (16:1n-7), oleic acid (18:1) and linoleic acid (18:2n-6) were dominated in biofloc system which is due to the viability of distillery spent wash as carbon source and dominance of heterotrophic bacteria and zooplankton, these results agreed with the findings of Crab *et al.* (2010). Total n-3 (linolenic acid, ecosapentaenoic acid, docosahexaenoic acid) FAs found to be lower which is mainly due to the lack of microalgal communities and substantial increment of heterotrophic bacteria, the results were consonance with Anand *et al.* (2014) results in biofloc system and also Aderme vega *et al.* (2012) found microalgae played a significant role in omega-3 fatty acid production.

Table 1: Proximate composition and fatty acid profile of dried biofloc.

Nutrient profile	Values (%)
Crude protein	16.61 ± 0.02
Crude lipid	1.40 ± 0.03
Crude fibre	16.03 ± 0.11
Total ash	37.53 ± 3.3
Myristic acid (14:0)	2.10 ± 0.1
Palmitic acid (16:0)	27.31 ± 0.02
Stearic acid (18:0)	6.80 ± 1.00
Oleic acid (18:1)	31.21 ± 0.01
Linoleic acid (18:2n-6)	15.30 ± 0.01
Linolenic acid (18:3n-3)	2.32 ± 0.01
Arachidic acid (20:1)	1.60 ± 1.00
Behenic acid (22:0)	3.14 ± 0.02
Ecosapentaenoic acid (20:5n-3)	2.14 ± 0.01
Docosahexaenoic acid (22:6n-3)	2.92 ± 0.02
Palmitoleic Acid (16:1n-7)	9.91 ± 0.02
Others	1.97 ± 0.03

The nutrient profile of GIFT tilapia (whole-body)

Proximate and fatty acid profile results of biofloc have been given in Table 2. No significant difference (P>0.05) was observed in whole-body proximate composition except ether extract whilst slightly higher crude protein found in biofloc compared to control fish which is due to the positive influence floc protein consumption by the fishes. This result was in agreement with Azim and little (2008) analyzed the nutrient profile in Nile tilapia from an indoor biofloc system. The ether extract was significantly (P<0.01) lower in biofloc fed fish than control fish which is due to the availability of live food and probiotic bacteria which have been balanced the lipid content of the supplemental diet. Azim and Little (2008) found similar finding in biofloc based tilapia culture. No significant difference (P>0.05) was observed between the crude fibre of biofloc and control. But crude fibre was slightly higher in biofloc fish rather than control fish which is due to the ingestion of biofloc (16.03 ± 0.11% of CF). A similar result was reported in biofloc based culture of *Penaeus vannamei* by Panigrahi *et al*. (2017). Ash content was increased in biofloc fed fish than control fish, no significant difference (P>0.05) was observed between them. Similar report has been given by Verma *et al*. (2016) in rohu fish body from

Table 2: Proximate composition and fatty acid profile of dried whole fish body acquired from control and biofloc system.

Nutrient profile	Control (%)	Biofloc (%)	P value
Crude protein	51.06 ± 0.46	52.79 ± 0.58	0.213
Crude fibre	0.98 ± 0.01	1.25 ± 0.24	0.128
Ether extract	28.13 ± 0.86	20.88 ± 0.04	0.009**
Total ash	9.50 ± 0.04	10.87 ± 1.06	0.085
Myristic acid (14:0)	1.21 ± 0.03	2.05 ± 0.012	0.04*
Palmitic acid (16:0)	18.54 ± 0.41	18.79 ± 0.17	0.103
Stearic acid (18:0)	4.81 ± 0.26	4.54 ± 0.36	0.224
Oleic acid (18:1)	37.64 ± 1.03	39.10 ± 1.06	0.032*
Linoleic acid (18:2n-6)	26.22 ± 2.03	27.72 ± 3.54	0.421
Linolenic acid (18:3n-3)	1.50 ± 0.3	1.28 ± 0.08	0.086
Arachidic acid (20:1)	1.22 ± 0.17	4.06 ± 0.33	0.019*
Behenic acid (22:0)	2.68 ± 0.51	0.81 ± 0.08	0.026*
Ecosapentaenoic acid (20:5n-3)	0.48 ± 0.4	1.81 ± 0.14	0.042*
Docosahexaenoic acid (22:6n-3)	2.08 ± 0.22	1.42 ± 0.23	0.156
Palmitoleic acid (16:1n-7)	3.10 ± 0.3	5.65± 0.73	0.015*
Others	1.16 ± 0.08	1.02 ± 0.08	0.135

Note: ***P<0.001, ** P<0.01 and * P<0.05 - significant and P>0.05 - Not significant.

biofloc system (sugar bagasse as carbon source). Out of twelve fatty acids, lower values of stearic acid (18:0), behenic acid (22:0) and docosahexaenoic acid (22:6n-3) have been found in biofloc fed fish body compared to control fish which is due to the lack of phytoplankton and dominance of heterotrophs in the culture water. Similarly, Aderme vega *et al*. (2012) reported the importance of the microalgal role in the production of omega-3 fatty acids. Current study found significantly ($P<0.05$) rich fatty acids (myristic acid, arachidic acid and ecosapentaenoic acid) in biofloc than control fish which is due to FAs of biofloc positively influenced the FAs of the fish body through probiotic effect. Similar result was obtained in rohu when the probiotics supplemented with diet (Sinha and Pandey, 2013; Das *et al*., 2021).

CONCLUSION

The present study revealed that DSW is an alternative carbon source for molasses for biofloc production in terms of cost-effectiveness and a low protein diet sufficient for biofloc based GIFT culture in the outdoor lined pond. Bioflocs did not significantly reflect on whole-body nutritional composition because those energy utilized for better growth whilst biofloc fed fish whole-body fatty acids showed the significance of myristic acid, arachidic acid and ecosapentaenoic acid.

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Conflict of interest

The authors declare that they have no conflict of interest.

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