Fermented soybean meal can partially replace fishmeal and improve the intestinal condition of goldfish juveniles reared in a biofloc system

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Abstract

This study evaluated the effects of different dietary inclusion levels of fermented soybean meal (FSM) as replacement for fish meal and their effects on the productive performance and intestinal condition of goldfish (*Carassius auratus*) produced in biofloc (BFT) system. Five isoproteic (39.5% crude protein) and isoenergetic diets (4250 kcal kg−1 of crude energy) were formulated with FSM inclusion levels of 0%, 7%, 14%, 21% and 28% (0, 11, 22, 32 and 43 fish meal replacement). A total of 400 goldfish (0.25 ± 0.02 g) were weighed and distributed in 20 glass aquariums (15 L). Fish were fed twice daily for 56 days. The diet with FSM inclusion level of 28% reduced the weight gain and the specific growth rate and increased the feed conversion of the goldfish. Diets with FSM inclusion levels of 21% and 28% increased the α-amylase activity in the fish intestine. The diet with FSM inclusion level of 21% increased total height of the intestinal villi of the goldfish. In conclusion, the inclusion of up to 21% of FSM can replace fishmeal without affecting the growth of goldfish juveniles reared in BFT system. Fish fed 21% of FSM showed evidence of improvement in the intestinal health.

1. INTRODUCTION

The biofloc technology (BFT) system is an intensive rearing environment with low water renewal that is successfully used in the sustainable production of fish and shrimp (Crab et al., 2012). The input of sources rich in organic carbon in BFT units stimulates the proliferation of heterotrophic microorganisms (Avnimelech, 2007). Heterotrophic bacteria transform nitrogenous compounds present in the cultivation water into microbial biomass that can serve as a supplementary food source for the cultivated species (Avnimelech, 1999; Azim & Little, 2008; Li et al., 2018; Najdegerami et al., 2016). The BFT system has already been validated for different species of fish such as tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*), South American catfish (*Rhamdia quelen*), goldfish (*Carassius auratus*) (Bakar et al., 2015; Battisti et al., 2020; Cunha et al., 2020; Long et al., 2015; Wang et al., 2015). Positive results were obtained in terms of growth, feed conversion (Ahmad et al., 2016; Luo et al., 2014) and survival rate (Ekasari et al., 2015; Pérez-Fuentes et al., 2016).

The intensification of aquaculture production systems can lead to stressful conditions for animals. Prolonged stress may influence the health of the target organism and cause outbreaks of diseases and mortality (Mohapatra et al., 2013). Similar to other intensive systems, the BFT system is prone to husbandry conditions that can cause stress to the animals. These conditions include peaks in nitrogen compounds (Fischer et al., 2020; Serra et al., 2015), low dissolved oxygen levels (Fischer et al., 2020) and an excess in suspended solids (Long et al., 2015; Ray et al., 2010; Romano et al., 2020). In this scenario, the aquaculture feed industry has given focus to developing additives and functional ingredients and meals as a strategy to mitigate the

harmful effects of stress (Dawood et al., 2019, 2020). The use of fermented aquaculture feeds was recently developed and has shown promising results related to fish health in standard rearing systems (Azarm & Lee, 2014; Dawood et al., 2020; Novriadi et al., 2018; Sotoudeh et al., 2016). However, no information exists regarding the use of fermented feeds in BFT systems. The microbial fermentation of foods produces bioactive compounds that offer several health benefits (Hayes & GarcíaVaquero, 2016). During the fermentation process, microorganisms degrade protein macromolecules into soluble compounds of low molecular weight, which are more easily absorbed and digested (Azarm & Lee, 2014). These microorganisms break down protein chains into small chains of amino acids and peptides, which can bring health benefits to the organism (Sanjukta & Rai, 2016). In addition, microbial fermentation produces secondary metabolites with prebiotic action and promotes the growth of beneficial microorganism populations with probiotic functions (Dawood & Koshio, 2020; Mukherjee et al., 2016; Nwachukwu et al., 2019; Zhang et al., 2014). In this scenario, soybean meal is considered an excellent substrate to produce functional feeds due to its low cost and high nutritional value (Olmos et al., 2015). Fermented soybean meal (FSM) has been evaluated as a substitute for fishmeal and as a functional ingredient in aquafeeds (Liet al., 2020; Rahimnejad et al., 2019; Wang et al., 2016). The partial replacement of fishmeal by FSM has shown no negative effects on fish growth (Hassaan et al., 2015; Jiang et al., 2018; Lee et al., 2016; Li et al., 2020; Liang et al., 2017; Rahimnejad et al., 2019). FSM has better digestibility and has shown higher protein content and higher levels of amino acids when compared with raw soybean meal (Liet al., 2019; Olmos et al., 2015; Shiu, Wong, et al., 2015), which may result in better animal growth performance and feed efficiency parameters (Hassaan et al., 2015; Jiang et al., 2018). In addition, numerous beneficial effects of FSM inclusion in diets on fish health have been found, such as: increased antioxidant capacity (Azarm & Lee, 2014; Hassaan et al., 2015; Jiang et al., 2018; Lee et al., 2016; Wang et al., 2016), modulation of microbiota, decreased inflammation and increased intestinal villi size (Li et al., 2020; Novriadi et al., 2018; Rahimnejad et al., 2019; Refstie et al., 2005; Yamamoto et al., 2010), as well as modulating the activity of digestive enzymes (Shiu et al., 2015b; Sotoudeh et al., 2016). Ornamental fish farming generates annually more than 300 million dollars (Mohammad et al., 2018) and is an important source of income for small producers (Goswami & Zade, 2015). The goldfish (*Carassius auratus*) is one of the most popular and produced ornamental fish in the world. This is an omnivorous species (Sales & Janssens, 2003), and its feeding preferences consist mainly of plant materials, debris, benthic organisms, mosquito larvae, microalgae and zooplankton (Penttinen & Holopainen, 1992; Sarbahi, 1951). The replacement of animal protein was not evaluated in the goldfish diet, but for a similar species (*Carassius auratus gibeo*), the inclusion of soybean meal even at lower levels (14.6%) compromised performance (Liu et al., 2016). Goldfish production in BFT systems has shown better control of water quality and improved animal growth when compared with the clear water production system (Besen et al., 2021; Faizullah et al., 2015; Wang et al., 2015). The BFT system has recently shown to increase the activity of digestive enzymes of goldfish (Yu et al., 2020), but the use of alternative feed ingredients for replacing conventional fish meal, as well as ingredients that may promote intestinal health, remains to be evaluated. The potential of using fermented foods for goldfish in a BFT system remains unknown, information that would be of value when testing new functional feed ingredients under sustainable farming systems. Thus, the objective of the present study was to evaluate the effects of different inclusion levels of FSM in the diets as a replacement for fish meal and its effects on the productive performance and intestinal health of goldfish produced in a BFT system.

2. MATERIAL S AND METHODS

The experiment was carried out over a period of 56 days at the Pisciculture Laboratory of the University of the State of Santa Catarina (UDESC), Lages, SC, Brazil. The study was approved by the Ethics Committee on the Use of Animals (CEUA) of UDESC (protocol number 8490131020). The experimental design was completely randomized with five treatments and four replications. The treatments corresponded to the FSM inclusion levels in the diets (0%, 7%, 14%, 21% and 28%).

2.1. Fermented soybean meal

Soybean meal was fermented with inoculants of the lactic acid bacteria *Lactobacillus acidophilus* using the methodology adapted from Azarm and Lee (2014). Isolated inoculum from the brand Aché® was used (*Lactobacillus acidophilus* LA14; 1 × 109 UFC g−1). Soybean meal was purchased from a local supplier and the bacteria from a commercial establishment. Deionized water was added to autoclaved samples (100°C for 20 min) of soybean meal for 50% humidity and inoculated with 44 g of bacteria (109 CFU) for each kilogram of soybean meal. The samples were mixed and placed in trays, maintaining a maximum height of two centimetres of sample per tray. The fermentation of soybean meal was carried out in an incubator at 36°C for 48 h. The humidity was corrected every 12 h, and the samples were mixed again. Afterwards, the fermented material was dried in an incubator (36°C) until reaching constant weight and subsequently kept in a freezer (−20°C). Samples of FSM were collected to analyse the nutritional composition (AOAC, 2000) and their amino acid profile (White et al., 1986) (Table 1). Samples were also collected for analysis of total lactic acid bacteria (Vieira & Tôrres, 2004), Ph (AOAC, 2000), enzyme activity (García-Careño & Haard, 1993; Métais & Bieth, 1968) and soluble protein (Bradford, 1976) (Table 2).

2.2. Experimental diets

Five isoprotein (39.5% crude protein) and isoenergetic diets (ca.4250 kcal kg−1) were formulated (Table 3) according to the nutritional requirements of the goldfish (Bandyopadhyay et al., 2005). FSM was added to diets to substitute fishmeal at the inclusion levels of 0%, 7%, 14%, 21% and 28%, which represented 0%, 11%, 22%, 32% and 43% fish meal replacement respectively. Dietary levels of crude lipids ranged from 12.1% to 8.4%. The adjustment in the lipids levels was necessary to balance the energy levels of the diets. In addition to fermented soybean meal, diets were formulated using fishmeal and soybean meal as the main protein sources and using soybean oil, corn meal and wheat flour as energy sources. Marine fishmeal was purchased from Agroforte® (Laguna, Santa Catarina, Brazil), and the other ingredients were purchased from local suppliers. The diets were supplemented with the amino acids of arginine, lysine (Infinity Pharma®, Campinas, São Paulo, Brazil) and methionine (Florien Fitoativos®, Piracicaba, São Paulo, Brazil) to meet the amino acid requirements of goldfish (Gatlin III, 1987) (Table 4). All of the ingredients were ground in a blender and sieved to obtain particles smaller than 0.71 mm. The ingredients were mixed and pelleted with water (30%) and dried at 36°C in an incubator for 48 h.

TABLE 1 Nutritional composition of the fermented soybean meal (FSM)

	Raw soybean meal	FSM
Centesimal composition (%)		
Crude protein	44.83	43.99
Crude lipid	5.23	4.19
Dry matter	88.35	90.64
Mineral matter	7.42	7.09
Amino acids (%)		
Arginine	3.43	3.47
Histidine	1.23	1.31
Isoleucine	2.18	2.00
Leucine	3.59	3.84
Lysine	2.87	3.14
Methionine	0.62	0.60
Phenylalanine	2.39	2.73
Threonine	1.82	1.98
Valine	2.27	2.38
Sum of the amino acids	20.40	21.45

TABLE 2 Characteristics of the fermented soybean mean (FSM)

	Raw soybean meal	FSM
LAB (log CFU g^{-1})	0.00	5.81
pH	6.70	6.50
EA α-amylase (U g FSM^{-1})	0.00	2.94
EA total alkaline proteases (U g FSM^{-1})	0.16	0.22
SP (mg g FSM^{-1})	24.38	33.49

Abbreviations: EA, enzyme activity; LAB, total lactic acid bacteria; SP, soluble protein.

The diets were then crushed and sieved to obtain the desired particle size for feeding (~1.5 mm) and were stored in a freezer (−20°C) until use in the experiment. The chemical composition of the diets was analysed according to the AOAC (2000).

2.3. Animals and facilities

A total of 400 goldfish (0.25 ± 0.02 g, mean ± standard deviation) were obtained from our laboratory broodstock held at Pisciculture Laboratory (UDESC, Brazil). Goldfish were acclimated to the experimental conditions for 14 days. During this period, the fish were kept in aquaria (30 L of functional volume; 40 fish per aquarium) connected to a water recirculation system equipped with a mechanical and biological filter. Fish were fed twice daily (at 10:00 and 16:00) until apparent satiation with the control diet formulated for the experiment (Table 1). At the end of the acclimatization period, goldish were weighed individually and distributed in 20 glass aquariums (15 L of functional volume) at a density of 20 fish per aquarium. Fish were fed twice daily as previously described. Biometric analyses were performed every 2 weeks to adjust the feed ration in relation to the stocked biomass. Feed was administered at a rate of 10% of the live weight per day for the first 2 weeks, 8% of the live weight per day for the following 2 weeks and 6% of the live weight per day in the last 2 weeks (Wang et al., 2015). Bioflocs were produced prior to the experimental period to obtain a mature culture media and stable water quality. The production of the heterotrophic medium was carried out in a 500 L tank with Nile tilapia (*Oreochromis niloticus*) that were fed with commercial feed (40% crude protein and 5 mm pellet diameter; Supra, Alisul Alimentos S.A., Brazil). The water temperature was kept constant (±22°C) using heaters equipped with thermostats, and a compressor system provided the aeration. This system was assembled to provide an up-flow air injection in the tank, which gentle stirred the water and kept the bioflocs in suspension. The C:N ratio was 20:1, which is considered as ideal for the Nile Tilapia (PérezFuentes et al., 2016). The system was fertilized daily with molasses (38% carbon) as the organic carbon source as described in Schryver et al. (2008). This methodology is based on the assumption that fish assimilate approximately 25% of the nitrogen from the feed and that the remaining 75% are converted to total ammonia nitrogen (TAN) in the water. At the beginning of the experiment, 50% of the useful volume of the aquaria was water obtained from a BFT system in which Nile tilapia was cultured, and the other 50% of the volume was clear water. Molasses (38% carbon) was added every 3 days (at 14:00) in each aquarium as

an additional carbon source to maintain a C/N ratio of 20:1 (Wang et al., 2015), according to the calculations proposed by Schryver et al. (2008). No water renewal was performed, but water was added to compensate for evaporation. The temperature was kept constant using heaters with thermostats, and the water was oxygenated using porous stones connected to silicone hoses (4 mm) and an air compressor. The hoses were glued to the bottom of the aquariums to force air to be injected from the bottom up, allowing for greater movement of the water. The photoperiod was 12 h of light and 12 h of darkness.

TABLE 3 Formulation and nutritional composition of the experimental diets for goldish (*Carassius auratus*) reared in biofloc

	Experimental diets				
Ingredients (% dry matter)	0%	7%	14%	21%	28%
FSM	0.00	7.00	14.00	21.00	28.00
Fishmeal	65.00	58.00	51.50	45.00	39.00
Cornmeal	7.70	7.70	8.10	8.10	8.40
Wheat bran	12.00	12.00	12.00	12.00	12.00
Soybean meal	7.00	7.00	7.00	7.00	7.00
Soybean oil	6.50	6.50	5.50	5.00	3.50
Premix[a]	1.00	1.00	1.00	1.00	1.00
Arginine	0.80	0.70	0.60	0.50	0.50
Lysine	0.00	0.00	0.00	0.10	0.20
Methionine	0.00	0.10	0.30	0.30	0.40
Total	100	100	100	100	100
Nutritional composition					
Dry matter (%)	92.37	92.61	92.75	92.95	93.16
Crude protein (%)	40.64	40.01	39.79	39.43	39.47
Crude energy (kcal kg^{-1})	4241.33	4282.86	4261.21	4272.91	4217.44
Crude lipid (%)	12.07	11.84	10.67	9.98	8.35

[a]Folic acid – 2400 mg, nicotinic acid – 48 g, pantothenic acid – 24 g, biotin – 96 mg, vit. A – 2400,000 IU, vit. D3–400,000 IU, vit. E – 24,000 IU, vit. B1 – 9600 mg, vit. B2 – 9600 mg, vit. B6 – 9600 mg, vit. B12 – 9600 mg, vit K3 – 4800 mg, vit. C – 96 g, iron – 100 g, manganese – 40 g, zinc – 6000 mg, cobalt – 20 mg, iodine – 200 mg, selenium – 200 mg, antioxidant – 19.6 g.

TABLE 4 Amino acid composition of experimental diets for goldish (*Carassius auratus*) reared in biofloc

	Experimental diets					
Amino acids (%)	0%	7%	14%	21%	28%	Requirements[a]
Arginine	2.88	2.83	2.80	2.77	2.85	2.72
Histidine	1.17	1.15	1.14	1.13	1.12	1.04
Isoleucine	2.62	2.50	2.41	2.31	2.23	1.59
Leucine	3.74	3.65	3.60	3.54	3.50	2.99
Lysine	3.82	3.66	3.52	3.48	3.47	3.43
Methionine	1.25	1.26	1.39	1.31	1.34	1.23
Phenylalanine	2.02	2.02	2.04	2.06	2.09	1.64
Threonine	1.96	1.91	1.87	1.84	1.84	1.84
Valine	2.75	2.65	2.57	2.48	2.42	1.79

[a]Requirements of amino acids for the goldfish (Gatlin III, 1987).

2.4. Water quality

Temperature, pH (Hanna HI98130) and dissolved oxygen (Hanna HI9147-10) were monitored daily. Total ammonia nitrogen (TAN), nitrite, nitrate, total suspended solids (TSS) (Rice et al., 2012) and turbidity (Hanna HI93703C) were monitored weekly. Salinity was maintained at around 4 g−1 (Luz et al., 2008) in all aquariums throughout the experimental period. The water quality parameters remained stable throughout the experiment, and the average values are listed in Table 5. All parameters remained within the recommended ranges for fish farming (Avnimelech, 2012; Boyd, 1998).

TABLE 5 Water quality parameters (mean ± standard deviation)

	Level of FSM inclusion in experimental diets				
	0%	7%	14%	21%	28%
Temp.	25.5 ± 0.7	25.3 ± 0.4	25.4 ± 0.8	25.7 ± 0.6	25.5 ± 0.9
DO	7.2 ± 0.3	7.1 ± 0.3	7.4 ± 0.1	7.1 ± 0.2	7.2 ± 0.3
pH	8.3 ± 0.0	8.3 ± 0.1	8.4 ± 0.1	8.4 ± 0.0	8.4 ± 0.1
TAN	0.1 ± 0.2	0.1 ± 0.1	0.2 ± 0.2	0.3 ± 0.3	0.1 ± 0.1
NO_2^-	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
NO_3^-	249.7 ± 180.0	248.2 ± 179.7	244.1 ± 179.6	233.5 ± 166.6	255.0 ± 188.3
Turb.	264.5 ± 168.7	268.1 ± 169.5	277.9 ± 189.2	281.5 ± 212.0	270.4 ± 191.1
TSS	538.4 ± 424.8	536.0 ± 418.6	556.4 ± 415.7	530.2 ± 392.4	570.9 ± 446.1

Note: Temp. (°C) = temperature, DO ($mg\,L^{-1}$) = dissolved oxygen, TAN ($mg\,L^{-1}$) = total ammonia nitrogen, NO_2^- ($mg\,L^{-1}$) = nitrite, NO_3^- ($mg\,L^{-1}$) = nitrate, Turb. (NTU) = turbidity, TSS ($mg\,L^{-1}$) = total suspended solids.

2.5. Productive performance and sample collection

At the beginning of the experiment and at 56 days, all fish were fasted for 24 h, anesthetized with eugenol (50 mg L−1) (Bittencourt et al., 2012) and weighed individually. Productive performance was analysed based on the following parameters: weight gain (WG = final average weight (g) − initial average weight (g)), specific growth rate (SGR, % day−1 = [(ln final weight (g) − ln initial weight (g))/experimental period] * 100) and apparent feed conversion rate (FCR = feed administration in grams/total weight gain). Mortality was recorded to assess the survival rate (S, % = [total animals at the end/total animals at the beginning] * 100). After weighing at 56 days, some animals were anesthetized with eugenol (50 mg L−1) and then euthanized by spinal section to collect biological materials for the analyses described below.

2.6. Microbiological analyses

The digestive tract of one fish from each aquarium (*n* = 4 fish per treatment) was sampled to assess the intestinal microbiota using classical methods adapted from Vieira and Tôrres (2004). The intestines were removed, weighed, ground, homogenized and diluted serially (1:10) in test tubes containing sterile saline (0.65%). Then, the intestinal homogenates were seeded in Petri dishes with MRS (Man Rogosa Sharpe) agar and TSA (tryptic soy agar) to quantify total lactic acid and heterotrophic bacteria respectively. The intestinal homogenates seeded in the Petri dishes were placed in an incubator at 35°C. Colony-forming units (log CFU) were counted after 24 h of incubation in the TSA medium and after 48 h in the MRS medium.

2.7. Intestinal morphometry

Histological analysis of the intestine was performed on two fish from each aquarium (*n* = 8 fish per treatment). Portions of approximately 3 cm in length were collected from the midgut, and each sample was fixed in a 10% buffered formalin solution for 24 h, dehydrated in an ascending series of alcohols, diaphanized in xylene, embedded in paraffin and cut into sections of 5 µm for slide preparation. These samples were then stained according to the PAS (periodic acidSchiff) staining method. The slides were observed under an optical microscope (OptiCam, 10×) and photographed using a digital camera (Moticam 2300, 3 MP, resolution 3264 × 2448 pixels). Eight villi per animal were selected by integrity criteria. The total height and width of the villi (Figure 1) were measured using the ToupTek ToupView- x64 image analyser software, version 2270/07/03.

2.8. Enzymatic analyses

The activities of α-amylase and total alkaline proteases were assessed using the intestines of two fish from each aquarium (n = 8 fish per treatment). After euthanasia, fish were immediately placed on ice and dissected to separate the intestine. The intestines were washed with distilled water and immediately frozen at −80°C until the analysis. At the time of analysis, the intestines were cut into small pieces and placed in 2 ml Eppendorf tubes, where they were diluted in cold distilled water (1:10, w/v). The Eppendorf tubes were submitted to ultrasonic baths for 5 min (5 times of 1 min with intervals of 1 min in an ice bath) for rupture of intestinal cells and release of digestive enzymes. Subsequently, the intestinal homogenates were centrifuged at 7000 rpm for 10 min (4°C), and the supernatants were separated and used to determine the activity of digestive enzymes.

Alpha-amylase activity was measured at λ = 580 nm using soluble starch dissolved (0.3%) in a Na2HPO4 buffer solution (pH 7.4) as substrate (Métais & Bieth, 1968). A unit of α-amylase activity (U) was defined as the amount of enzyme that catalyses the hydrolysis of 1 mg of starch in 30 min at 37°C per millilitre of enzymatic extract. Total alkaline protease activity was determined after 30 min of incubation at 25°C, using 0.5% (w/v) casein as a substrate in 50 mM Tris–HCl (pH 8.0). The reaction was stopped with trichloroacetic acid (20% w/v), the extract was centrifuged (5000 rpm, 20 min), and the absorbance of the supernatant was measured at λ = 280 nm at room temperature. A unit of protease activity (U) was defined as the amount of enzyme needed to catalyse the hydrolysis of 1 µmol of hydrolysed casein per minute per millilitre of enzymatic extract (García-Careño & Haard, 1993). The same procedures were used for quantifying the activity of both enzymes in FSM.

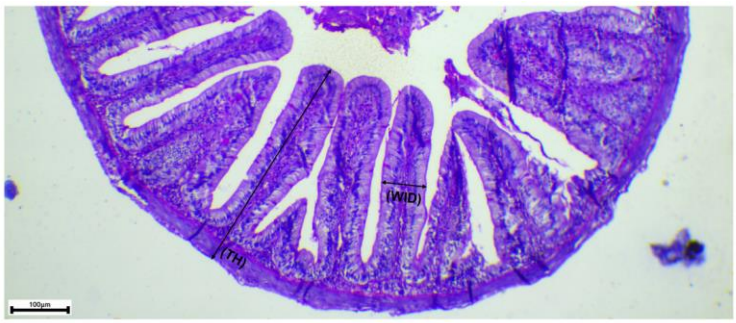


FIGURE 1 Method used to measure the total height (TH) and width (WID) (µm) of intestinal villi of goldfish.

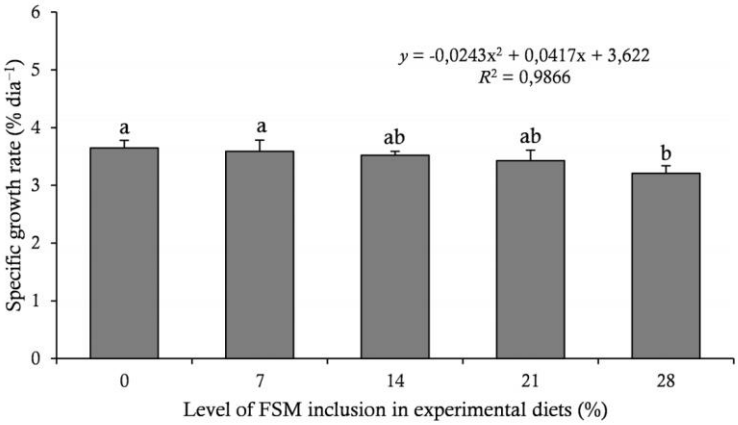


FIGURE 2 Specific growth rate (% day^{-1}) (mean ± standard deviation; n = 4) of goldfish fed with diets containing different inclusion levels of FSM in a BFT system for 56 days. Means followed by different letters differed by Duncan's test (p < 0.05).

2.9. Statistical analyses

All data were subjected to tests of error normality (Shapiro–Wilk) and variances homoscedasticity (Levene). The percentage values were arcsine transformed prior their analysis. The results were analysed using the parametric analysis of variance (ANOVA) and using a corresponding test to compare the means at the 5% level of significance. When significant difference was shown, the parameters and inclusion levels of FSM in the diet were analysed using polynomial regression. These analyses were performed using the statistical software Statistic 7® (STATSOFT, 2005).

3. RESULTS

3.1. Productive performance

Fish performance indicators in terms of growth and feed efficiency were significantly affected by the level of FSM in diets for goldfish reared in a BFT system. In particular, the 28% FSM negatively impacted on goldfish weight gain and the specific growth rate, whereas it also increased the apparent feed conversion when compared with the control diet (Figure 2; Table 6; $p < 0.05$). Fish survival rate was over 95% in all dietary groups, and no significant differences in this parameter were found between treatments ($p > 0.05$).

3.2. Levels of lactic acid and heterotrophic bacteria

In the microbiological analysis of the intestine (Table 7), the amount of lactic acid bacteria showed a tendency not statistically significant ($p = 0.1163$) to increase in treatments with the inclusion of FSM. The inclusion of FSM in the diets had no influence on the concentration of total heterotrophic bacteria in the fish intestine ($p > 0.05$).

3.3. Intestinal morphometry and digestive enzyme activity

The highest values of total villi height were observed in goldfish fed the diet with 21% FSM, whereas the lowest ones were found in the 0% and 28% FSM groups. The rest of the experimental groups showed intermediate values (Figure 3; $p < 0.05$). The inclusion of FSM in the diets had no influence ($p > 0.05$) on the width of the intestinal villi of the fish.

Diets with the FSM inclusion levels of 21% and 28% increased the total activity of α-amylase in the intestine of goldfish (Figure 4; $p < 0.05$). The inclusion of FSM in the diets had no influence on the activity of total alkaline proteases among dietary groups ($p > 0.05$).

TABLE 6 Productive performance of the goldfish (mean ± standard deviation; $n = 4$) fed with diets containing different inclusion levels of FSM in a BFT system for 56 days

	Level of FSM inclusion in experimental diets					
	0%	7%	14%	21%	28%	*p* value
WG	1.58 ± 0.13a	1.51 ± 0.28ab	1.43 ± 0.10ab	1.35 ± 0.13ab	1.16 ± 0.13b	0.0272
FCR	1.82 ± 0.09b	1.94 ± 0.11b	2.00 ± 0.07ab	2.00 ± 0.11ab	2.17 ± 0.08a	0.0019
S	98.75 ± 2.50	98.75 ± 2.50	97.50 ± 2.89	100.00 ± 0.00	96.25 ± 4.79	0.4735

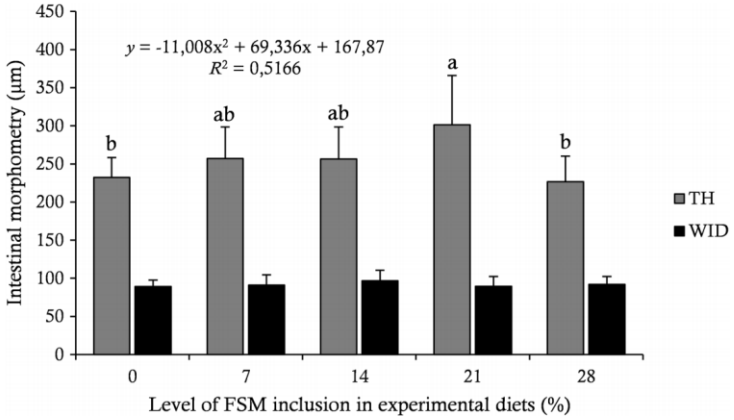
Note: Different letters in the same row represent statistically significant differences among dietary groups (Tukey post-hoc test; $p < 0.05$). WG (g): weight gain ($y = -3.4985x^2 - 0.449x + 1.5717$; $R^2 = 0.9886$), FCR: apparent feed conversion rate ($y = 0.5831x^2 + 0.9224x + 1.8397$; $R^2 = 0.9054$) and S (%): survival.

TABLE 7 Concentration of bacteria per gram of intestine (log CFU g^{-1}) (mean ± standard deviation; $n = 4$) of goldfish fed with diets containing different inclusion levels of FSM in a BFT system for 56 days

	Level of FSM inclusion in experimental diets					
	0%	7%	14%	21%	28%	*p* value
MRS	1.50 ± 0.33	3.22 ± 1.03	2.78 ± 0.80	2.64 ± 0.76	2.40 ± 0.35	0.1163
TSA	6.56 ± 0.34	6.83 ± 1.28	5.82 ± 0.52	5.93 ± 0.40	6.34 ± 0.51	0.4082

Note: No differences were shown between the means according to the ANOVA test ($p > .05$). Abbreviations: MRS, Man Rogosa Sharpe (lactic acid bacteria); TSA, tryptic soy agar (total heterotrophic bacteria).

FIGURE 3 Average intestinal morphometry (μm) (mean ± standard deviation; $n = 8$) of goldfish fed with diets containing different inclusion levels of FSM in a BFT system for 56 days. Means followed by different letters differed by Duncan's test ($p < 0.05$).



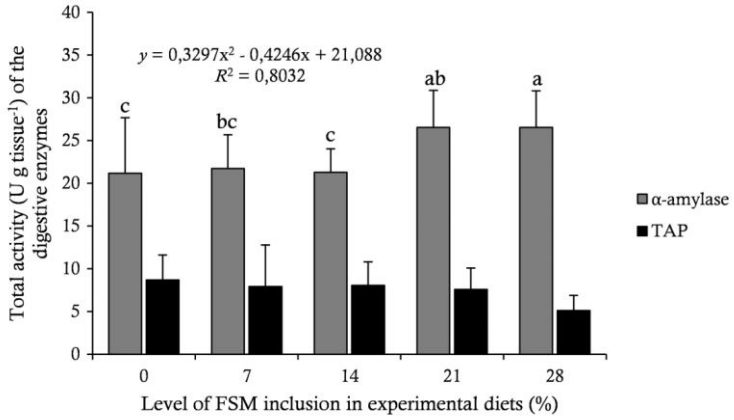


FIGURE 4 Total activity (U g $tissue^{-1}$) of the digestive enzymes (mean ± standard deviation; $n = 8$) in the intestine of goldfish intestine fed with diets containing different inclusion levels of FSM in a BFT system for 56 days. Means followed by different letters indicate differences by Duncan's test ($p < 0.05$). TAP, Total alkaline protease.

4. DISCUSSION

The water quality parameters remained within adequate levels for the production of goldfish in a BFT system (Cunha et al., 2020; Wang et al., 2015; Yu et al., 2020). The addition of mature BFT inoculants in the experimental units prevented the occurrence of peaks of nitrogen compounds.

Nitrate, turbidity and SST increased gradually over the course of the experiment, but with no evident negative impact on fish health. All water quality parameters were similar between the experimental groups throughout the experimental period. This is evidence that the results obtained in the present study are due to the supplementation of FSM in the diets and were not influenced by the conditions of the BFT system. In the present experimental conditions using BFT, the inclusion of up to 21% of FSM (32% fish meal replacement) in goldfish diets had no influence on the growth performance and apparent feed conversion values. This is the first study evaluating the inclusion of FSM in diets for this ornamental fish species and validates the hypothesis that fermentation is a valuable strategy for enabling higher dietary levels of inclusion of soybean meal in substitution for fish meal in aquafeeds. During fermentation, there were changes in the characteristics of soybean meal that may have contributed to the maintenance of results similar to those obtained with fish meal. There was an increase in intestinal lactic acid bacteria, which are used as a probiotic in fish feed (Selle et al., 2014). The increase in soluble protein indicates a higher proportion of lowmolecular-eight peptides, which can positively affect health and performance (Ha et al., 2019; Khosravi et al., 2015). Furthermore, it should be noted that the effects of the FSM may have been enhanced using the BFT system, and this still needs to be better understood. In addition to the effects of direct ingestion by the fish, probiotic microorganisms and bioactive compounds present in fermented products can also affect the quality of bioflocs, with secondary effects on fish performance and health (Bañuelos-Vargas et al., 2021; Kathia et al., 2018). The diet with the highest level of inclusion of FSM (28%) reduced growth performance and increased feed conversion values in goldfish. Similarly, FSM inclusion levels between 21% and 39% resulted in decreased performance and increased feed conversion of other fish species, such as the grouper (*E. coioides*) (Shiu et al., 2015b), the Japanese sea bass (*Lateolabrax japonicus*) (Rahimnejad et al., 2019), the rainbow trout (*Oncorhynchus mykiss*) (Choi et al., 2020) and the largemouth bass (*Micropterus salmoides*) (He et al., 2020). Considering that the amino acid requirements were met in all treatments, indicating no problems in relation to possible deficiencies, we hypothesize that an excess of soluble protein in the diet from the greater inclusion of FSM may have caused the decrease in goldfish performance. High inclusions of soluble peptides and amino acids may have led to saturation of the intestinal transport mechanisms (Cahu et al., 1999; Tonheim et al., 2005; Wei et al., 2020), which can result in an unbalanced absorption of amino acids and a reduction in the use of dietary protein (Aragão et al., 2004; Kolkovski & Tandler, 2000). Further research regarding the effects of high dietary levels of FSM on nutrient absorption and digestibility needs to be conducted in order to further understand the poorer results obtained by the 28% FSM, with regard to growth and feed utilization indices. Goldfish fed diets with FSM showed a tendency, even though not significant, to increase the amount of *Lactobacillus* sp. in the intestine when compared with the control treatment. Recent studies suggest that supplementing diets with FSM improves the composition of intestinal microbiota in fish (Catalán et al., 2018; He et al., 2020; Li et al., 2020). Although most fermentation bacteria are killed during the drying process of FSM, residues of dead bacteria and their metabolites may promote improvements in the intestinal microbiota in fish and potentially have a pre and probiotic effect on the host (Dawood & Koshio, 2020; He et al., 2020). The use of inactivated microorganisms, known as paraprobiotics, has shown similar results when compared with the administration of live probiotics (Choudhury & Kamilya, 2019). The microbiological results of the present study can be considered preliminary, with the need for further validation. The small number of samples (n = 4) limited the degrees of freedom of the ANOVA and may have influenced the absence of significant differences between treatments. However, the potential beneficial effects of rearing fish using BFT (Abakari et al., 2021; Santos et al., 2021) may have also masked the positive effects of FSM on enhancing acid lactic bacterial

populations in goldfish gut due to the microbial characteristics of the BFT system (Avnimelech, 2007). In this sense, further studies with more robust microbiological analyses using massive sequencing techniques are necessary to describe the different types of microorganisms and confirm the results obtained.

The FSM inclusion level of 21% increased the height of the intestinal villi of the goldfish. This results in a larger surface for nutrient absorption and may improve the integrity of the intestinal epithelium of fish. Previous studies have shown that fermentation of soybean meal protects fish from possible morphological damage to the intestine (i.e., enteritis), which is usually induced by antinutritional compounds and allergens present in raw soybean meal (Choi et al., 2020; He et al., 2020; Wang et al., 2016). The present study is the first to observe an improvement in the intestinal morphometry of fish fed with FSM. The increase in the height of the villi may be attributed to the possible presence of metabolites with prebiotic function in the FSM (Dawood & Koshio, 2020). In this sense, the increase in villi height may be linked to an improvement in intestinal immunity barriers (Dawood, 2021; Pirarat et al., 2011). The BFT system can also improve intestinal health (Long et al., 2015), and the relationship between the intestinal and environmental bacteria may have optimized the results. It is still necessary to evaluate the effect of fermented products on the composition and quality of bioflocs. Fish fed diets with FSM inclusion levels of 21% and 28% showed higher α-amylase activity when compared with the control diet. The activity of digestive enzymes in fish is influenced by several factors, such as feeding habits, diet composition and presence of antinutritional factors (Hidalgo et al., 1999; Li et al., 2019; Penttinen & Holopainen, 1992; Sarbahi, 1951). The enzymatic activity of α-amylase increased with the use of FSM when compared with raw soybean meal, which may be attributed to a reduction in ANFs in FSM. In addition, previous studies reported that the use of exogenous enzymes in diets increases the intestinal enzymatic activity of fish (Kumar et al., 2006; Lin et al., 2007; Zhou et al., 2013). Thus, in this context, the higher amylolytic activity in FSM in comparison to the raw soybean meal may also be responsible for the higher α-amylase activity values in goldfish fed diets containing the higher inclusion levels of FSM (21% and 28%). Supplementation with FSM in diets had no effects on the activity of total alkaline proteases in goldfish. This is a positive result when considering that raw soybean meal contains antinutritional factors and allergens that can decrease the activity of proteolytic enzymes in fish (Li et al., 2019; Liu et al., 2017; Shiu et al., 2015b; Zhang et al., 2018). Fermentation of soybean meal may reduce antinutritional factors (Li et al., 2019), but FSM inclusion levels of over 14% decreased the protease activity in the intestine of the rainbow trout (Choi et al., 2020). Physiological and feeding habit differences between species and quality and composition of FSM between studies may explain the differences among studies. Further investigation is needed to understand how fermentation of ingredients affects antinutritional factors and intestinal enzyme activity in fish.

5. CONCLUSION

The inclusion of up to 21% of FSM can replace fishmeal up to 32%without affecting the growth performance and feed efficiency values of goldfish juveniles reared in BFT system, whereas higher FSM inclusion levels negatively affected growth and feed performance variables. Replacing fish meal by 21% inclusion of improved the intestinal condition of goldfish as data on enzyme activity and intestinal morphometry indicated.

AUTHOR CONTRIBUTIONS

Larissa da Cunha: Conceptualization; Methodology; Validation; Formal analysis; Investigation; Writing - Original Draft; Writing- Review and Editing and Visualization. **Kayane Pereira Besen**: Methodology; Validation and Investigation. **Nandara Soares de Oliveira**: Methodology; Validation and Investigation. **Fernanda Regina Delziovo**: Methodology; Validation and Investigation. **Rafaela Gomes**: Methodology; Validation and Investigation. **Júlia Montibeller da Cruz**: Investigation. **Fernanda Picoli**: Methodology; Validation and Investigation. **Enric Gisbert**: Methodology; Validation; Writing - Review and Editing. **Everton Skoronski**: Methodology; Validation; Writing - Review and Editing; Investigation and Resources. **Thiago El Hadi Perez Fabregat**: Conceptualization; Methodology; Validation; Formal analysis; Resources; Writing - Original Draft; Writing - Review and Editing; Supervision and Project administration.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

Abakari, G., Luo, G., Shao, L., Abdullateef, Y., & Cobbina, S. J. (2021). Effects of biochar on microbial community in biofloc and gut of *Oreochromis niloticus* reared in a biofloc system. *Aquaculture International*, *29*, 1295–1315. https://doi.org/10.1007/s10499-021-00697-3

Ahmad, H. I., Verma, A. K., Babitha Rani, A. M., Rathore, G., Saharan, N., & Gora, A. H. (2016). Growth, non-specific immunity and disease resistance of *Labeo rohita* against *Aeromonas hydrophila* in biofloc systems using different carbon sources. *Aquaculture*, *457*, 61–67. https://doi.org/10.1016/j.aquaculture.2016.02.011

Aragão, C., Conceição, L. E. C., Fyhn, H. J., & Dinis, M. T. (2004). Estimated amino acid requirements during early ontogeny in fish with different life styles: Gilthead seabream (*Sparus aurata*) and Senegalese sole (*Solea senegalensis*). *Aquaculture*, *242*, 589–605. https://doi.org/10.1016/j.aquaculture.2004.09.015

Association of Official Analytical Chemists (AOAC). (2000). *Official methods of analysis* (17th ed.). Author. Avnimelech, Y. (1999). Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, *176*, 227–235. https://doi.org/10.1016/s0044-8486(99)00085-x

Avnimelech, Y. (2007). Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. *Aquaculture*, *264*, 140–147. https://doi.org/10.1016/j.aquaculture.2006.11.025

Avnimelech, Y. (2012). *Biofloc technology: A practical guide book* (2nd ed.). The World Aquaculture Society https://lib.ugent.be/catalog/rug01:002180973

Azarm, H. M., & Lee, S. (2014). Effects of partial substitution of dietary fish meal by fermented soybean meal on growth performance, amino acid and biochemical parameters of juvenile black sea bream *Acanthopagrus schlegeli*. *Aquaculture Research*, *45*, 994–1003. https://doi.org/10.1111/are.12040

Azim, M. E., & Little, D. C. (2008). The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *283*, 29–35. https://doi.org/10.1016/j.aquaculture.2008.06.036

Bakar, N. S. A., Nasir, N. M., Lananan, F., Hamid, S. H. A., Lam, S. S., & Jusoh, A. (2015). Optimization of C/N ratios for nutrient removal in aquaculture system culturing African catfish, (*Clarias gariepinus*) utilizing bioflocs technology. *International Biodeterioration & Biodegradation*, *102*, 100–106. https://doi.org/10.1016/j.ibiod.2015.04.001

Bandyopadhyay, P., Swain, S., & Mishra, S. (2005). Growth and dietary utilization in goldfish (*Carassius auratus* Linn.) fed diets formulated with various local agro-produces. *Bioresource Technology*, *96*, 731–740. https://doi.org/10.1016/j.biortech.2004.06.018

Bañuelos-Vargas, I., de Oca, G. A. R. M., Martínez-Montaño, E., PérezJiménez, A., Mendoza-Gamboa, O. A., Estrada-Godínez, J. A., & Hernández, C. (2021). Antioxidant and immune response of juvenile red tilapia (*Oreochromis* sp) cultured at different densities in sea water with biofloc plus probiotics. *Aquaculture*, *544*, 737112. https://doi.org/10.1016/j.aquaculture.2021.737112

Battisti, E. K., Rabaioli, A., Uczay, J., Sutili, F. J., & Lazzari, R. (2020). Effect of stocking density on growth, hematological and biochemical parameters and antioxidant status of silver catfish (*Rhamdia quelen*) cultured in a biofloc system. *Aquaculture*, *524*, 735213. https://doi.org/10.1016/j.aquaculture.2020.735213

Besen, K. P., Cunha, L., Delziovo, F. R., Melim, E. W. H., Cipriani, L. A., Gomes, R., Skoronski, E., & Fabregat, T. E. H. P. (2021). Goldfish (*Carassius auratus*) larviculture in biofloc systems: Level of *artemia* nauplii, stocking density and concentration of the bioflocs. *Aquaculture*, *540*, 736738. https://doi.org/10.1016/j.aquaculture.2021.736738

Bittencourt, F., Souza, B. E., Boscolo, W. R., Rorato, R. R., Feiden, A., & Neu, D. H. (2012). Benzocaine and eugenol as anesthetics for golden fish (*Carassius auratus*). *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *64*, 1597–1602. https://doi.org/10.1590/S0102-09352012000600028

Boyd, C. E. (1998). *Water quality for pond aquaculture*. Auburn University, ICAAE.

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Cahu, C. L., Zambonino Infante, J. L., Quazuguel, P., & Le Gall, M. M. (1999). Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass *Dicentrarchus labrax* larvae. *Aquaculture*, *171*, 109–119. https://doi.org/10.1016/S0044-8486(98)00428-1

Catalán, N., Villasante, A., Wacyk, J., Ramírez, C., & Romero, J. (2018). Fermented soybean meal increases lactic acid bacteria in gut microbiota of Atlantic Salmon (*Salmo salar*). *Probiotics and Antimicrobial Proteins*, *10*, 566–576. https://doi.org/10.1007/s12602-017-9366-7

Choi, D. G., He, M., Fang, H., Wang, X. L., Li, X. Q., & Leng, X. J. (2020). Replacement of fish meal with two fermented soybean meals in diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*, *26*, 37–46. https://doi.org/10.1111/anu.12965

Choudhury, T. G., & Kamilya, D. (2019). Paraprobiotics: An aquaculture perspective. *Reviews in Aquaculture*, *11*, 1258–1270. https://doi.org/10.1111/raq.12290

Crab, R., Defoirdt, T., Bossier, P., & Verstraete, W. (2012). Biofloc technology in aquaculture: Beneficial effects and future challenges. *Aquaculture*, *356-357*, 351–356. https://doi.org/10.1016/j.aquaculture.2012.04.046

Cunha, L., Besen, K. P., Ha, N., Uczay, J., Skoronski, E., & Fabregat, T. E. H. P. (2020). Biofloc technology (BFT) improves skin pigmentation ofgoldfish (*Carassius auratus*). *Aquaculture*, *522*, 735132.https://doi.org/10.1016/j.aquaculture.2020.735132

Dawood, M. A. O. (2021). Nutritional immunity of fish intestines: Important insights for sustainable aquaculture. *Reviews in Aquaculture*, *13*, 642–663. https://doi.org/10.1111/raq.12492

Dawood, M. A. O., Eweedah, N. M., Khalafalla, M. M., Khalid, A., El Asely, A., Fadl, S. E., Amin, A. A., Paray, B. A., & Ahmed, H. A. (2020). *Saccharomyces cerevisiae* increases the acceptability of Nile tilapia (*Oreochromis niloticus*) to date palm seed meal. *Aquaculture Reports*, *17*, 100314. https://doi.org/10.1016/j.aqrep.2020.100314

Dawood, M. A. O., & Koshio, S. (2020). Application of fermentation strategy in aquafeed for sustainable aquaculture. *Reviews in Aquaculture*, *12*, 987–1002. https://doi.org/10.1111/raq.12368

Dawood, M. A. O., Koshio, S., Abdel-Daim, M. M., & Doan, H. V. (2019). Probiotic application for sustainable aquaculture. *Reviews in Aquaculture*, *11*, 907–924. https://doi.org/10.1111/raq.12272

Ekasari, J., Rivandi, D. R., Firdausi, A. P., Surawidjaja, E. H., Zairin, M., Jr., Bossier, P., & De Schryver, P. (2015). Biofloc technology positively affects Nile tilapia (*Oreochromis niloticus*) larvae performance. *Aquaculture*, *441*, 72–77. https://doi.org/10.1016/j.aquaculture.2015.02.019

Faizullah, M., Rajagopalsamy, C. B. T., Ahilan, B., & Francis, T. (2015). Impact of biofloc technology on the growth of goldfish young ones. *Indian Journal of Science and Technology*, *8*, 1–8. https://doi.org/10.17485/ijst/2015/v8i13/54060

Fischer, H., Romano, N., Renukdas, N., Egnew, N., Sinha, A. K., & Ray, A. J. (2020). The potential of rearing juveniles of bluegill, *Lepomis macrochirus*, in a biofloc system. *Aquaculture Reports*, *17*, 100398. https://doi.org/10.1016/j.aqrep.2020.100398

García-Careño, F. L., & Haard, N. F. (1993). Characterization of proteinase classes in langostilla (*Pleuroncodes planipes*) and crayfish (*Pacifastacus astacus*) extracts. *Journal of Food Biochemistry*, *17*, 97–113. https://doi.org/10.1111/j.1745-4514.1993.tb00864.x

Gatlin, D. M., III. (1987). Whole-body amino acid composition and comparative aspects of amino acid nutrition of the goldfish, golden shiner and fathead minnow. *Aquaculture*, *60*, 223–229. https://doi.org/10.1016/0044-8486(87)90289-4

Goswami, C., & Zade, V. S. (2015). Analysis of international trade and economical & commercial scope of ornamental fishes. *International Journal of Applied Science and Engineering*, *2*, 257929 https://media.neliti.com/media/publications/257929-analysis-of-internationaltrade-and-econ-294f9c14.pdf

Ha, N., Jesus, G. F. A., Goncalves, A. F. N., Oliveira, N. S., Sugai, J. K., Pessatti, M. L., Mourino, J. L. P., & Fabregat, T. E. H. P. (2019). Sardine (*sardinella* spp.) protein hydrolysate as growth promoter in south American catfish (*Rhamdia quelen*) feeding: Productive performance, digestive enzymes activity, morphometry and intestinal microbiology. *Aquaculture*, *500*, 99–106. https://doi.org/10.1016/j.aquaculture.2018.10.004

Hassaan, M. S., Soltan, M. A., & Abdel-Moez, A. M. (2015). Nutritive value of soybean meal after solid state fermentation with *Saccharomyces cerevisiae* for Nile tilapia, *Oreochromis niloticus*. *Animal Feed Science and Technology*, *201*, 89–98. https://doi.org/10.1016/j.anifeedsci.2015.01.007

Hayes, M., & García-Vaquero, M. (2016). Bioactive compounds from fermented food products. In K. Ojha & B. Tiwari (Eds.), *Novel food fermentation technologies*. Spring International Publishing. https://doi.org/10.1007/978-3-319-42457-6_14

He, M., Li, X., Poolsawat, L., Guo, Z., Yao, W., Zhang, C., & Leng, X. (2020). Effects of fish meal replaced by fermented soybean meal on growth performance, intestinal histology and microbiota of largemouth bass (*Micropterus salmoides*). *Aquaculture Nutrition*, *26*, 1058–1071. https://doi.org/10.1111/anu.13064

Hidalgo, M. C., Urea, E., & Sanz, A. (1999). Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. *Aquaculture*, *170*, 267–283. https://doi.org/10.1016/S0044-8486(98)00413-X

Jiang, Y., Zhao, P. F., Lin, S. M., Tang, R. J., Chen, Y. J., & Luo, L. (2018). Partial substitution of soybean meal with fermented soybean residue in diets for juvenile largemouth bass, *Micropterus salmoides*. *Aquaculture Nutrition*, *24*, 1213–1222. https://doi.org/10.1111/anu.12659

Kathia, C. M., del Carmen, M. D. M., Aida, H. P., Jorge, C. M., Félix, A. G. J., & Amadeo, B. M. J. (2018). Effect of two probiotics on bacterial community composition from biofloc system and their impact on survival and growth of tilapia (*Oreochromis niloticus*). *International Journal of Fisheries and Aquatic Studies*, *6*(2), 525–533.

Khosravi, S., Bui, H. T., Rahimnejad, S., Herault, M., Fournier, V., Kim, S. S., Jeong, J. B., & Lee, K. J. (2015). Dietary supplementation of marine protein hydrolysates in fish-meal based diets for red sea bream (*Pagrus major*) and olive flounder (*Paralichthys olivaceus*). *Aquaculture*, *435*, 371–376. https://doi.org/10.1016/j.aquaculture.2014.10.019

Kolkovski, S., & Tandler, A. (2000). The use of squid protein hydrolysate as a protein source in microdiets for gilthead seabream *Sparus aurata* larvae. *Aquaculture Nutrition*, *6*, 11–15. https://doi.org/10.1046/j.1365-2095.2000.00125.x

Kumar, S., Sahu, N. P., Pal, A. K., Choudhury, D., & Mukherjee, S. C. (2006). Studies on digestibility and digestive enzyme activities in *Labeo rohita* (Hamilton) juveniles: Effect of microbial α-amylase supplementation in non-gelatinized or gelatinized corn-based diet at two protein levels. *Fish Physiology and Biochemistry*, *32*, 209–220. https://doi.org/10.1007/s10695-006-9002-z

Lee, S. M., Azarm, H. M., & Chang, K. H. (2016). Effects of dietary inclusion of fermented soybean meal on growth, body composition, antioxidant enzyme activity and disease resistance of rockfish (*Sebastes schlegeli*). *Aquaculture*, *459*, 110–116. https://doi.org/10.1016/j.aquaculture.2016.03.036

Li, C., Zhang, B., Liu, C., Zhou, H., Wang, X., Mai, K., & He, G. (2020). Effects of dietary raw or *enterococcus faecium* fermented soybean meal on growth, antioxidant status, intestinal microbiota, morphology, and inflammatory responses in turbot (*Scophthalmus maximus* L.). *Fish & Shellfish Immunology*, *100*, 261–271. https://doi.org/10.1016/j.fsi.2020.02.070

Li, H. D., Han, D., Zhu, X. M., Yang, Y. X., Jin, J. Y., Xie, S. Q., & Liu, H. K. (2018). Effect of biofloc technology on water quality and feed utilization in the cultivation of gibel carp (*Carassius auratus gibelio* var. CAS III). *Aquaculture Research*, *49*, 2852–2860. https://doi.org/10.1111/are.13748

Li, J., Zhou, R. L., Ren, Z. Q., Fan, Y. W., Hu, S. B., Zhuo, C. F., & Deng, Z. Y. (2019). Improvement of protein quality and degradation of allergen in soybean meal fermented by *Neurospora crassa*. *LWT – Food Science and Technology*, *101*, 220–228. https://doi.org/10.1016/j.lwt.2018.10.089

Liang, X. F., Hu, L., Dong, Y. C., Wu, X. F., Qin, Y. C., Zheng, Y. H., Shi, D. D., Xue, M., & Liang, X. F. (2017). Substitution of fish meal by fermented soybean meal affects the growth performance and flesh quality of Japanese seabass (*Lateolabrax japonicus*). *Animal Feed Science and Technology*, *229*, 1–12. https://doi.org/10.1016/j.anifeedsci.2017.03.006

Lin, S., Mai, K., & Tan, B. (2007). Effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, *Oreochromis niloticus* x *O. aureus*. *Aquaculture Research*, *38*, 1645–1653. https://doi.org/10.1111/j.1365-2109.2007.01825.x

Liu, H., Jin, J., Zhu, X., Han, D., Yang, Y., & Xie, S. (2017). Effect of substitution of dietary fish meal by soybean meal on different sizes of gibel carp (*Carassius auratus gibelio*): Digestive enzyme gene expressions and activities, and intestinal and hepatic histology. *Aquaculture Nutrition*, *23*, 129–147. https://doi.org/10.1111/anu.12375

Liu, H., Zhu, X., Yang, Y., Han, D., Jin, J., & Xie, S. (2016). Effect of substitution of dietary fishmeal by soya bean meal on different sizes of gibel carp (*Carassius auratus gibelio*): Nutrient digestibility, growth performance, body composition and morphometry. *Aquaculture Nutrition*, *22*, 142–157. https://doi.org/10.1111/anu.12239

Long, L., Yang, J., Li, Y., Guan, C., & Wu, F. (2015). Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*). *Aquaculture*, *448*, 135–141. https://doi.org/10.1016/j.aquaculture.2015.05.017

Luo, G., Gao, Q., Wang, C., Liu, W., Sun, D., Li, L., & Tan, H. (2014). Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system. *Aquaculture*, *422*, 1–7. https://doi.org/10.1016/j.aquaculture.2013.11.023

Luz, R. K., Martínez-Álvarez, R. M., Pedro, N., & Delgado, M. J. (2008). Growth, food intake regulation and metabolic adaptations in goldfish (*Carassius auratus*) exposed to different salinities. *Aquaculture*, *276*, 171–178. https://doi.org/10.1016/j.aquaculture.2008.01.042

Métais, P., & Bieth, J. (1968). Détermination de l'a-amylase. *Annales de Biologie Clinique*, *26*, 133–142.

Mohammad, T., Moulick, S., & Mukherjee, C. K. (2018). Economic feasibility of goldfish (*Carassius auratus* Linn.) recirculating aquaculture system. *Aquaculture Research*, *49*, 2945–2953. https://doi.org/10.1111/are.13750

Mohapatra, S., Chakraborty, T., Kumar, V., DeBoeck, G., & Mohanta, K. N. (2013). Aquaculture and stress management: A review of probiotic intervention. *Journal of Animal Physiology and Animal Nutrition*, *97*, 405–430. https://doi.org/10.1111/j.1439-0396.2012.01301.x

Mukherjee, R., Chakraborty, R., & Dutta, A. (2016). Role of fermentation in improving nutritional quality of soybean meal - review. *AsianAustralasian Journal of Animal Sciences*, *29*, 1523–1529. https://doi.org/10.5713/ajas.15.0627

Najdegerami, E. H., Bakhshi, F., & Lakani, F. B. (2016). Effects of biofloc on growth performance, digestive enzyme activities and liver histology of common carp (*Cyprinus carpio* L.) fingerlings in zero-water exchange system. *Fish Physiology and Biochemistry*, *42*, 457–465. https://doi.org/10.1007/s10695-015-0151-9

Novriadi, R., Rhodes, M., Powell, M., Hanson, T., & Davis, D. A. (2018). Effects of soybean meal replacement with fermented soybean meal on growth, serum biochemistry and morphological condition of liver and distal intestine of Florida pompano *Trachinotus carolinus*. *Aquaculture Nutrition*, *24*, 1066–1075. https://doi.org/10.1111/anu.12645

Nwachukwu, U., George-Okafor, U., Ozoani, U., & Ojiagu, N. (2019).Assessment of probiotic potentials of *lactobacillus plantarum* CS and *Micrococcus luteus* CS from fermented milled corn-soybean waste-meal. *Scientific African*, *6*, e00183. https://doi.org/10.1016/j.sciaf.2019.e00183

Olmos, A. R., Bru, E., & Garro, M. S. (2015). Optimization of fermentation parameters to study the behavior of selected lactic cultures on soy solid state fermentation. *International Journal of Food Microbiology*, *196*, 16–23. https://doi.org/10.1016/j.ijfoodmicro.2014.11.030

Penttinen, O. P., & Holopainen, I. J. (1992). Seasonal feeding activity and ontogenetic dietary shifts in crucian carp, *Carassius carassius*. In W. Wieser, F. Schiemer, A. Goldschmidt, & K. Kotrschal (Eds.),

Environmental biology of European cyprinids. Springer Development Environment and Biology Fishes. https://doi.org/10.1007/978-94-011-2544-4_19

Pérez-Fuentes, J. A., Hernández-Vergara, M. P., Pérez-Rostro, C. I., & Fogel, I. (2016). C:N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. *Aquaculture*, *452*, 247–251. https://doi.org/10.1016/j.aquaculture.2015.11.010

Pirarat, N., Pinpimai, K., Endo, M., Katagiri, T., Ponpornpisit, A., Chansue, N., & Maita, M. (2011). Modulation of intestinal morphology and immunity in nile tilapia (*Oreochromis niloticus*) by *lactobacillus rhamnosus* GG. *Research in Veterinary Science*, *91*, e92–e97. https://doi.org/10.1016/j.rvsc.2011.02.014

Rahimnejad, S., Lu, K., Wang, L., Song, K., Mai, K., Davis, D. A., & Zhang, C. (2019). Replacement of fish meal with *bacillus pumillus* SE5 and *Pseudozyma aphidis* ZR1 fermented soybean meal in diets for Japanese seabass (*Lateolabrax japonicus*). *Fish & Shellfish Immunology*, *84*, 987–997. https://doi.org/10.1016/j.fsi.2018.11.009

Ray, A. J., Lewis, B. L., Browdy, C. L., & Leffler, J. W. (2010). Suspended solids removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems. *Aquaculture*, *299*, 89–98. https://doi.org/10.1016/j.aquaculture.2009.11.021

Refstie, S., Sahlström, S., Bråthen, E., Baeverfjord, G., & Krogedal, P. (2005). Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). *Aquaculture*, *246*, 331–345. https://doi.org/10.1016/j.aquaculture.2005.01.001

Rice, E. W., Baird, R. B., Eaton, A. D., & Clesceri, L. S. (2012). *Standard methods for the examination of water and wastewater* (22nd ed.). American Public Health Association, American Water Works, Water Environment Federation. Romano, N., Surratt, A., Renukdas, N., Monico, J., Egnew, N., & Sinha, A. K. (2020). Assessing the feasibility of biofloc technology to largemouth bass *Micropterus salmoides* juveniles: Insights into their welfare and physiology. *Aquaculture*, *520*, 735008. https://doi.org/10.1016/j.aquaculture.2020.735008

Sales, J., & Janssens, G. P. J. (2003). Nutrient requirements of ornamental fish. *Aquatic Living Resources*, *16*, 533–540. https://doi.org/10.1016/j.aquliv.2003.06.001

Sanjukta, S., & Rai, A. K. (2016). Production of bioactive peptides during soybean fermentation and their potential health benefits. *Trends in Food Science & Technology*, *50*, 1–10. https://doi.org/10.1016/j.tifs.2016.01.010

Santos, D. K. M., Kojima, J. T., Santana, T. M., de Castro, D. P., Serra, P. T., Dantas, N. S. M., Fonseca, F. A. L., Mariúba, L. A. M., & Gonçalves, L. U. (2021). Farming tambaqui (*Colossoma macropomum*) in static clear water versus a biofloc system with or without *Bacillus subtilis* supplementation. *Aquaculture International*, *29*, 207–218. https://doi.org/10.1007/s10499-020-00618-w

Sarbahi, D. S. (1951). Studies of the digestive tracts and the digestive enzymes of the goldfish, *Carassius auratus* (Linnaeus) and the largemouth black bass, *Micropterus salmoides* (Lacépède). *The Biological Bulletin*, *100*, 244–257. https://doi.org/10.2307/1538534

Schryver, P., Crab, R., Defoirdt, T., Boon, N., & Verstraete, W. (2008). The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture*, *277*, 125–137. https://doi.org/10.1016/j.aquaculture.2008.02.019

Selle, K. M., Klaenhammer, T. R., & Russel, W. M. (2014). Lactobacillus acidophilus. In C. A. Batt & M. L. Tortorello (Eds.), *Encyclopedia of food microbiology* (2nd ed., pp. 412–417). Academic Press Elsevier. https://doi.org/10.1016/B978-0-12-384730-0.00179-8

Serra, F. P., Gaona, C. A., Furtado, P. S., Poersch, L. H., & Wasielesky, W. (2015). Use of different carbon sources for the biofloc system adopted during the nursery and grow-out culture of *Litopenaeus vannamei*. *Aquaculture International*, *23*, 1325–1339. https://doi.org/10.1007/s10499-015-9887-6

Shiu, Y. L., Hsieh, S. L., Guei, W. C., Tsai, Y. T., Chiu, C. H., & Liu, C. H. (2015). Using *Bacillus subtilis* E20-fermented soybean meal as replacement for fish meal in the diet of orange-spotted grouper (*Epinephelus coioides*, Hamilton). *Aquaculture Research*, *46*, 1403–1416. https://doi.org/10.1111/are.12294

Shiu, Y. L., Wong, S. L., Guei, W. C., Shin, Y. C., & Liu, C. H. (2015). Increase in the plant protein ratio in the diet of white shrimp, *Litopenaeus vannamei* (Boone), using *Bacillus subtilis* E20-fermented soybean meal as a replacement. *Aquaculture Research*, *46*, 382–394. https://doi.org/10.1111/are.12186

Sotoudeh, E., Moghaddam, J. A., Shahhosseini, G., & Aramli, M. S. (2016). Effect of dietary gamma-irradiated and fermented soybean meal on the growth performance, body composition, and digestive enzymes activity of Caspian brown trout, *Salmo trutta caspius*, juvenile. *Journal of the World Aquaculture Society*, *47*, 830–842. https://doi.org/10.1111/jwas.12297

Statsoft, I. (2005). *STATISTICA (data analysis software system), version 7.1*. Statsoft, Inc. www.statsoft.com

Tonheim, S. K., Espe, M., Hamre, K., & Rønnestad, I. (2005). Pre-hydrolysis improves utilisation of dietary protein in the larval teleost Atlantic halibut (*Hippoglossus hippoglossus* L.). *Journal of Experimental Marine Biology and Ecology*, *321*, 19–34. https://doi.org/10.1016/j.jembe.2004.12.036

Vieira, R. H. S. F., & Tôrres, R. C. O. (2004). Contagem padrão em placas (CPP) de microrganismos aeróbios, viáveis. In R. H. S. F. Vieira (Ed.), *Microbiologia, higiene e qualidade do pescado: teoria e prática*. São Paulo, SP.

Wang, G., Yu, E., Xie, J., Yu, D., Li, Z., Luo, W., Qiu, L., & Zheng, Z. (2015). Effect of C/N ratio on water quality in zero-water exchange tanks and the biofloc supplementation in feed on the growth performance of crucian carp, *Carassius auratus*. *Aquaculture*, *443*, 98–104. https://doi.org/10.1016/j.aquaculture.2015.03.015

Wang, L., Zhou, H., He, R., Xu, W., Mai, K., & He, G. (2016). Effects of soybean meal fermentation by *lactobacillus plantarum* P8 on growth, immune responses, and intestinal morphology in juvenile turbot (*Scophthalmus maximus* L.). *Aquaculture*, *464*, 87–94. https://doi.org/10.1016/j.aquaculture.2016.06.026

Wei, Y., Liang, M., & Xu, H. (2020). Fish protein hydrolysate affected amino acid absorption and related gene expressions of IGF-1/AKT pathways in turbot (*Scophthalmus maximus*). *Aquaculture Nutrition*, *26*, 145–155. https://doi.org/10.1111/anu.12976

White, A., Hart, R. J., & Fry, J. C. (1986). An evaluation of the waters Picotag system for the amino-acid analysis of food materials. *Journal of Automatic Chemistry*, *8*, 170–177. https://doi.org/10.1155/S1463924686000330

Yamamoto, T., Iwashita, Y., Matsunari, H., Sugita, T., Furuita, H., Akimoto, A., Okamatsu, K., & Suzuki, N. (2010). Influence of fermentation conditions for soybean meal in a non-fish meal diet on the growth performance and physiological condition of rainbow trout *Oncorhynchus mykiss*. *Aquaculture*, *309*, 173–180. https://doi.org/10.1016/j.aquaculture.2010.09.021

Yu, Z., Li, L., Zhu, R., Li, M., Duan, J., Wang, J. Y., Liu, Y., & Wu, L. F. (2020). Monitoring of growth, digestive enzyme activity, immune response and water quality parameters of Golden crucian carp (*Carassius auratus*) in zero-water exchange tanks of biofloc systems. *Aquaculture Reports*, *16*, 100283. https://doi.org/10.1016/j.aqrep.2020.100283

Zhang, C., Rahimnejad, S., Wang, Y. R., Lu, K., Song, K., Wang, L., & Mai, K. (2018). Substituting fish meal with soybean meal in diets for Japanese seabass (*Lateolabrax japonicus*): Effects on growth, digestive

enzymes activity, gut histology, and expression of gut inflammatory and transporter genes. *Aquaculture*, *483*, 173–182. https://doi.org/10.1016/j.aquaculture.2017.10.029

Zhang, S., Shi, Y., Zhang, S., Shang, W., Gao, X., & Wang, H. (2014). Whole soybean as probiotic lactic acid bacteria carrier food in solid-state fermentation. *Food Control*, *41*, 1–6. https://doi.org/10.1016/j.foodcont.2013.12.026

Zhou, Y., Yuan, X., Liang, X. F., Fang, L., Li, J., Guo, X., Bai, X., & He, S. (2013). Enhancement of growth and intestinal flora in grass carp: The effect of exogenous cellulase. *Aquaculture*, *416-417*, 1–7. https://doi.org/10.1016/j.aquaculture.2013.08.023